

RESEARCH/INVESTIGACIÓN

OCCURRENCE, DISTRIBUTION, AND ABUNDANCE OF PLANT-PARASITIC NEMATODES ASSOCIATED WITH KHAT (*CATHA EDULIS* FORSK) IN EAST HARARGHE ZONE, ETHIOPIA

Awol Seid^{1,3*}, Muluken Gofishu¹, Lemma Degebassa¹, and Tesfamariam Mekete²

¹Haramaya University, College of Agriculture and Environmental Sciences, School of Plant Sciences, P. O. Box 138, Dire Dawa, Ethiopia; ²University of Florida, Department of Entomology and Nematology, Gainesville, FL 32607, USA; ³Ghent University, Faculty of Sciences, Department of Nematology, K.L. Ledeganckstraat 35, 9000 Gent, Belgium. *Corresponding author: awolseid07@gmail.com

ABSTRACT

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Plant-parasitic nematodes have received little research attention in the tropics particularly in sub-Saharan Africa as compared to temperate countries. Despite the daily use and consumption of khat (*Catha edulis* Forsk) by millions of people across Ethiopia and other countries, very little is known about the associated plant-parasitic nematodes. Consequently, this survey was initiated to study the occurrence, distribution, and abundance of plant-parasitic nematodes associated with *C. edulis* in East Hararghe Zone, Ethiopia. A total of 300 composite soil and root samples (150 each) were collected from 15 major khat-growing districts in East Hararghe Zone during the 2013/14 growing season. This survey documented eight plant-parasitic nematode genera: *Criconema* spp., *Helicotylenchus* spp., *Hemicyclophora* spp., *Longidorus* spp., *Meloidogyne* spp., *Paratylenchus* spp., *Pratylenchus* spp., and *Rotylenchulus* spp. associated with khat crop. Among these, *Pratylenchus*, *Meloidogyne*, *Helicotylenchus*, and *Longidorus* were the most frequently encountered and abundant plant-parasitic nematode genera with 80, 60, 53.3, and 46.6% frequency of occurrence from soil, respectively. This diversity could be attributed to the cropping pattern of khat, which is mostly intercropped with solanaceous and other vegetables (mainly tomato, pepper, cabbage, potato, and sweet potato), cereals (mainly maize and sorghum), and groundnut by Hararghe farmers. These findings suggest that producers need to monitor and carefully select their cropping pattern. Furthermore, research should focus on the damage potential of these nematode species on khat crop performance to determine the economic threshold level and estimate a cost-benefit analysis on management practices.

Key words: abundance, cropping pattern, distribution, khat, occurrence, prominence value.

RESUMEN

Seid, A., M. Gofishu, L. Degebassa, y T. Mekete. 2015. Presencia, distribución, y abundancia de nematodos parásitos de plantas asociados con Qat (*Catha edulis* Forsk) en la zona este de Hararghe, Etiopía. *Nematropica* 45:208-214.

Los nematodos parásitos de plantas han sido poco estudiados en los trópicos, y en particular en el África subsahariana, en comparación con los países de clima templado. A pesar del consumo y uso diario del qat (*Catha edulis* Forsk) por millones de personas en Etiopía y otros países, se conoce muy poco sobre sus nematodos parásitos de plantas asociados. En consecuencia, este muestreo se inició para estudiar la presencia, distribución, y abundancia de los nematodos parásitos de plantas asociados a *Catha edulis* en la zona este de Hararghe, Etiopía. Un total de 300 muestras compuestas de suelo y raíces (150 de cada) se recolectaron en 15 distritos productores de qat en la zona este de Hararghe durante el periodo de cultivo 2013/14. En este muestreo se documentan ocho géneros de nematodos parásitos de plantas: *Criconema* spp., *Helicotylenchus* spp., *Hemicyclophora* spp., *Longidorus* spp., *Meloidogyne* spp., *Paratylenchus* spp., *Pratylenchus* spp., y *Rotylenchulus* spp. asociados al cultivo del qat. Entre ellos, *Pratylenchus*, *Meloidogyne*, *Helicotylenchus*, y *Longidorus* fueron los más frecuentemente encontrados, así como los géneros de nematodos parásitos de plantas más abundantes con 80, 60, 53.3, y 46.6% de frecuencia de aparición en suelo, respectivamente. Esta diversidad puede ser atribuida al modelo de cultivo del qat seguido por los agricultores de Hararghe, el cual se cultiva intercalado con solanáceas

