Detection and molecular identification of some plant parasitic nematodes associated with ornamental plants from Jimma, Ethiopia

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Abstract

There has been a concerning presence of phytoparasitic nematodes on annual and biennial ornamental plants at Jimma University's agriculture campus. Rhizosphere soil samples were collected to elucidate the presence of plant parasitic nematodes associated with ornamental plants. The samples were collected from three locations of eleven different ornamental species, including *Agapanthus africanus, Canna generalis, Cuphea ignea, Dahlia hybrids, Dianthus barbatus, Gerbera jamesonii, Impatiens balsamina, Impatiens hybrids, Pelargonium* spp., *Tagetes erecta* and *Zinnia elegans.* Nematodes were extracted from 100 ml aliquot soil using the modified Baermann tray method. The study identified ten genera of plant parasitic nematodes, including *Helicotylenchus*, *Hemicycliophora*, *Hoplotylus*, *Meloidogyne*, *Mesocriconema*, *Paratylenchus*, *Paratrichodorus*, *Scutellonema*, *Telotylenchus, and Trichodorus*. *Helicotylenchus* was the most dominant genus with a mean population density of 126 individuals/100 ml soil, a frequency of occurrence (FO) of 64%, and a prominence value (PV) of 101 among all sampled ornamental plant species. *Meloidogyne* and *Scutellonema* followed with mean population densities of 129 and 70 individuals/100 ml soil, FOs of 48% and 39%, and PV of 89 and 44, respectively. The least important genera were *Paratylenchus* and *Trichodorus*, with an FO of 3% and mean nematode population densities of 10 and 17 individuals/100 ml soils, respectively. Molecular identification based on 18S small subunit (SSU) ribosomal DNA (rDNA) gene sequences revealed the species identity of *Scutellonema bradys*, *Hemicycliophora conida* and *Telotylenchus ventralis*. This study provides valuable information on nematodes associated with ornamental plant species vital for developing management plans in future landscape maintenance.

Keywords: Population density, *Helicotylenchus*, Landscape management, *Meloidogyne*, Phylogeny

Introduction

The global value of ornamental production is estimated at around USD 55 billion. However, sustainability in production and marketing remains a significant challenge for the industry (Vasanthakumar and Bulti, 2017). Ethiopia has various ornamental plant species, including roses, gypsophila, carnations,

chrysanthemums, limonium, hypericum, dracaena, and Gerbera. In 2015, Ethiopia produced 49000 tons of roses and exported 714.5 million cut flowers, which generated US$ 276 million (FloralDaily, 2016). However, the sector is constrained by multiple factors (Vasanthakumar and Bulti, 2017), including the damage caused by pests.

Plant-parasitic nematodes seriously threaten highly valued crops, including landscape ornamentals, flowering bulbs, herbs, vegetables, fruit trees, and palms, particularly in tropical and subtropical countries (Benson and Barker, 1985; Bala and Hosein, 1996; Hagan, 2005; Asmaa, 2014; Abebe et al., 2015; Meressa et al., 2015). The damage caused by these nematodes is severe and diverse, affecting many ornamentals grown in nurseries, home gardens, commercial farms, greenhouses, and the beauty of landscapes (Salawu and Darabidan, 2010; El-Sherbiny, 2011; Meressa et al., 2014; Nibedita, 2016; Aseffa et al., 2018; Meressa et al., 2018). The plant-parasitic nematodes of the genera *Criconemoides*, *Helicotylenchus*, *Hoplolaimus*, *Meloidogyne*, *Pratylenchus*, *Telotylenchus*, *Tetylenchus* and *Xiphinema* are known to be associated with ornamental plants (Mohammad et al., 2008; Nibedita, 2016; Aasia, 2014; Aseffa et al., 2018). Nematode infected plants show some common symptoms that include a decrease in overall health, root galling, tissue necrosis, yellowing of the leaves, stunted growth of the shoots, premature shedding of leaves, and decreased crop production. An instance of this is *Meloidogyne*, which causes root galls induced by the nematode's stylets puncturing the root surface; the root galls contain root-knot nematode females (Moens et al., 2009).

Previous studies in Ethiopia have mainly focused on identifying and managing fungal and bacterial diseases in ornamental plants, while limited studies have been done on nematodes affecting commercial cut flowers and landscapes (Meressa et al., 2014; Aseffa et al., 2018). As a result, plant-parasitic nematodes have not been considered a significant economic pest in ornamental plants (Meressa et al., 2012). Therefore, this study was conducted to generate preliminary data on the identity, prevalence and abundance of plant-parasitic nematodes associated with selected ornamental plants on the landscape of Jimma, Ethiopia.

