Research Article

Distribution of Plant Parasitic Nematodes Associated with Carrot (*Daucus carota* L., subsp. *sativus*) in North Showa Zone, Ethiopia

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Abstract | Carrot (*Daucus carota* L. subsp. sativus (Hoffm.)) is one of the most important vegetable crops nutritionally and economically worldwide as well in Ethiopia. Plant-parasitic nematodes (PPNs) reduce up to 12.3% of global food production on an annual basis. They account for about 25–50% of losses on carrots. The North Showa Zone is one of the major carrot-producing areas in Ethiopia yet the distribution of PPNs on carrots in this area remains unknown. Therefore, this survey study was carried out to know the distribution of PPNs on carrots in the North Showa Zone. The study detected a total of seven PPN genera from carrot crops in Ethiopia viz. *Helicotylenchus, Hoplolaimus, Pratylenchus, Meloidogyne, Rotylenchulus, Scutellonema, and Tylenchorhynchus*. The genus *Meloidogyne* was the most frequent, abundant, and prominent genus with a 62% frequency of occurrence, 217 juveniles/100 ml soil mean density, and a 174.14 prominence value, followed by *Pratylenchus*. *M. incognita* was more prevalent than *M. javanica*, with a 62.9% absolute frequency and a 70.83% relative frequency. This study would suggest future research efforts to focus on the determination of damage threshold, extent of yield loss, and extent of pathogenicity to the carrot in the country.

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Introduction

Carrot (*Daucus carota* L., subsp. *sativus* (Hoffm.)) is one of the most important vegetable crops nutritionally and economically worldwide *as well in Ethiopia*. It has a high market value as a vegetable and as a food for processing. Carrots are among the top ten vegetable crops after tomatoes, onions, cabbage, cucumbers, and eggplant, with an annual world production of about 428 million tons grown on about 11.5 million hectares (FAO, 2021). Carrots are excellent sources of nutrients like potassium, antioxidants, vitamin A, and Beta-carotene (Khyati and Kirti, 2022).

Diseases caused by different pathogens, including plant parasitic nematodes (PPNs), are among the major reasons for the low productivity of carrots.

Plant-parasitic nematodes (PPN) account for about 25–50% of losses on carrots (Anwar and McKenry, 2012; Singh and Kumar, 2015). PPNs enter plant cells using their stylet, a feeding device that creates a wound that will serve as a route for secondary pathogens such as *Rhizoctonia solani, Alternaria duci,* and *Pectobacerium carotovorum* (Sowmya and Rao, 2011). The significance of disease complexes has been observed, whereby *M. incognita* increased bacterial wilt (*Ralstonia solanacearum*) disease severity in potatoes (Tasew *et al*., 2021). More than 90 species of PPNs that infect umblliferous crops have been identified. These include but are not limited to *Meloidogyne, Pratylenchus, Longidorus, Paratylenchus, Belonalaimus, Paratrichodorus, Rotylenchus,* and *Ditylenchus* (Davis and Raid, 2002). *Meloidogyne* spp., *Pratylenchus* spp., *Paratylenchus* spp., and *Paratrichodorus* spp. are the most important and widespread on carrots (Noling, 2012). Carrot is the most vulnerable crop to plant-parasitic nematodes with a damage threshold density of less than 1 egg/ml soil for RKNs. Nematode infected carrots are forked, stubby,and malformed and their increased root production would drastically lower the taproot's marketable quality (Vrain and Belair, 1981).

Plant parasitic nematodes are also one of the most important groups of pests affecting vegetable production in Ethiopia (Seid and Hailu, 2019). O'Bannon (1975) reported the presence of *Helicotylenchus* spp., *Heterodera* spp., *M. incognita*, *M. ethiopica, Pratylenchus* spp., and *Tylenchus* spp. associated with tomato, lettuce, sweet pepper, onion, and sweet basil. Root-knot nematodes (RKN) were found to be the most widespread and reported to cause severe damage and yield reduction in vegetable production (Stewart and Yirgu, 1967; O'Bannon, 1975; Mandefro and Mekete, 2000, 2002; Seid and Hailu, 2019; Miheret *et al*., 2019). In Ethiopia, the available research information on nematodes is very limited and restricted both by crops and geographical coverage as compared to other diseases and pest problems. The North Showa zone is among the major carrot-producing areas in Ethiopia yet the occurrence, distribution, and abundance of plant parasitic nematodes on carrots in this area remain unknown. Therefore, the present study was carried out with the objective of identifying plant-parasitic nematode genera associated with carrots in the major carrot-growing areas of the North Showa Zone of Ethiopia.