y otras verduras (principalmente tomate, pimiento, col, patata, y boniato), cereales (principalmente maíz y sorgo), y cacahuete. Estos resultados sugieren que los productores necesitan monitorizar y seleccionar cuidadosamente sus modelos de cultivo. Además, se necesitan investigaciones centradas en el daño potencial que estas especies de nematodos puedan causar en el cultivo del qat para determinar el umbral económico de daño y estimar un análisis coste-beneficio de las prácticas de manejo.

Palabras clave: abundancia, modelo de cultivo, distribución, qat, presencia, valor de prominencia.

INTRODUCTION

Khat (*Catha edulis*) is an evergreen perennial shrub that belongs to the Celastraceae family. Khat originated from Ethiopia, specifically in Hararghe zones with a gradual expansion to different areas of Ethiopia, Yemen, and other parts of the world (Huffnagel, 1961). High clay content soil is not suitable for khat production. It needs well-drained, dark red-brown, sandy loam with a low percentage of clay (Murphy, 1959). In Ethiopia, khat is an important and potentially lucrative cash crop. Currently, it is the fourth largest export earnings crop accounting for 200 million USD annually (Central Statistics Authority, 2012/13). Although non-users both in rural and urban areas condemn the practice of chewing khat, the number of people who use this plant is increasing, particularly among the youth. In urban areas, chewing khat is becoming a common leisure activity (Dechassa, 2001). Millions of Ethiopians, regardless of religion, education, profession, philosophy, occupation, and gender consume khat (Gessesse, 2013).

The total land area under khat cultivation in Ethiopia was estimated at 78,570 ha (Central Statistics Authority, 1997/98). The state of Oromia, mainly the east and west Hararghe zones, are the most important center of khat production. The East Hararghe zone alone contributes 53.4% of the total production in Ethiopia (Dechassa, 2001). Hararghe is considered to be the most important producer of quality khat in the world (Peter, 1952). East Hararghe is also known for its extensive khat production and the dependence of the people on the crop. East Hararghe has a well-developed khat infrastructure due to the development of a khat culture that includes adequate roads and proximity to market centers that are vital for successful production of the commodity. Thus, the employment opportunities such as growing, harvesting, sorting, packing, transporting, loading, and unloading the commodity created through khat cultivation is very high (Dechassa, 2001; Gessesse, 2013).

Despite the daily use and consumption of khat by millions of people in Ethiopia and other countries

and its growing importance as a cash crop, very little is known about the associated plant-parasitic nematodes. Hence, the objective of this study was to assess the distribution, presence, and abundance of plant-parasitic nematodes associated with *C. edulis* in East Hararghe Zone, Ethiopia.

MATERIALS AND METHODS

Study site and sampling

A total of 300 composite soil and root samples (150 each) were collected from 15 major khat-growing districts in East Hararghe Zone of Ethiopia (Fig. 1) during the 2013/14 growing season (from October 2013 to March 2014). Sample numbers were evenly distributed and 10 fields were randomly chosen per district. Soil samples of 1.5 kg consisting of 15 to 20 soil cores were taken in a zigzag pattern from 0 to 40 cm deep. The cores were combined per field to form a single composite sample. About 200 g of adventitious roots were sampled from



Fig. 1. The major khat (*Catha edulis*) growing districts in East Hararghe Zone, Ethiopia, from which root and soil samples were collected during the 2013/2014 growing season.

ten individual plants randomly selected per field (Talwana *et al.*, 2008). Then, the samples were brought to the plant nematology laboratory at Haramaya University, Ethiopia, for extraction and further characterization of the nematodes.