Material and Methods

Sampling

Soil samples from the rhizosphere of ornamental plants grown in Jimma University College of Agriculture and Veterinary Medicine (JUCAVM) campus were collected in 2020. JUCAVM is located at 7°68'N, 36°83'E, and 1752 m.a.s.l. Eleven species of ornamental plants grown along pedestrian roadsides, namely, *Canna generalis*, *Impatiens balsamina*, *Tagetes erecta*, *Dahlia hybrids*, *Impatiens hybrids*, *Zin-*

nia elegans, Pelargonium spp., *Gerbera jamesonii, Cuphea ignea, Dianthus barbatus,* and *Agapanthus africanus* were considered. The sampling area was first divided into three sub-areas based on the abundance of ornamental species and considered each sub-sampling site as a replication. From each sub-sampling area, 500 ml of a soil sample was collected from the top 25 cm around the plant root of ten individual plants of each species, using a 3 cm diameter sampling tube. After thoroughly mixing, a subsample of about 200 ml was put into a labeled plastic bag and stored in a refrigerator at 5ºC until nematode extraction.

Nematode extraction and enumeration

Nematodes were extracted from aliquots of 100 ml soil using the modified Baermann tray method (Hooper et al., 2005). Nematodes were collected after 48 hrs on a 38 µm aperture stainless sieve and transferred to plastic cups to store in a refrigerator at 5ºC until nematode counting. Then, nematodes were identified and enumerated to a genus level from a 1 ml capacity counting slide under AmScope 3.7 compound microscope (AmScope, Irvine, China). Siddiqi's (2000) and Manzanilla-Lopez and Marban-Mendoza (2012) nematode identification keys were used for morphological identification.

DNA extraction

Total DNA was extracted from a female nematode for a total of 167 samples that were recovered, handpicked using a needle and placed in 5 µl double deionized water in a 1.5 ml Eppendorf tube. Tubes were centrifuged for 5 seconds and left open at room temperature until the water evaporated (Meressa et al., 2015). Next, 10 µl of worm lysis buffer containing 2 µl of 20 mg/ml Proteinase K (Waeyenberge et al., 2000) was added to the nematode and thoroughly stirred with a sterilized toothpick (Meressa et al., 2015). The lysate was then incubated, for 3 hr at 60°C, followed by 10 min incubation at 95°C. After centrifuging the tube for 3 minutes, the lysate was stored at -20°C until needed for PCR.

SSU rDNA amplification

The primer sets used to amplify the two fragments of 18S ribosomal DNA were 1813F/2646R and 988F/1912R for the first and second fragment, respectively, as Holterman et al. (2006) described. Briefly, the PCR mix (25 µl) contained 1 µl DNA template, 2.5 µ2 mM dNTPs, 2.5 µl 25 mM $MgCl_2$, 0.6 µl 10 µM of each primer, 5 µl 5× Go Taq® buffer and 0.5 u/µl of Go Taq® DNA polymerase (Fisher Scientific Inc., Schwerte, Germany). The PCR reaction was set for heating at 95°C for 5 min followed by the first 5 cycles of amplification at 94°C for 30s, 45°C for 30s, and 72°C for 1 min; and a second 35 cycles of amplification for 30s 94°C, 30s 54°C, 30s 72°C, with a final incu-

bation for 5 min at 72°C. All PCR reactions were run in Applied Biosystem® Thermal cycler (Applied Biosystems, Foster City, CA, USA). A 5 µl of the amplified products with 1 µl lading dye were separated on 1.0% agarose gel in 0.5× TBE buffer at 80 V 34 Am for 85 min, stained with ethidium bromide for 30 min, and visualized at UV-light. 1 kb plus DNA ladder was used as a marker.

Sequencing

The PCR products of three selected nematode genera that are lesser-known in the country, namely *Hemicycliophora, Scutellonema* and *Telotylenchus* were successfully sequenced directly in one direction. Before sequencing, the PCR product was purified using the Wizard® SV Gel and PCR Clean-Up System according to the manufacturer's instructions for PCR-product purification. A 5 µl of the PCR products and 5 µl of 10 pmole μl^{-1} of the respective forward primer of both fragments were mixed and sequenced at the Macrogene sequencing facility service (Amsterdam, The Netherlands).

Phylogenetic analysis

Newly obtained raw sequences were edited using FinchTV. A BLAST search at NCBI was performed using sequences of the two fragments of the 18S rDNA sequence of the nematodes to identify the most closely related species for three selected genera (*Scutellonema, Hemicycliophora* and *Telotylenchus*). Sequences revealing high similarity to the present sequences were downloaded separately for each nematode. Sequences from the present study, the corresponding outgroup sequences and those most closely related sequences retrieved from the NCBI nucleotide database were aligned with MEGA11 version 11.0.13 software for multiple sequence alignment using Muscle alignment (Tamura et al., 2021). An independent phylogenetic tree was generated using the aligned sequences in MEGA11 by the Maximum Likelihood (ML) model with the 1000 replicates and revealed the phylogenetic relationships of the nematode species.