Materials and Methods

Description of the study area, sampling, and nematode isolation and quantification

The survey was conducted in the North Showa zone at Angolelana tera, Menz gera midir, Menz mama midir, Basoena werena, and Debre birhan districts from June to July during the 2022 rainy season. The geographical coordinates of the sampled locations across the five districts were mapped with ArcView 3.2 (Figure 1).

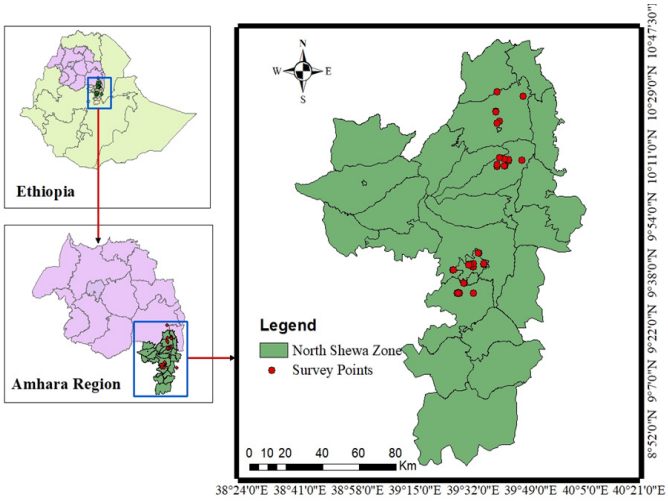

Figure 1: *Map of North showa zone showing sampling points.*

From the North Showa Zone, five major carrot-producing districts were selected. From each district, three kebele were selected, and from each kebele, three carrot fields were assessed. A total of 45 samples were collected from all surveyed sites (districts). Twenty soil cores were collected from each farm in a zig-zag pattern using an augur with associated carrot root, and the soil cores collected from a particular field were mixed to make a 1.5–2 kg composite sample.

At each sampling point, the top dry soil of about 1-2 cm was removed, and the remaining soil was collected up to a depth of 30 cm. The collected samples were placed into labeled plastic bags, kept in an icebox, and transported to the Ambo Agricultural Research Center, Nematology Laboratory for diagnosis.

Nematodes were extracted separately from roots and soil for each of the collected samples. Each composite soil sample was placed on separate, clean paper, and soil clumps were broken into fine soil particles. Crop residue and stones were removed. The remaining soil was mixed and sieved using a 2 mm sieve. A

sub-sample of 100 ml of sieved soil was used to extract nematodes using the extraction tray method (Whitehead and Hemming, 1965).

The roots were washed carefully by placing them in a separate bucket of water. Root gall and egg-mass index were given based on a 0 to 5 scale, where 0 = no egg masses/galls; 1 = 1-2 egg masses/galls; 2 = 3–10 egg masses/galls; 3 = 11–30 egg masses/galls; 4 = 31–100 egg masses/galls; and 5 = >100 egg masses/galls (Taylor and Sasser, 1978). Roots were sliced finely into 0.5 cm root pieces using a surgical knife, and nematodes were extracted from a sub-sample of 10 g roots using the extraction tray method (Whitehead and Hemming, 1965). After a 48 hrs incubation period nematodes were recovered from both soil and root samples. The extracted nematodes were collected separately in a 38 µm sieve and transferred to a graduated cylinder. Then the extracts were allowed to settle for three hours, and the supernatant was carefully siphoned, a final volume of 50 ml was adjusted for each sample for assessment the nematode population density.

Nematode population densities were calculated by taking three replicates a 2 ml aliquot of nematode suspension from the original 50 ml homogenized nematode suspension and pipetted out into a nematode counting slide using a pasture pipette under a compound microscope (Olympus CX33). The means of the three readings were multiplied by the original volume of 50 ml to express the density of plant-parasitic nematodes in 100 ml of soil or a 10 g carrot root.