For each sampling site, cropping history, cropping patterns, crops grown, frequency of khat production, and irrigation system employed were recorded. Field location and altitude were documented with GPS coordinates while soil texture was determined through a soil association map. During the sampling period, khat was found intercropped with different crops across the major sampling districts. In Adele (with tomato, cabbage, beans, and maize), Babile (tomato, cabbage, beans, maize, and groundnut), Dere Teyara (tomato, cabbage, beans, groundnut, maize, potato, and sorghum), Erer (tomato, cabbage, maize, potato, and sweet potato), Fedis (tomato, maize, groundnut beans, and sweet potato), Fenekele (onion and maize), Gendeji (maize), Gursum (tomato, maize, pepper, and groundnut), Haramaya (maize and beans), Kersa (tomato, maize, sorghum, cabbage, potato, and pepper), Kombolcha (tomato, maize, potato, sweet potato, and cabbage) where as in Aweday, Battee, Bombase, and Hamaressa khat was grown as a sole crop.

Extraction and processing of nematodes

Each soil sample was thoroughly mixed, and a subsample of 100 cm³ soil was assayed for nematodes using the modified Baermann funnel technique (Southey, 1970). For the root samples, a subsample of 10 g roots were washed and chopped into 1- to 2-cm pieces and macerated 3× for 10 sec, separated by 5-sec intervals using a blender (Orisajo and Fademi, 2012). The population density of the plant-parasitic nematode genera was enumerated from a subsample of the extracted nematode suspension using a stereomicroscope. Three subsamples with equal volume (3 ml) were counted three times, and the average was used to describe the population densities. For detailed morphological analysis and identification, ten adult females from all the genera except (*Meloidogyne* and *Rotylenchulus*) were fixed in formalin, processed in glycerol, and mounted on a glass slide based on a standard procedure (Coyne *et al.*, 2007). Whenever possible, different life stages such as for *Meloidogyne* spp. (second-stage juveniles and females) and *Rotylenchulus* spp. (males and vermiform juvenile females) were considered for identification. Characterization of the plant-parasitic nematodes was done using identification keys developed by Mai and Mullin (1996) and Mekete *et al.* (2012).

Data collected

Nematode numbers were expressed as the number of nematodes per 100 cm³ soil or 10 g fresh root weight (FRW). The prominence value (PV) was calculated as: absolute density × $\sqrt{\text{absolute frequency of occurrence}/10}$ (De Waele and Jowaan, 1988). Frequency is expressed as the number of sites where a genus occurred. Genera were considered widespread when they occurred in more than 30% of the sites. A genus whose mean density was more than 10 individuals/100 g of root was considered abundant (Adikom, 1988; Khashaija *et al.*, 1994; Talwana *et al.*, 2008). The proportion of roots with no symptoms or those that were necrotic, dead, galled, or forked were expressed as a percentage of the total roots collected.

RESULTS

Eight plant-parasitic nematode genera: *Criconema*, *Helicotylenchus*, *Hemicyclophora*, *Longidorus*, *Meloidogyne*, *Paratylenchus*, *Pratylenchus*, and *Rotylenchulus* with their respective infective stages and females were recovered from the soil samples. Among these, only *Pratylenchus* and *Meloidogyne* were recovered from the root samples. Out of the 150 composite soil samples, the prevalent nematodes encountered were *Pratylenchus*, *Meloidogyne*, *Helicotylenchus*, and *Longidorus* with 80, 60, 53.3, and 46.6% frequency of occurrence, respectively. *Pratylenchus* followed by *Meloidogyne* with 80 and 60% frequency of occurrence, respectively, were recovered from the 150 collected root samples (Tables 1 and 2). Erer, Fedis, Dere Teyara, and Kersa were the four districts where a higher plant-parasitic nematode density was detected in both soil and root samples. Soil samples collected from Aweday, Battee, Haramaya, and Gendeji (with almost intensive khat monoculture production) showed a low plant-parasitic nematode density and less root lesion damage from the respective root samples.