Statistical analyses

Norton's (1978) method was used to analyze the population density (PD) and frequency of occurrence (FO) of nematodes. The nematode data were checked for normality with the Kolmogorov-Smirnov and Shapiro-Wilk tests to ensure accuracy. The nematode count was transformed using log(x+1), and all descriptive statistics and data normality tests were performed using SPSS version 20. The prominence value (PV) was calculated using the method described by De Waele et al. (1998).

Results

The plant parasitic nematodes found on different ornamental plants were *Helicotylenchus*, *Hemicycliophora*, *Hoplotylus*, *Meloidogyne*, *Mesocriconema*, *Paratrichodorus*, *Paratylenchus*, *Scutellonema*, *Telotylenchus* and *Trichodorus*. We observed the association of different ornamental plant species with different genera of plant-parasitic nematodes (Table 1). *Cuphea ignea* had the highest number of nematode genera (seven), while *Gerbera jamesonii* had the lowest (two) (Table 2; Fig. 1).

Not all the ornamental species hosted every nematode genus identified. *Helicotylenchus* and *Meloidogyne* were the two nematode genera found in all ornamental plant species sampled except on *G. jamsoni*, *C. ignea*, *T. erecta,* and *D. hybrids*. *Zinnia elegans* particularly appeared susceptible to the root-knot nematode *Meloidogyne* (Table 2; Fig. 2). *Hoplotylus*, *Trichodorus*, and *Paratylenchus* were found in only one host each. In contrast, *C. ignea* hosted both *Trichodorus* and *Hoplotylus*, while *I. balsamina* hosted *Paratylenchus*. The remaining ornamental hosted two or more nematode genera (Table 2).

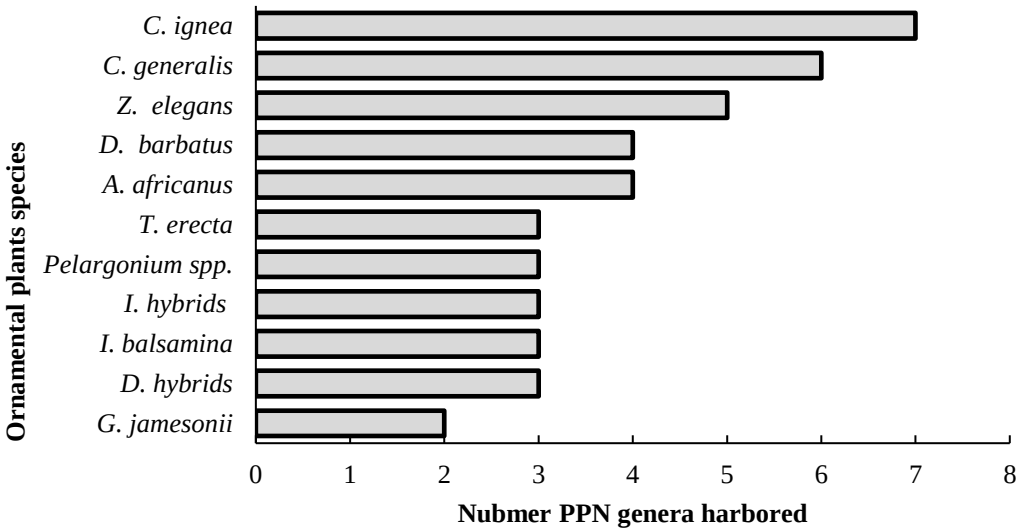

Figure 1. Number of plant parasitic nematode genera identified from the rhizosphere soil sample collected from ornamental plants grown in JUCAVM.

Table 1. Ornamental plant species and associated prominent nematode genera identified.

Common name	Scientific name	Family name	Life span	Prominent nematode genera
African lily	*Agapanthus africanus*	Amaryllidaceae	Perennial	*Helicotylenchus*
African marigold	*Tagetes erecta*	Asteraceae	Annual	*Meloidogyne*
Canna lily	*Canna generalis*	Cannaceae	Annual/ biennial	*Helicotylenchus*
Cigar plant	*Cuphea ignea*	Lythraceae	Annual/ biennial	*Hemicycliophora*
Common zinnia	*Zinnia elegans*	Asteraceae	Annual	*Meloidogyne*
Dahlia	*Dahlia hybrids*	Asteraceae	Annual	*Hemicycliophora*
Garden balsam	*Impatiens balsamina*	Balsaminaceae	Annual	*Helicotylenchus*
Gerbera daisy	*Gerbera jamesonii*	Asteraceae	Annual/ biennial	*Scutellonema*
Impatiens	*Impatiens hybrids*	Balsaminaceae	Annual	*Scutellonema*
Pelargoniums	*Pelargonium* spp.	Geraniaceae	Annual	*Meloidogyne*
Sweet William	*Dianthus barbatus*	Caryophyllaceae	Annual/ biennial	*Helicotylenchus*