The frequency of the nematodes was determined from the relationship between the number of samples where the nematode was detected divided by the total number of samples taken, and multiplied by 100 to express the percentage of the prevalence (Sawadogo *et al*., 2009). The prominence value (PV) was calculated according to De Waele and Jowaan (1988). The absolute, and relative frequency of RKN species were calculated according to the formulas below (Norton *et al*., 1978).

$$Prominence\ Value(PV) = \frac{\text{Absolute density} \times \sqrt{\text{Absolute frequency of}}}{10}$$

$$\%\ \text{Occurrence} = \frac{\text{Number of fields with root} - \text{knot nematodes}}{\text{Total number of fields surveyed}} \times 100$$

$$\text{Absolute frequency} = \frac{\text{Number of samples containing species}}{\text{Number of samples collected}} \times 100$$

$$\text{Relative frequency} = \frac{\text{Frequency occurrence of species}}{\text{The sum of the frequency of all Meloidogyne species}} \times 100$$

Nematode identification

Plant-parasitic nematode genera were examined and identified to the genus level based on morphological characteristics including body shape, body size, stylet type, stylet length, mouth type, lip region, pharyngeal overlap, vulva position, and tail type under a compound microscope (Olympus CX33) (Mai *et al*., 1996). Identification of *Meloidogyne* species to species level was conducted using adult female perineal patterns in each district. Adult females were carefully removed from root tissues using a needle and forceps under a stereomicroscope (Euromex PB 4161 6803 ED) and kept in a solution of 45% lactic acid to remove the body contents and clear the perineal regions of adult females. Neck regions were excised, and the posterior end was trimmed and mounted to a drop of glycerin on a microscope slide (Taylor and Netscher, 1974; Hartman and Sasser, 1985). For each infected carrot root sample, ten perineal patterns were prepared and mounted in glycerin for microscopic observation. In root samples where females were not present, nematodes were identified at the genus level only. The species were identified based on the technique described by Eisenback *et al*. (1981).

Results and Discussion

Of the total 45 samples collected from five major carrot-growing districts in the North Showa zone, 40 of the soil samples and 36 of the root samples were found to be infested with different genera of plant parasitic nematodes. A total of seven genera of plant parasitic nematodes were detected, viz., *Meloidogyne*, *Helicotylenchus, Pratylenchus, Rotylenchulus, Scutellonema, Hoplolaimus,* and *Tylenchorynchus* (Table 1). A photomicrograph of plant parasitic nematode genera recovered from samples (Figure 3). The genera *Pratylenchus* and *Meloidogyne* were recovered from root samples, while *Helicotylenchus, Rotylenchulus, Scutellonema, Hoplolaimus,* and *Tylenchorynchus* were recorded on soil samples (Table 1). The distribution of these plant parasitic nematodes fluctuates across the five districts. *Meloidogyne, Pratylenchus, Helicotylenchus,* and *Hoplolaimus* were detected in all five surveyed districts of the North Showa Zone (Table 1). *Rotylenchulus* was recorded only in four of the districts. *Scutellonema* and *Tylenchorynchus* were observed in the three surveyed districts.

Table 1: *The average, minimum, and maximum density of plant parasitic nematode genera in carrot (Daucus carota L., subsp. sativus (Hoffm.)) across the surveyed district of North Showa zone during the 2022 growing season.*

No	Nematode genera	Menz gera midir		Menz mama midir		Debre birhan		Angolelana tara		Basoena werena	
		Soil	Root	Soil	Root	Soil	Root	Soil	Root	Soil	Root
1	*Meloidogyne*	295 (240-390)	227 (173-325)	278 (244-300)	201 (148-237)	185 (60-269)	122 (10-191)	101 (11-214)	50 (5-128)	252 (120-500)	168 (66-351)
2	*Helicotylenchus*	43 (10-90)	-	102 (89-116)	-	95 (88-103)	-	82 (12-153)	-	170 (*)	-
3	*Pratylenchus*	103 (65-125)	49 (20-75)	105 (85-125)	48 (28-68)	88 (76-100)	32 (15-50)	92 (32-152)	96	194 (200-212)	138 (114-170)
4	*Rotylenchulus*	34 (34-35)	-	67 (*)	-	34 (*)	-	-	-	12 (*)	-
5	*Scutellonema*	60 (*)	-	-	-	10 (*)	-	25 (*)	-	-	-
6	*Haplolaimus*	30 (20-40)		68 (56-80)	-	62 (*)	-	61 (43-80)	-	29 (*)	-
7	*Tylenchorhychus*	26 (*)	-	-	-	-	-	16 (*)	-	18 (*)	-

– =Absent PPN, Numbers with (*) indicate density when the genera are found in only one sample in surveyed districts, Numbers outside the parentheses indicate average density of the genera, and numbers within the parentheses indicate the minimum and maximum density of the genera in each districts.