The highest plant-parasitic nematode population densities with 5,830 individuals per 100 cm³ soil and 1,790 individuals per 10 g fresh root weight were recovered from Erer district. Out of 5,830 individuals per 100 cm³ soil, *Pratylenchus*, *Meloidogyne*, *Helicotylenchus*, and *Longidorus* occurred at densities of 2,300, 1,400, 1,130, and 200 individuals respectively (Fig. 4). Out of 1,790 individual plant-parasitic nematodes, 52 and 48% were *Pratylenchus* and *Meloidogyne*, respectively. There were no plant-parasitic nematodes recovered from root samples collected from Battee and Hamaressa districts. The lowest plant-parasitic nematode density was

Table 1. Prominence value (PV), frequency of occurrence (FO) and abundance of predominant plant-parasitic nematodes recovered from soils and roots of khat (*Catha edulis*) from East Hararghe Zone during 2013/14 growing season.

Nematode genera	Soil (100 cm ³)			Root (10 g)		
	Abundance ^x	FO (%) ^y	PV ^z	Abundance	FO (%)	PV
<i>Criconema</i>	173	26.66	89.33	-	-	-
<i>Helicotylenchus</i>	353	53.33	257.79	-	-	-
<i>Hemicyclophora</i>	51	26.66	26.33	-	-	-
<i>Longidorus</i>	180	46.66	122.88	-	-	-
<i>Meloidogyne</i>	668	60	517.43	390	60	302.1
<i>Paratylenchus</i>	35	20	15.65	-	-	-
<i>Pratylenchus</i>	787	80	703.91	450	80	402.5
<i>Rotylenchulus</i>	103	26.66	53.18	-	-	-

^xAbundance is mean number of individuals of a genus over the sampling sites where the genus was detected.
^yFrequency of occurrence (%) = number of sites where a genus detected/total number of sites sampled* 100.
^zProminence value (PV) = Mean population density *(Frequency of occurrence)^{1/2} *10⁻¹.

Table 2. Occurrence of plant-parasitic nematode genera in soil samples from 15 major khat growing districts in East Hararghe Zone, Ethiopia, during the 2013/14 growing season.

District	<i>Criconema</i>	<i>Helicotylenchus</i>	<i>Hemicyclophora</i>	<i>Longidorus</i>	<i>Meloidogyne</i>	<i>Paratylenchus</i>	<i>Pratylenchus</i>	<i>Rotylenchulus</i>
Adele		+		+	+		+	
Aweday	+	+		+				+
Babile			+		+	+	+	+
Battee	+		+			+		
Bombase	+						+	
Dere Teyara		+		+	+		+	
Erer		+		+	+		+	+
Fedis		+	+		+		+	
Fenekele			+		+	+	+	
Gendeji							+	
Gursum		+		+	+		+	
Hamaressa	+							
Haramaya							+	+
Kersa		+		+	+		+	
Kombolcha		+		+	+		+	

recorded from Aweday district with 880 nematodes per 100 cm³ soil (Fig. 2). *Longidorus* were high in density from Dere Teyara (900/100 cm³ soil) and Gursum, Kombolcha, and Kersa (each 400/100 cm³ soil) where khat was intensively intercropped with maize (Fig. 4). All the recovered plant-parasitic genera were abundant from collected soil samples but not from all root samples (Table 1). Considering all the sampling districts, *Pratylenchus* followed by *Meloidogyne* were the highest in population density from root and soil samples analyzed (Fig. 2 and 3).

The highest PV was recorded for *Pratylenchus* (703.91) and *Meloidogyne* (517.43) followed by *Helicotylenchus* (257.79) from soil samples. The PV of *Pratylenchus* (402.5) and *Meloidogyne* (302.1) were calculated from root samples (Table 1). Root necrosis was observed on 60% of the samples, while

14% of the samples were dead. Root galling was seen on 12.9% of the root samples collected (Fig. 5). The highest percentage of dead roots was observed from samples collected from Erer district. The altitude of the sampled areas ranged from 1,320 m above sea level (Erer) to 2,189 m above sea level (Kombolcha).

