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Survey and Identification of Plant Parasitic Nematodes Associated with Pepper in Ethiopia

Belay Feyisa Fikremariam Yimer Gemechu Kebede
Nematology Research, Ambo Agricultural Research Center, P. O. Box 37, Ethiopia
Email address: belay22feyisa@gmail.com

Abstract
A survey was conducted to determine abundance, frequency of occurrence and population density of plant parasitic nematodes associated with pepper in Ethiopia, during 2020 growing season. A total of one hundred seventeen soil (composite) and root samples were randomly collected from three regions of Ethiopia; i.e. Oromia, SNNP and Amhara. An extraction tray method was used for nematode extraction from these samples. According to the result, six nematode genera; viz. *Scutellonema, Meloidogyne, Hoplolaimus, Helicotylenchus, Tylenchorhynchus* and *Criconema* were detected during the survey. The most dominant nematode genera were *Meloidogyne* and *Helicotylenchus* followed by *Scutellonema*, with 38.5%, 25.6%, and 15.3% frequency of Occurrence, respectively. Except *Hoplolaimus* and *Criconema*, the other genera were considered as the widely distributed parasites of pepper as they were occurred in all regions. *Criconema* was the only nematode genus that was detected in Oromia region only. This study encourages more intensive and repeated survey is needed to establish the economic importance and management of pepper nematodes.
Keywords: Nematode, Parasitic, Pepper, Survey
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Introduction

Pepper (*Capsicum* spp.) is a new world crop that belongs to the family Solanaceae. The genus *Capsicum* is the world's second most important of the family after tomatoes. Locally known as "barbare" is both a vegetable and spice crop of significant economic value to Ethiopia. It is one of the leading vegetable crops noted for its export potential and used daily in most homes. Pepper is grown in many countries of the world and its production for culinary and vegetable uses has been increased from time to time. Today, in Ethiopia it is extensively produced and used. It is actually considered as a national spice crop. Its production is a good source of income for small-scale producers or out growers and it is a significant source of foreign exchange among other vegetable crops (Bonsu *et al.,* 2003).

As a food, pepper has little energy value (25 Kcal/100g), but it is an excellent source of vitamin A (530 IU/100g) and C (128 mg/100g) and a good source of vitamin B2 (0.05mg/100g), potassium (195 mg/100g), phosphorus (22 mg/100g), and calcium (6 mg/100g) (Bosland, 2000). The high nutritive and culinary value of pepper makes it a high demand in the market year round. *Capsicum* spp. is used fresh or dried, whole or ground into powder and alone or in combination with other flavoring agents. According to (FAOSTAT, 2015), the world production was 45.25 million tons, of which 5.031 million tons was from African countries. In Ethiopia, 14,672.74 and 76,202.62 ha of land was covered by green and red pepper, respectively (CSA, 2014). Regardless of its importance, hot pepper production for green and dry pod has a low yield (national average yield of 7.6 t/ha for green pod) whereas for dry pod, the yield was 1.6 t/ha.

Occurrence of pests and wide range of diseases including plant parasitic nematodes particularly root-knot nematodes (Eshetu *et al.,* 2006) contributed in reducing yield/unit of production and quality of produce. In order to get comparative world average production per hectare in Ethiopia, it is a need to alleviate these constraints of production including plant parasitic nematodes. To meet this objective, it is obvious that first identification and prioritization of plant parasitic nematodes associated with pepper is crucial. Therefore, the objective of this study is to identify, document and prioritize plant parasitic nematodes associated with pepper in Ethiopia and to map the geographical distribution of economically important plant parasitic nematodes on pepper.