Table 2: *The frequency occurrence (F.O), abundance, and prominence value (PV) of plant parasitic nematode genera in carrot (Daucus carota L., subsp. sativus (Hoffm.)) across the surveyed district of North Showa zone during 2022 growing season.*

No	Nematode genera	F.O (%)		Abundance		PV	
		Soil	Root	Soil (100ml)	Root (10 g)	Soil	Root
1	*Meloidogyne*	62.2(28)	60(27)	217	153	171.14	118.5
2	*Helicotylenchus*	26.6(12)	-	86	-	44.35	-
3	*Pratylenchus*	33.3(15)	29.9(13)	124	82	71.55	44.08
4	*Rotylenchulus*	11(5)	-	36	-	11.93	-
5	*Scutellonema*	6.6(3)	-	32	-	8.22	-
6	*Haplolaimus*	17.7(8)	-	51	-	21.45	-
7	*Tylenchorhychus*	6.6(3)	-	20	-	5.13	-

*The value in the parenthesis are number of samples containing a genus; F.O=Frequency of Occurrence; *Total number of sample=45, 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}.*

The genus *Meloidogyne* was the most frequently encountered genus, observed in 28 of the samples with a frequency of occurrence of 62.2%, followed by *Pratylenchus* with a frequency of 33.3% (Table 2). Among all the genera, *Scutellonema and Tylenchorynchus* were detected with the least equal frequency of occurrence (6.6%). The highest mean abundance of 217 juveniles/100 ml soil and 153 juveniles/10g root was recorded with *Meloidogyne,* followed by *Pratylenchus* (124 nematodes/100 ml soil) and 82 nematodes/10g root (Table 2). The genera *Scutellonema* and *Tylenchorynchus* showed the least abundance, with 32 and 20 nematodes per 100 ml of soil, respectively (Table 2). *An elevated* population density of 500 J2s per 100 ml of soil was also observed for the genus *Meloidogyne* in a particular field in the Basona werena district (Table 1).

The highest prominence values of 174.14 and 118.5 in soil and root samples, respectively, were recorded with the genus *Meloidogyne* (Table 2). *Pratylenchus* was the next most prominent nematode genus on carrots, with prominence values of 71.55 and 44.08 in soil and root samples, respectively.

This study is in line with the previous study by Mandefro and Mekete (2000), who reported *Meliodogyne* spp. as the most widely spread and predominant plant parasitic nematode affecting vegetable crops in Ethiopia. In agreement with the present study, Nordalyn *et al.* (2013) reported *Meloidogyne* and *Pratylenchus* to be the most predominant genera in semi-temperate vegetable crops. A similar result was obtained in other survey studies conducted in Nigeria and India (Kabuk

et al., 2020; Anupriya *et al.*, 2019). In another study conducted in northern Tasmania, Australia, *Meloidogyne* spp. was reported as the second most dominant species next to *Pratylenchus* spp. on carrots (Hay and Pethybridge, 2005).