DISCUSSION

This study documented the frequency of occurrence and abundance of a diversity of ectoparasitic and endoparasitic nematode genera across the major khat growing districts in the East Hararghe Zone, Ethiopia. *Criconeema*, *Helicotylenchus*, *Hemicyclophora*, *Longidorus*, *Meloidogyne*, *Paratylenchus*, *Pratylenchus*, and *Rotylenchulus* have all been associated with yield

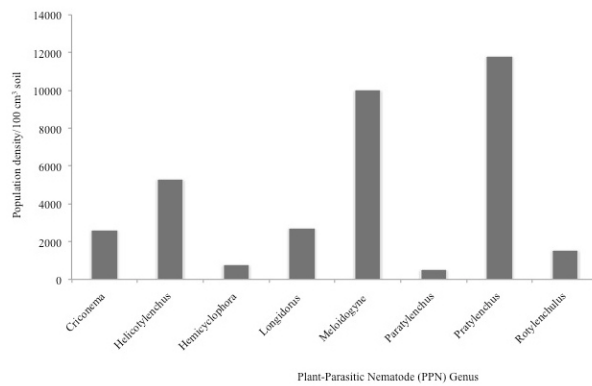


Fig. 2. The plant-parasitic nematode population density recovered from soil samples in all the 15 major khat growing districts of East Hararghe Zone, Ethiopia, during the 2013/14 growing season.

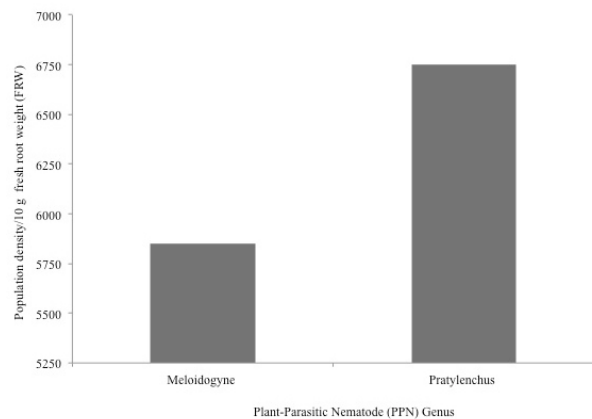


Fig. 3. The plant-parasitic nematode population density from root samples in all 15 major khat growing districts of East Hararghe Zone, Ethiopia, during the 2013/14 growing season.

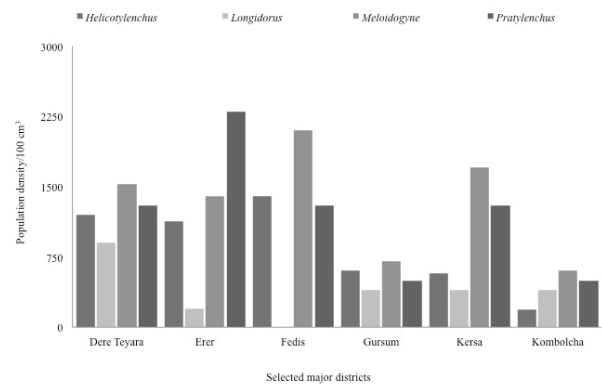


Fig. 4. The most frequent and abundant plant-parasitic nematodes recovered from soil from six major districts separately in East Hararghe Zone, Ethiopia.

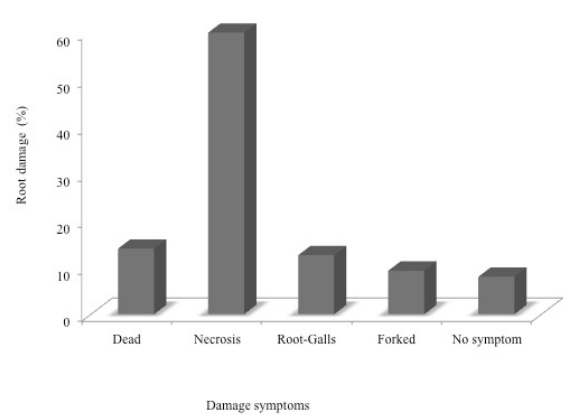


Fig. 5. Root damage in samples collected from the major khat growing districts of East Hararghe Zone, Ethiopia, during the 2013/14 growing season.

reduction in many crops worldwide (Afolami *et al.*, 2014), but little is known about their impact on the yield of khat.