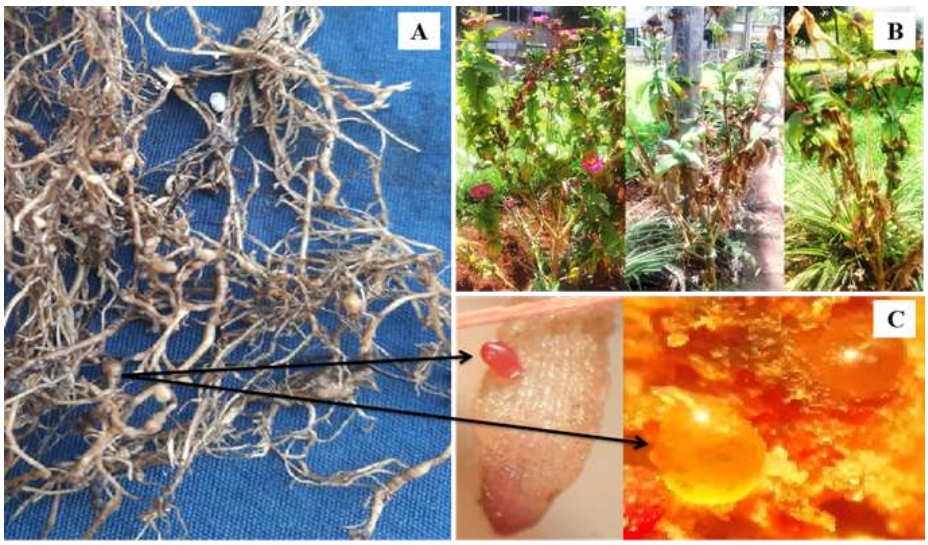

Figure 2. Root-knot nematode (*Meloidogyne*) disease symptoms on *Zinnia elegans.* Root galls (A), wilting symptoms (B); root-knot nematode females after staining with red food colorant (C).

Table 2. Occurrences of plant-parasitic nematode genera associated with each annual and biennial ornamental plant species from soil samples collected in JUCAVM.

Nematode Genera	Ornamental host species										
	C. ignea	*C. generalis*	*Z. elegans*	*A. africanus*	*D. barbatus*	*D. hybrids*	*I. balsamina*	*I. hybrids*	*Pelargonium* spp.	*T. erecta*	*G. jamesonii*
Helicotylenchus	-	**	***	**	***	**	***	***	***	-	-
Meloidogyne	-	***	***	**	*	-	*	**	***	* *	-
Scutellonema	**	**	**	*	-	-	-	***	-	*	**
Mesocriconema	**	*	*	-	**	*	-	-	-	**	-
Hemicycliophora	***	*	*	-	-	*	-	-	*	-	-
Paratrichodorus	*	-	-	*	*	-	-	-	-	-	-
Telotylenchus	*	*	-	-	-	-	-	-	-	-	*
Trichodorus	**	-	-	-	-	-	-	-	-	-	-
Hoplotylus	*	-	-	-	-	-	-	-	-	-	-
Paratylenchus	-	-	-	-	-	-	*	-	-	-	-

(*) Number of samples containing the nematode genus:- (*** all the three samples positive, ** two samples positive, * one sample positive composite per host plant) and (-) nematode undetected.

Different types of ornamental plants hosted various types of nematode genera in a varying population density. *Pelargonium* sp. had the highest mean nematode population for *Meloidogyne*, while *C. generalis, C. ignea*, and *I. hybrids* host *Helicotylenchus*, *Hemicycliophora*, and *Scutellonema*, respectively. The density of these four genera ranged from 110 to 327 individuals per 100 ml of soil. On the other hand, the nematode genera *Hoplotylus*, *Mesocriconema*, *Paratrichodorus*, *Paratylenchus*, *Telotylenchus* and *Trichodorus* were found at very low mean population densities ranging from 10 to 80 nematodes per 100 ml of soil (Fig 3). Moreover, *Dahlia hybrids*, *I. balsamina*, *T. erecta* and *Z.legans*, had lower mean nematode population densities than the other ornamental plants.

Meloidogyne, *Helicotylenchus*, and *Scutellonema* were found to have high population density and frequency of occurrence (Fig. 3). *Meloidogyne* had the highest mean population density of 129 individuals/100 ml of soil with a frequency of occurrence of 48%. *Helicotylenchus* and *Scutellonema* had population densities of 126 and 70 individuals/ 100 ml of soil, respectively, with 64% and 39% frequency of occurrence. The nematode genera were ranked by prominence value (PV), with *Helicotylenchus* having the highest PV of 101, followed by *Meloidogyne* with a PV of 89, and *Scutellonema* with a PV of 44 in all

sampled ornamental plants. The other genera (*Hemicycliophora, Hoplotylus, Paratrichodorus, Mesocriconema, Telotylenchus, Trichodorus,* and *Paratylenchus*) had relatively lower density and frequency of occurrence, except for *Hoplotylus*, which had a relatively higher density.