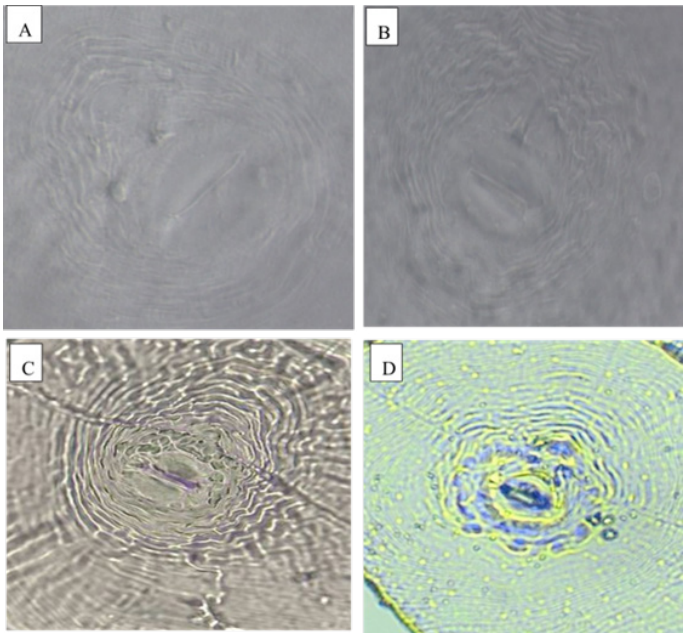

Figure 2: *Perineal pattern of RKN spp. Meloidogyne incognita (A-B) and Meloidogyne javanica (C-D).*

Moreover, the present study identified two *Meloidogyne* species, i.e., *M. incognita* and *M. javanica,* based on their perineal patterns (Figure 2). *Meloidogyne incognita* is oval to round with a squarish, high dorsal arch possessing a clear whorl around the tail terminus, and striae are typically smooth and wavy (Hunt and Handoo, 2009). *Meloidogyne javanica* has a low dorsal arch with oval to pear-shaped, smooth, and less wavy striae (Williams, 1972) and two lateral lines as a key to distinguishing it from other *Meloidogyne* species (Eisenback, 1985). *Meloidogyne incognita* was the most frequently encountered RKNs (Root-knot nematodes) species, observed in 17 of the root samples with a 62.9% and 70.89% absolute and relative frequency of occurrence, respectively (Table 3). *Meloidogyne javanica* was observed only in seven of the root samples, with a 25.9% and 29.1% absolute and relative frequency of occurrence, respectively.

The results agree with the previous study (Tadele and Mengistu, 2000; Mandefro and Mekete, 2002), who had reported *M. incogenta, and M. javanica* to be the most predominant species parasitizing vegetable crops, including carrot, in Ethiopia. According to the present survey study, *M. incognita* was more prevalent with a 62.9% absolute frequency and a 70.83% relative frequency than *M. javanica* which had an absolute frequency of 25.9% and a relative frequency of 29.1%.

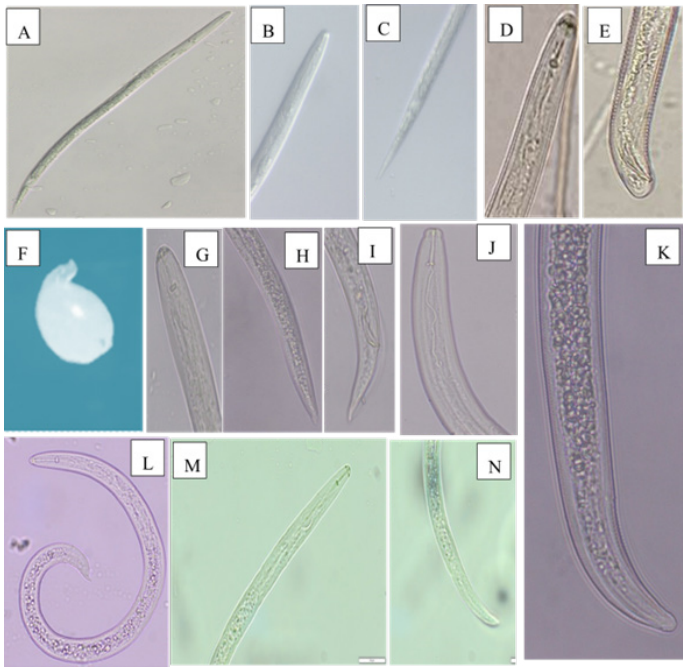

Figure 3: *Morphology of plant parasitic nematode genera associated with carrot in North Showa Zone; Entire morphology of Meloidogy spp. (A), Head region of Meloidogyne J2 (B), Tail region of Meloidogyne J2 (C), Head region of Meloidogyne male (D), Tail region of Meloidogyne male (E) Entire morphology of adult female Meloidogyne spp. (F), Head region of Pratylenchus spp. (G), Tail region of female Pratylenchus spp. (H), Tail region of male Pratylenchus spp. (I) Head region Tylenchorhynchus spp. (J), Tail region of Tylenchorhynchus spp. (K) Entire morphology of Helicotylenchus spp. (L), Head region of Rotylenchulus (M), Tail region of Rotylenchulus spp. (N).*