Materials and Methods

A contiguous survey of plant parasitic nematodes on major pepper growing areas of Ethiopia was conducted to identify, document and prioritize major plant parasitic nematodes associated with pepper in Ethiopia. One hundred seventeen soil and root samples were collected from the rhizosphere zone of pepper plants in 2018 growing season. Three regions, namely Oromia, Southern nation, nationality and peoples of Ethiopia and Amhara were assessed for the presence of plant parasitic nematodes on pepper. Soil samples were obtained by digging the soil through auger downward to a distance of about 15-30 cm in a random and zigzag sampling pattern. Samples were taken in white plastic bags and transported to nematology laboratory of Ambo Agricultural Research Center and kept in refrigerator at 4°C until nematode extraction (Talwana *et al.,* 2008).

Nematode extraction from the samples was done by using extraction tray method (Goodey JB 1957). Nematode identification at genus level was done based on the morphological characters of the nematodes from frequent aliquots (1ml/each) in each soil sample (Mai *et al*., 1975). The counting slide was used for determining the number of each nematode genus. Nematodes were also extracted from roots using a modified maceration and filtration technique according to (Hooper *et al*., 2005) and identified to genus level and subsequently enumerated. Population of nematode was expressed as the number of nematodes per 100ml of soil and per 10g of root. The prominence value (PV) was calculated as: absolute density × √absolute frequency of occurrence/10 (De Waele and Jowaan, 2000). 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/100g of root was considered abundant (Adikom, 1988; Khashaija *et al*., 1994; Talwana et *al*., 2008).

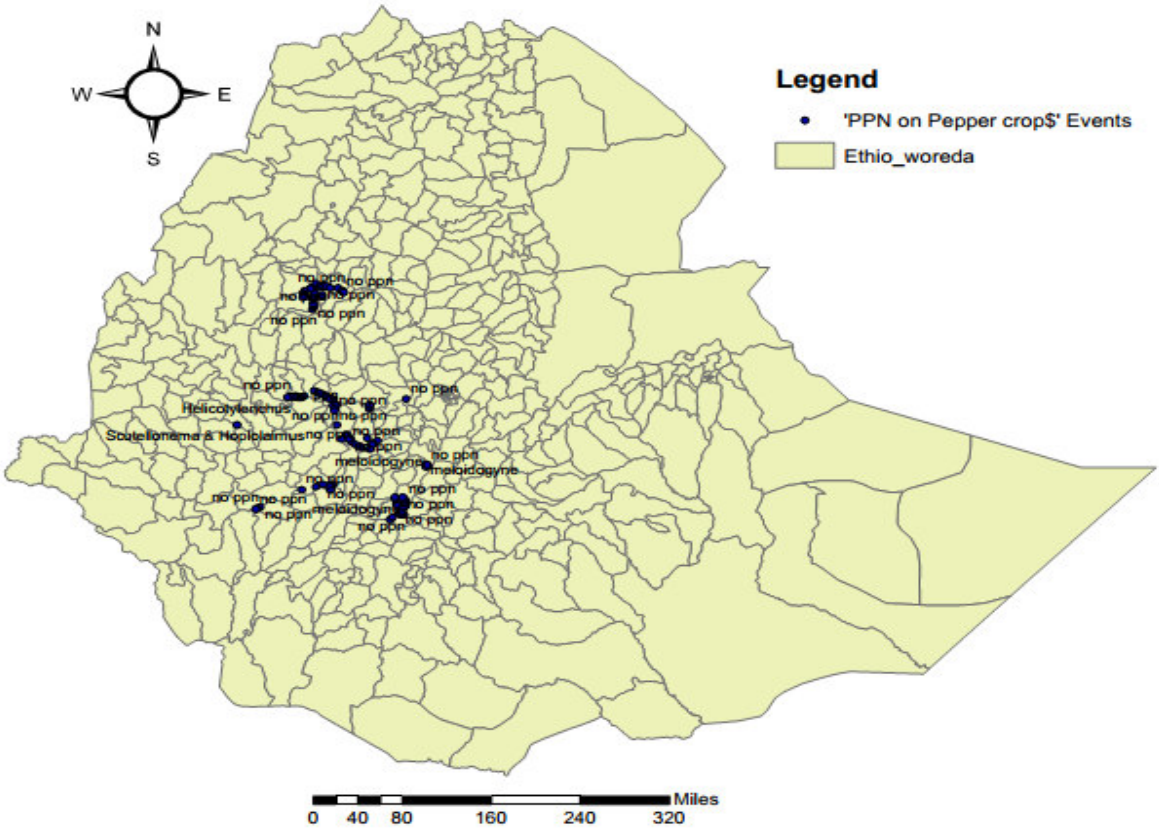

Figure 1. Map showing the major pepper growing districts in Ethiopia, from which soil and root samples were collected.