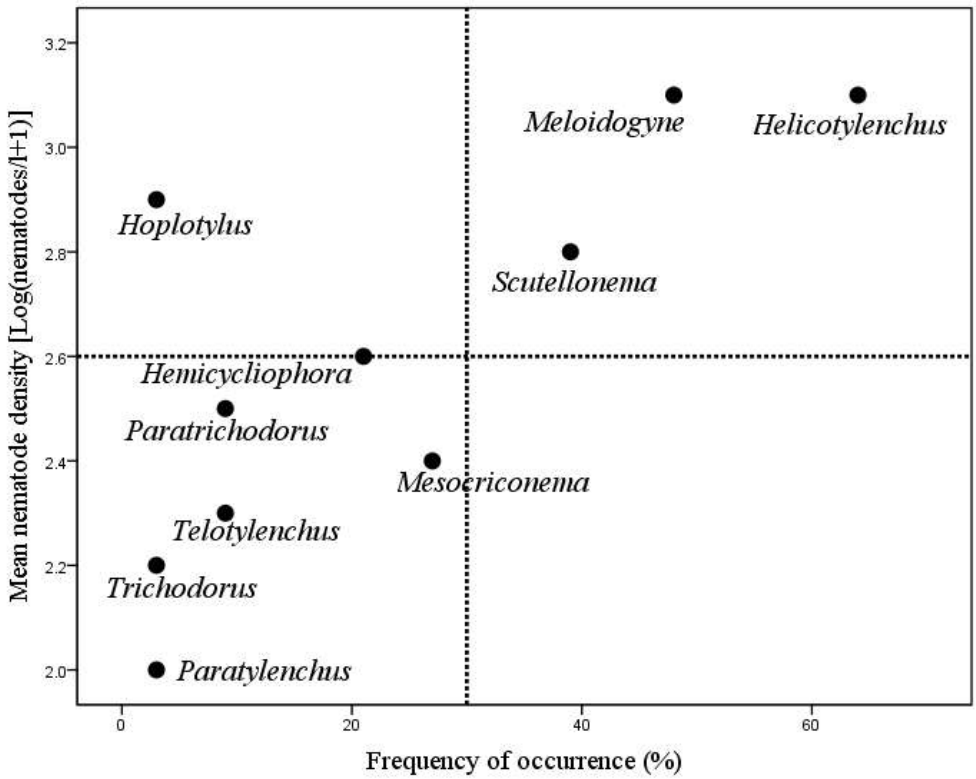

Figure 3. The overall frequency of occurrence and nematode mean population density associated with ornamental plant species.

Phylogenetic analysis

Based on the sequence of both fragments (Fig 4a-b) of the 18S rDNA, the Maximum Likelihood (ML) tree was constructed individually for three species (*Scutellonema bradys, Hemicycliophora conida* and *Telotylenchus ventralis*).

Scutellonema bradys

The PCR amplification of the two fragments of the 18S rDNA region of *S. bradys* yielded a fragment size of 1608 bp (OR262896). The BLAST analysis of our sequences revealed 96% and 95% similarity with the GenBank sequences of *S. bradys* (AJ966504 and AY271723), respectively. The phylogenetic tree was reconstructed from 46 sequences retrieved from NCBI. Based on ML trees, the present sequence (OR262896) clustered in the same clade together with the sequences of *S. bradys* (AJ966504 and AY271723), *R. iranicus* (KJ765352) and *R. robustus* (KJ636452 and KJ636365) (Fig. 5).

Hemicycliophora conida

The 18S rDNA region amplification resulted in a single sequence of 1628 bp (OR262897). The BLAST analysis of the present sequences showed 98% similarity with the Genbank sequences of *H. conida* (AJ966471 and KJ934172). The multiple sequence alignments contained 47 sequences. *M. ethiopica* was used as an outgroup taxon. The reconstructed ML phylogenetic tree exhibited the identity of *H. conida* in which the sequence was clustered together with the sequences of *H. conida* (KJ934172-KJ934173 and AJ966471), *Hemicycliophora* sp. (MF094951) and *H. subbotini* (MG701278 -MG701280) obtained from NCBI database (Fig. 6).

Telotylenchus ventralis

A single sequence of 18S rDNA was obtained with a length of 1673 bp (MK348061). The BLAST search result revealed that the present sequence showed 97% similarity with the sequences of *T. ventralis* (AY593905) retrieved from the Genbank. Multiple sequence alignments involved 22 sequences. *M. ethiopica* was used as outgroup taxa. Based on the phylogenetic tree (Fig. 7), the sequence of the present *T. ventralis* formed a sister clade with *T. ventralis* (AY593905), *Macrotrophurus arbusticola* (AY284595-AY284596), *Neodolichorhynchus microphasmis* (EU669917), *Bitylenchus dubius* (AY284601), *Bitylenchus parvus* (KX789742) and *Bitylenchus parvulus* (MT226922).