Figure 4: *Photographs captured during the surveying carrot fields in the North Showa zone and laboratory processing at Ambo Agricultural research center. Where, A=Nematodes infected carrot field showing patchy growth, B&C=Misshapen carrot roots due to Pratylenchus and Meloidogyne spp, D=Microscopic examination of roots in the laboratory, E&F=nematode extraction from soil and root samples.*

Table 3: *The mean root gall, egg-mass index, overall frequency, absolute and relative frequency, of root-knot nematodes on carrot (Daucus carota L., subsp. sativus (Hoffm.)) across the surveyed districts of North Showa zone during the 2022 growing season.*

Districts	Mean index		RKN species as per the perineal pattern identification			
	Gall	Egg-mass				
Menz gera midir	3.16	2.26	*M. incognita*			
Menz mama midir	2.82	1.9	*M. incognita* & *M. javanica*			
Debre birhan	2.0	1.62	*M. incognita* & *M. javanica*			
Angolelana tara	1.57	1.37	*M. incognita* & *M. javanica*			
Basoenawerana	2.43	1.67				
Overall mean	2.39	1.76				
Overall, absolute and relative frequency of Meloidogyne species						
Total samples collected	RKN infested samples	*Meloidogyne* spp.	No samples infected with RKN spp	Occurrence (%)	Absolute frequency (%)	Relative frequency (%)
45	25	*M. incognita*	17	37.7	62.9	70.83
		M. javanica	7	15.5	25.9	29.1

In agreement with the current finding Cunha *et al.* (2021) have reported *M. incognita* to be the most frequent species, affecting 60% of carrot fields in Brazil. Another study conducted in Tamil Nadu, India (Wesly *et al.*, 2021) reported *M. incognita* to be found in association with a carrot but not *M. javanica*.

Moreover, the distribution of plant parasitic nematodes detected in this study varies across the five districts. As summarized by Wallace (1963), variability in densities of nematodes in the surveyed districts could be attributed to various factors present in the sampled location, including production systems, soil texture, the variety used, altitude, pH, and temperature.

The present results show an increased density and diversity of PPNs in areas where sandier soil types are dominant and no intercropping is practiced. This study would suggest future research efforts to focus on the determination of damage threshold, extent of yield loss, and extent of pathogenicity to the carrot in the country on the most predominant and widespread nematode genera.

Conclusions

This survey study documented the distribution of plant-parasitic nematode genera associated with carrots in the major carrot-producing districts of the North Showa Zone of Ethiopia. The present study reported the presence of 7 genera of plant parasitic nematodes viz. *Meloidogyne*, *Helicotylenchus, Pratylenchus, Rotylenchulus, Scutellonema, Hoplolaimus* and *Tylenchorynchus*. *Meloidogyne, Pratylenchus, Helicotylenchus,* and *Hoplolaimus*. *Meloidogyne* was the most abundant and frequent plant-parasitic nematode genus detected followed by *Pratylenchus and Helicotylenchus*. The genera including *Rotylenchulus, Scutellonema, Hoplolimus,* and *Tylenchorynchus* occurred relatively with lower frequency occurrence and abundance. Generally, the present survey study has generated information on the occurrence and distribution of plant-parasitic nematodes associated with carrots in the major carrot-producing districts of the North Showa Zone of Ethiopia. Carrot is the most sensitive crop to plant-parasitic nematode with a damage threshold density of less than 1 egg/ml soil for RKNs. The mean densities of RKNs in the surveyed districts were higher than the action threshold level of *Meloidogyne* on carrots. Therefore, it seems necessary to take immediate action to prevent significant inoculum buildup and spread of the root-knot nematodes in the carrot fields in the surveyed areas.

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Novelty Statement

This study was the inaugural report of plant parasitic nematodes associated with carrots from the North Showa Zone, Ethiopia.

Author's Contribution

The first author managed the research investigation, methodology, data collection, and preparation of the first draft, and the second and third authors provided feedback, a review, and edited the work.

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Conflict of interest
The authors have declared no conflict of interest.

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