Numerous factors could have contributed to the distribution of these nematodes in the 15 major khat growing districts, including soil type, cropping patterns, production systems, suitable climatic conditions and cultural practices employed. Soil type plays a significant role in nematode distribution. A sandy soil favors the abundance and greater damage of crops by many species of plant-parasitic nematodes in comparison with heavy clay soil (Taylor, 1971; Schenck and Holtzmann, 1990; Bond *et al.*, 2000; Afolami *et al.*, 2014). The soil requirement for optimum khat growth is a well-drained, dark red-brown, sandy or sandy loam soil, which is also ideal for nematode reproduction and infectivity (Murphy, 1959). This soil type was predominant in all of the sampling districts. The practice of furrow irrigation may also have played a significant role in nematode distribution across the sampling areas.

The practice of khat intercropping with other crops, coupled with the perennial nature of the plant, may tend to favor the development and buildup of a higher population density of nematode communities in khat fields. Continuous production without interruption could result in an increase in nematode population densities, particularly with k-strategists (Trudgill and Phillips, 1998; Eche *et al.*, 2013) such as *Longidorus* spp. as documented in this study. Similar results were documented in sugarcane plantations in many parts of the world (Hall and Irely, 1991; Afolami *et al.*, 2014). The minimum temperature of the East Hararghe zone ranges from 20 to 25°C, and the maximum range is from 30 to 35°C (Tegene *et al.*, 2014), which is typically suitable for nematode reproduction and infection. Plant-parasitic nematodes were recovered from soil samples in higher densities than from root samples, suggesting that not all nematodes that infect the intercrop may be able to infect the khat crop.

Farmers in East Hararghe Zone intercropped khat with solanaceous and other vegetable crops (mainly tomato, pepper, cabbage, potato, and sweet potato), cereals (maize and sorghum), and groundnut. *Pratylenchus*, *Meloidogyne*, and *Longidorus* were recovered at high population densities that could be detrimental to khat growth and biomass production. This was shown with the highest percentage of dead root samples from Erer district where *Pratylenchus* and *Meloidogyne* were present in higher densities. The high population density of these nematodes could be attributed either to the associated intercropping or to the suitability of khat as a host. The highest nematode density was recovered from both soil and root samples when

the field had a history of intensive and continuous intercropping with more than four crops on the same field for more than five consecutive years (Seid *et al.* unpublished). There appeared to be a relationship between the nematode genus that was present in a given locality and the intercropped crops that were grown. For instance, in Erer, Fedis, Dere Teyara, and Kersa districts, khat was highly infected by a mixture of *Meloidogyne*, *Pratylenchus*, and *Longidorus* and was intercropped with solanaceous crops (tomato, pepper, and cabbage) and cereals (mainly maize) (De Waele and Jowaan, 1988; Talwana *et al.*, 2008). *Criconema* seems to increase in population density in a khat field when it is grown as a monoculture.

In khat fields that were intercropped with solanaceous or cereals, either *Meloidogyne* or *Pratylenchus* and *Longidorus* were the dominant species, respectively. The PV of *Pratylenchus* (703.91, 402.5), *Meloidogyne* (517.43, 302.1), and *Longidorus* (122.88, -) was also higher from soil and root samples, respectively. Furthermore, the effect was manifested visually through the stunted growth and yellowing of leaves coupled with a severe root necrosis and dead roots. During an informal interview with farmers about their awareness of nematode problems, they stated that they were not even aware of the existence of plant-parasitic nematodes and were more concerned about leaf miner insect pests.

As a short-run solution, farmers should be educated regarding plant-parasitic nematodes and how their cropping pattern may impact them. Moreover, understanding the damaging potential of specific nematode species that occur in a given locality will be necessary to help us to know the economic threshold level and to estimate cost-benefit relationships involved in implementing management strategies.

This is the first report of plant-parasitic nematode genera associated with khat in Ethiopia. To our knowledge, the aggressiveness and pathogenicity of these individual plant-parasitic nematodes on khat crop have never been examined anywhere in the world. Further studies to identify the species within these genera, and to document their damage potential on the crop will be necessary to quantify the impact of nematodes in production systems.

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