Discussion

Helicotylenchus and *Meloidogyne* are well known parasites to ornamental plants in nurseries, greenhouses, and gardens due to their cosmopolitan nature in high mean population density (Benson and Barker, 1985; Hagan, 2005; Aseffa et al., 2018; Meressa et al., 2018). Landscapes often rely on planting materials raised in the nursery, which could be means of disseminating to nematode-free areas. Thus, nematode infection could be avoided initially at the nursery. *Helicotylenchus* is the most important genus affecting ornamental plants (Borgohain, 2016). *Meloidogyne* species, including *M. javanica, M. hapla* and *M. incognita*, have been widely reported to severely infect *Dianthus, Gerbera* and *Zinnia* in the USA (Chandel et al., 2010; Borgohain, 2016; Wheeler et al., 2018).). Gimenes et al. (2010) reported a noticeable plant wilting and stunted growth of some ornamentals, which can lead to reduced ornamental properties. Saeedizadeh (2016) reported that *Helicotylenchus* damage could cause a loss of beauty in various ornamental species. The present study, which attested to the occurrence of *Meloidogyne* and *Helicotylenchus* in higher density, strengthens the previous work of Assefa et al. (2018), who reported high population densities and the occurrence of these two nematode genera associated with perennial ornamental plants from the same study area. Additionally, Sigariova et al. (2015) found that *Telotylenchus* and *Paratylenchus* can cause severe damage to landscape plants, reducing their economic value.

In our study, *Meloidogyne* was detected from samples collected rhizosphere of *T. erecta*, though there were no visible symptoms of root galling. This suggests that *T. erecta* may not be a suitable host for this nematode. Mostafa et al. (2014) have also reported the absence of root galls in *M. incognita*-infected *T. erecta*. Low density and frequency of *Meloidogyne* was detected in *Dianthus barbatus*, a plant species known to suffer from *M. incognita* and *M. javanica* infection (Borgohain, 2016). This might be a less preferred host for other species of this genus (Walker et al., 1994; Johnson et al., 2003). Other nematodes, such as *Hemicycliophora* were more common in ornamentals and were observed in higher density compared to others. This nematode was also reported to be associated with greenhouse cut flowers (Meressa et al., 2014). Prominent root galling and leaf wilting on *Z. elegans* were evident, indicating that this plant is a suitable host for *Meloidogyne* spp. The work of McSorley and Frederick (1994) has indicated a high association between *M. javanica* and *Z. elegans.*

Previously, *M. arenaria* has been molecularly identified from *Z. elegans* roots (Mekuria et al., 2023). It should be noted that exporting planting materials may be prohibited due to nematode infection, even though no quarantine species were detected in this study.

This study identified three plant parasitic nematode species based on 18S rDNA sequence data. To our knowledge, this work is the first report on the molecular identification of *S. bradys*, *H. conida* and *T. ventralis* from ornamental plants in Ethiopia. Compared to other plant parasitic nematodes, there is limited information on ornamental plant-associated nematodes. While *M. hapla* parasitizing rose plants in commercial greenhouses has been molecularly characterized (Meressa et al., 2015), more research is still needed.