Results and Discussion

A total of one hundred seventeen soil and root samples were collected from the rhizosphere zone of pepper plants

in 2020 growing season. Out of one hundred seventeen samples, a total of six genera of plant parasitic nematodes belonging to four families were identified from the soil and root samples in the three regions (**Table 1**). These nematode genera were *Helicotylenchus, Scutellonema, Hoplolaimus, Tylenchorhynchus, Meloidogyne* and *Criconema* (**Figure 2**). Among these, *Meloidogyne* and *Helicotylenchus* were alike to be the widespread nematode genera with frequency of occurrence 38.5 and 25.6%, respectively (**Table 2**). Furthermore, the two nematode genera *Scutellonema* and *Tylenchorhynchus* showed modest distribution with frequency of occurrence 15 and 12%, respectively (**Table 2**). On the other hand, *Criconema* was the least widespread genus with frequency of occurrence 0.85% in Oromia region. However, it was not detected in the remaining two regions (**Table 2**). This study conforms to previous reports that root-knot nematodes (*Meloidogyne* spp.) are present in Ethiopia (Stewart and Dagnachew 1967, O'Bannon). This study also in line with the previous survey conducted by Mandfro & Mekete (2002) reported the presence of root-knot nematode (*Meloidogyne* spp.) associated with several vegetable crops in Ethiopia.

Plant-parasitic nematodes generally occur in poly specific communities. Competition between them especially between nematodes that have the same feeding sites (endo-parasitic nematodes and between ecto-parasitic nematodes) is generally mutually suppressive because of the contention for available feeding sites and physiological alterations; however, neutral and stimulatory interactions also occur (Eisenback, 1981). In this study, furthermore five plant parasitic nematode genera namely *Helicotylenchus*, *Scutellonema, Hoplolaimus, Tylenchorhynchus and Criconema* associated with pepper were detected from soil samples of the three regions (**Table 1**). Some of the detected genera by this survey were in agreement with the previous survey as (O'Bannon, 1975) reported *Meloidogyne*, *Helicotylenchus*, *Pratylenchus* and *Tylenchus* in Ethiopia. Mostly Vegetables are susceptible host for nematodes because of their flesh cortex and soft epidermal nature. Survey conducted by Belay and Fikremariam (2020) is in agreement with this study that the presence of plant parasitic nematode genera viz. *Meloidogyne, Pratylenchus, Xiphinema, Longidorus, Helicotylenchus and Rotylenchulus* on potato in Ethiopia.

During this survey, Most of above listed plant parasitic nematode genera were detected in the surveyed three regions. Among these, root-knot nematodes (*Meloidogyne*) were with high abundance, frequency of occurrence and prominence value followed by *Helicotylenchus*. This might be the wide host range and host preference of these genera (e.g tomato and pepper are called universal host for *Meloidogyne*) and also during the survey, most of the crops grown in the past growing season were vegetables which allow the nematodes to overwinter and reproduce faster and build their population in high amount in the soil. This shows also these nematodes are widely spread and densely populated in the soil affecting the tomato fields in the surveyed area. Once these nematodes are introduced in the soil, they can stay for several years persistently, particularly in irrigated fields of central rift valley of Ethiopia with continuous mono cropping of vegetables, reaching high population level in short time.