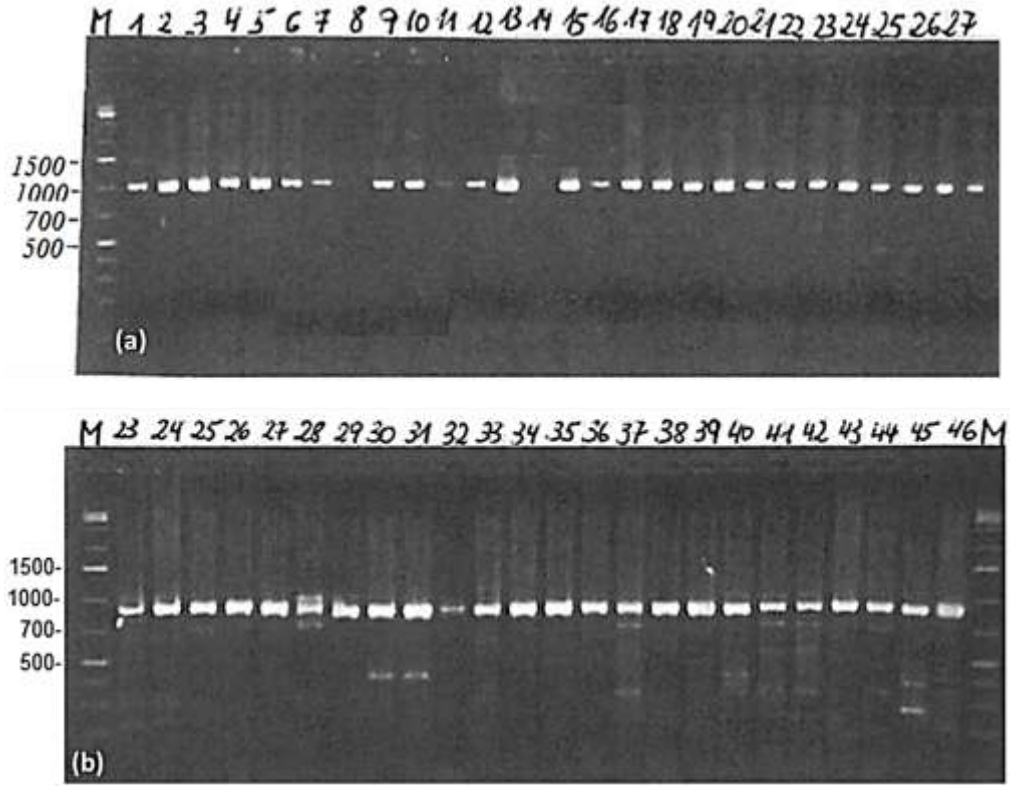

Figure 4. PCR gel electrophoresis of 18S small subunit ribosomal DNA fragments. A 5 ml amplicon was separated on a 1.5% gel and ran for 140 minutes at 80 V and 34 mA. Lanes on fragment 1: *H. conida 1-7; T. ventralis* 8-14; *S. bradys 15-27. (b) Lanes on fragment 2. H. conida 23-31; T. ventralis* 32-35; *S. bradys 36-46. M= 1 KB plus DNA ladder.*