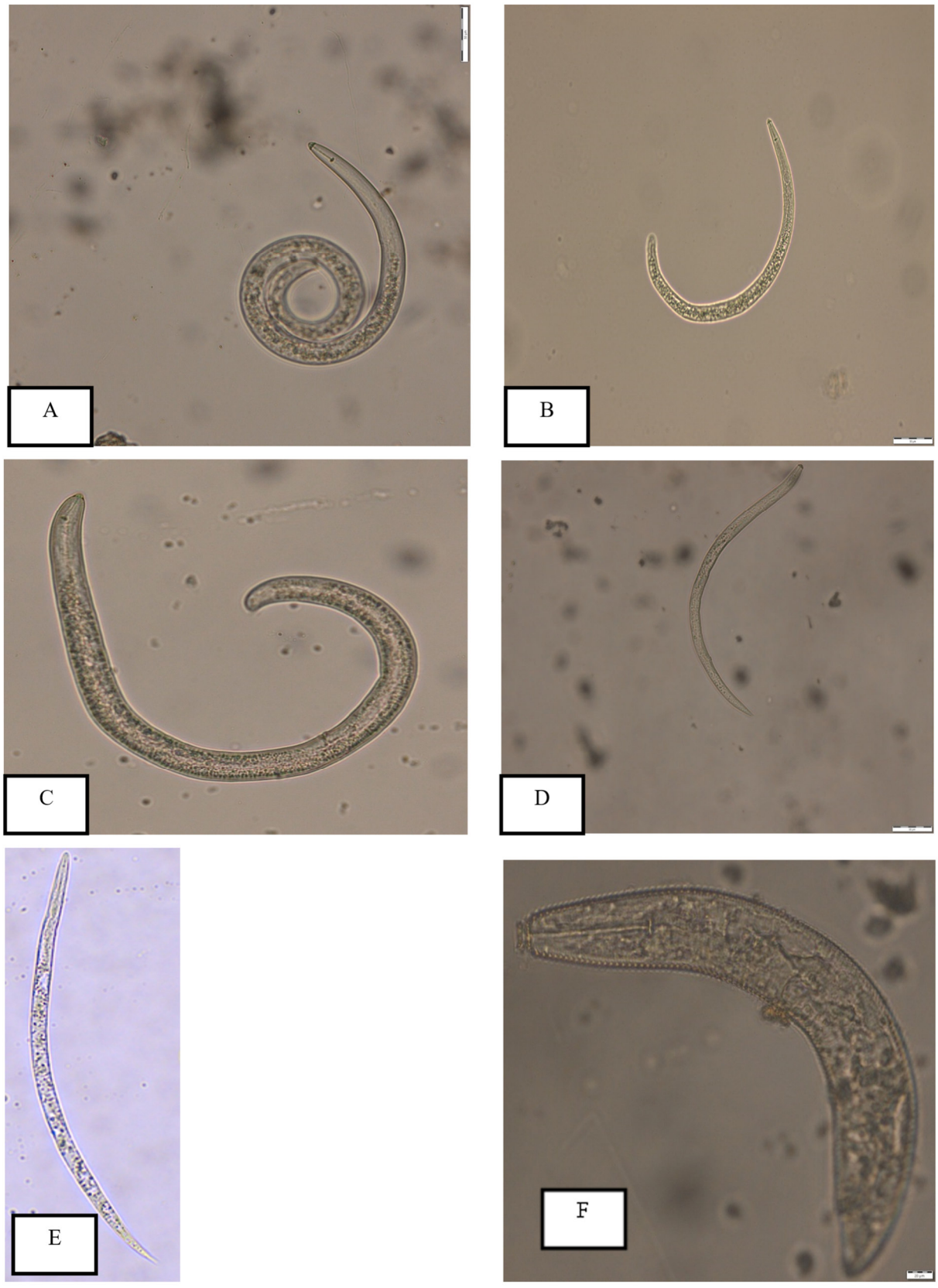

Figure 2. Morphology of plant parasitic nematode genera recovered from pepper root and soil samples: *Helicotylenchus* (A), *Scutellonema* (B), *Hoplolaimus* (C), *Tylenchorhynchus* (D), *Meloidogyne* (E) and *Criconema* (F).

Table 1. Plant parasitic nematode genera isolated from soils and roots of pepper in three regions

Order	Sub-order	Family	Genus
Tylenchida	Tylenchina	Hoplolaimidae	*Helicotylenchulus* *Scutellonema* *Hoplolaimus*
		Tylenchorhynchidae	*Tylenchorhynchus*
		Heteroderidae	*Meloidogyne*
		Criconematidae	*Criconema*

Table 2. Frequency of occurrence of plant parasitic nematode genera on pepper

No.	Nematode genera's	Frequency of occurrence of nematode genera on pepper	
		No	FO%
1	*Scutellonema*	18	15
2	*Meloidogyne*	45	38.5
3	*Hoplolaimus*	6	5
4	*Helicotylenchus*	30	25.6
5	*Tylenchorhynchus*	14	12
6	*Criconema*	1	0.85
Total		114	

No=Number of samples containing a genus. FO=Frequency of Occurrence

The PV of *Scutellonema* (50), *Hoplolaimus* (37) and *Tylenchorhynchus*(35) was higher from soil samples. The PV of meloidogyne was (42) from root samples.When the populations of different PPN genera in soil were evaluated for all mentioned regions, the population density ranged from 20 to 150 nematodes per 100 g of dry soil (Table:3). *Hoplolaimus* had a high mean density of 150 nematodes/100g soil. The predominant genera present were*Hoplolaimus*, *Scutellonema* and *Tylechorhynchus*across all the regions. The highest PV was recorded from Scutellonema(50) and *Hoplolaimus*(37) followed by *Tylenchorhynchus*(35) from soil samples and *Meloidogyne*(42) from root samples. The lowest PV was recorded from *Criconema*(2) followed by *helicotylenchus* (25) were calculated from soil samples (Table 3). The altitude of the sampled areas ranged from 1556(Oromia) to 2056 m (SNNP) region above sea level.

Table 3. Prominence value (PV), frequency of occurrence (FO) and abundance of predominant plant parasitic nematodes recovered from soils and roots of pepper

Nematode genera	Soil (100ml)			Root (10 g)		
	Abundance	**FO (%)**	**PV**	**Abundance**	**FO (%)**	**PV**
Scutellonema	118	18	50	-	-	-
Meloidogyne	-	-		62	45	42
Hoplolaimus	150	6	37	-	-	-
Helicotylenchus	46	30	25	-	-	-
Tylenchorhynchus	94	14	35	-	-	-
Criconema	20	1	2			

A=Abundance is mean number of individuals of a genus over the sampling sites where the genus was detected. Frequency of occurrence (FO %) = number of sites where a genus detected/total number of sites sampled*100. Prominence value (PV) = Mean population density *(Frequency of occurrence) $^{1/2}$ *10^{-1}.

All 6 nematode genera except *helicotylenchus* were found in Oromia region.Among that the highest population of nematode was *meloidogyne* (2780). *Helicotylenchus* nematode is the only nematode recorded from Amhara region. *Scutellonema*(2120) followed by *meloidogyne* (2780) were the highest nematode population found in both regions respectively. The highest nematode population found in the Oromia region was *Meloidogyne*(2780).The highest nematode population found in the Oromia and Amhara was *Hoplolaimus* (150/100cm^3) and *Scutellonema* (118/100cm3) of soil respectively.

Table 4. Occurrence of plant-parasitic nematode genera in soil and root samples from 3 major pepper growing regions of Ethiopia during the 2018 growing season

Nematode genera	*Oromia,*	SNNP	*Amhara*
Scutellonema	+	+	+
Meloidogyne	+	+	+
Hoplolaimus	+	-	-
Helicotylenchus	-	-	+
Tylenchorhynchus	+	+	+
Criconema	+	-	-

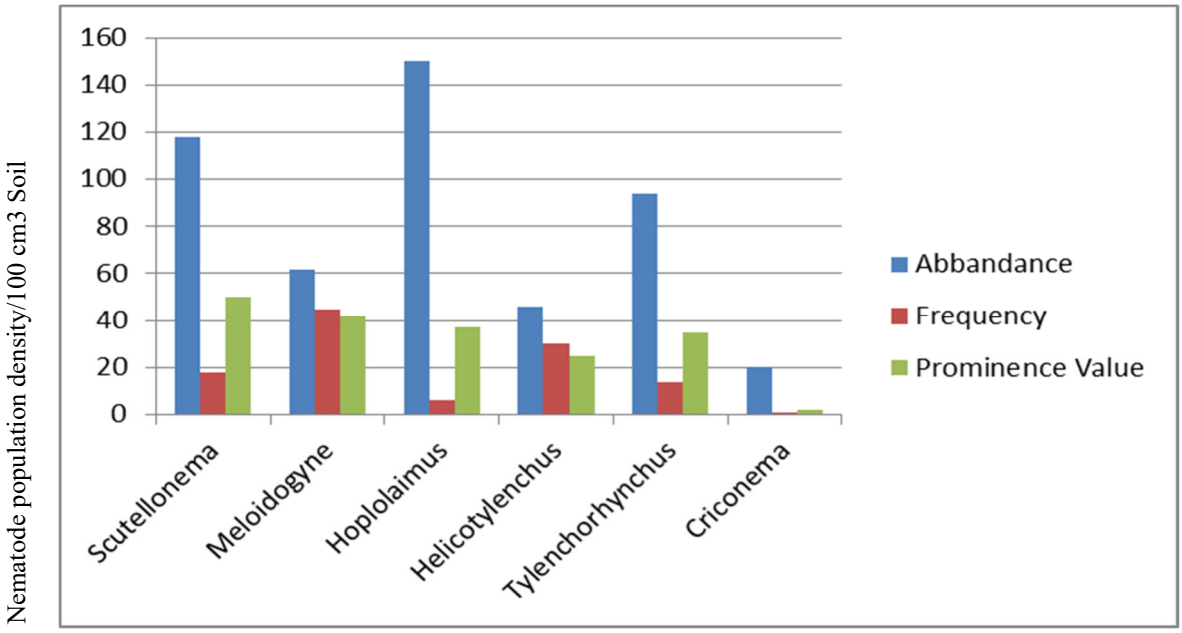

Figure 3. The plant-parasitic nematode abundance, frequency and prominence value recovered from soil samples in all the 3 major pepper growing regions of Ethiopia, during the 2020 growing season.

Conclusion and Recommendation

The survey was conducted in three regions of Ethiopia in 2018 growing season to determine the incidence, occurrence and abundance of plant parasitic nematode on pepper. In general, the study reported six genera of plant-parasitic nematode associated with pepper in the three regions of Ethiopia. All of these genera have been reported in association with pepper or other host plants in Ethiopia. Based on this survey, *Helicotylenchus* was the most frequently encountered and abundantly found genus next to *Melodogyne* in the three regions of Ethiopia. It can also be concluded that root-knot nematodes (*Meloidogyne* spp.) are considered the most important nematode genera that cause severe symptoms and severe damage on pepper in most locations of the three regions of Ethiopia. In Ethiopia, many pests, including nematodes, have been reported as production constraints of cereal, pulse and oil crops. However, survey of plant parasitic nematodes on various crops particularly on vegetables in Ethiopia is still untouched research area compared to survey on other diseases and pests. Therefore, there is a need to conduct a national and organized survey of plant parasitic nematodes in Ethiopia to establish baseline information for the economic importance of nematodes and their management. In general, the essence of this survey would facilitate in choosing planting systems in the selected locations and evade planting the susceptible hosts, as well as should use in design of nematode management programs.

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