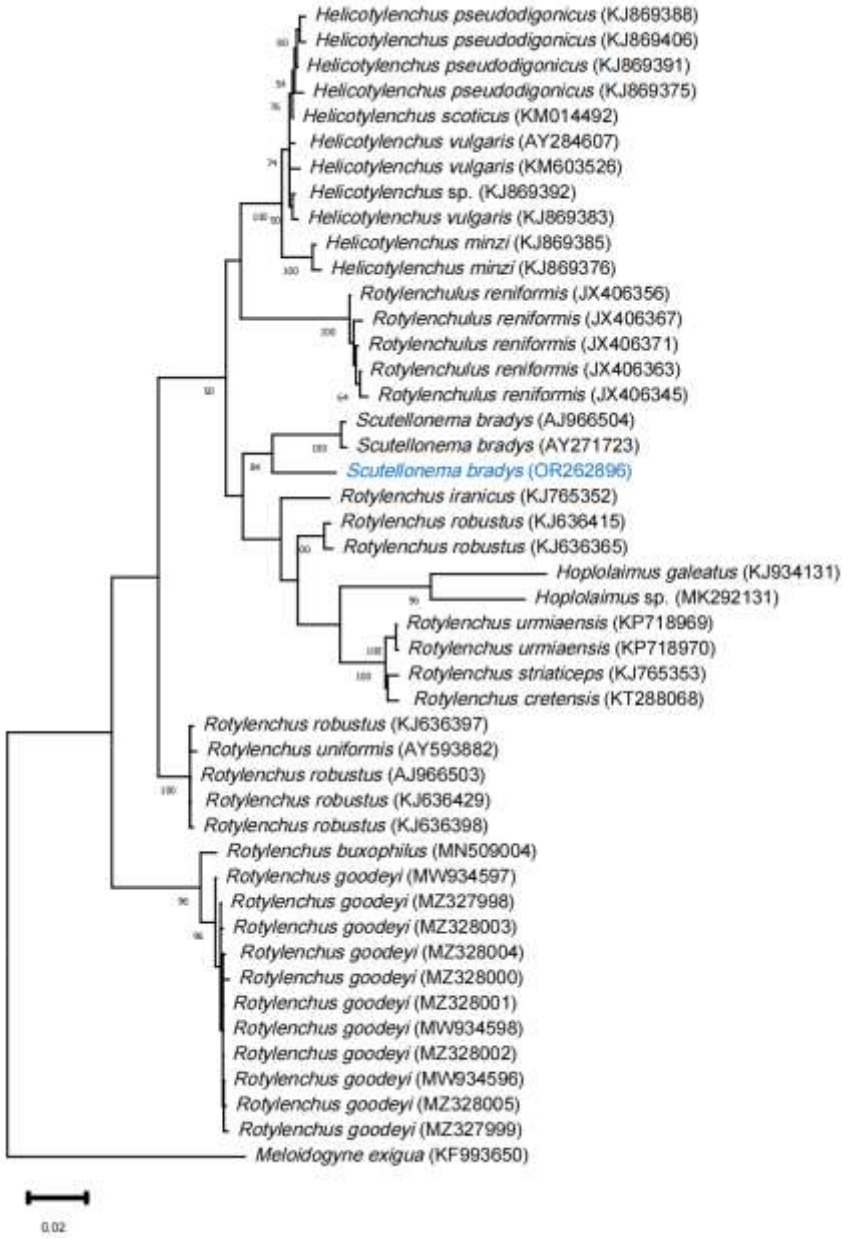

Figure 5. Phylogenetic relationships of *S. bradys* and other related nematode sequences of 18S rDNA based on Maximum likelihood (ML) phylogenetic tree using MEGA11. The analysis was performed using 1000 replicates. Bootstrap values exceeding 70% are given on the appropriate clade. The sequence from this study is indicated with the accession number OR262896. *M. exigua* was used as an outgroup taxon.

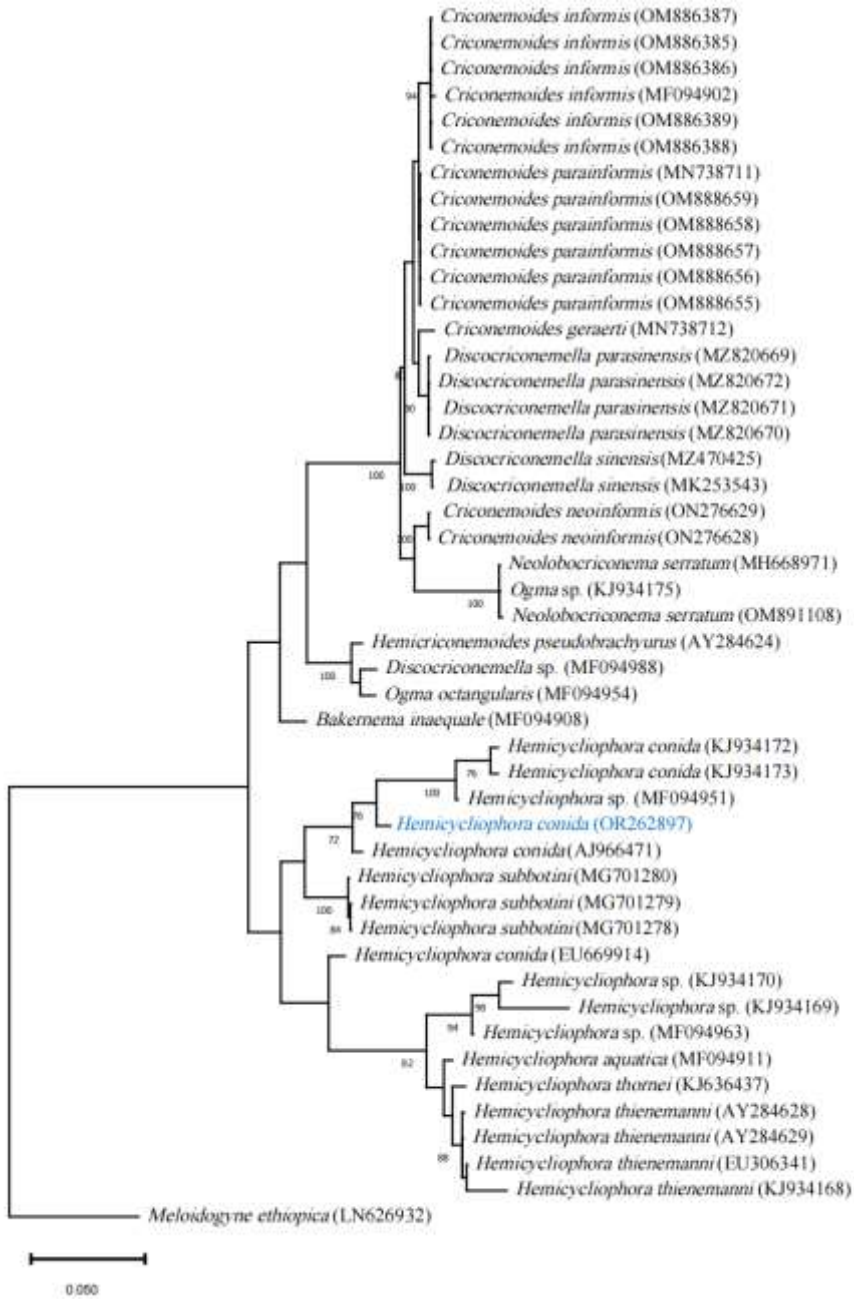

Figure 6. Phylogenetic relationships of *Hemicycliophora conida* and other related nematode sequences of 18S rDNA based on Maximum likelihood (ML) phylogenetic tree using MEGA 11. The analysis was performed using 1000 bootstrap. Posterior probability values exceeding 70% are given on the appropriate clade. The sequence from this study is indicated with the accession number OR262897.

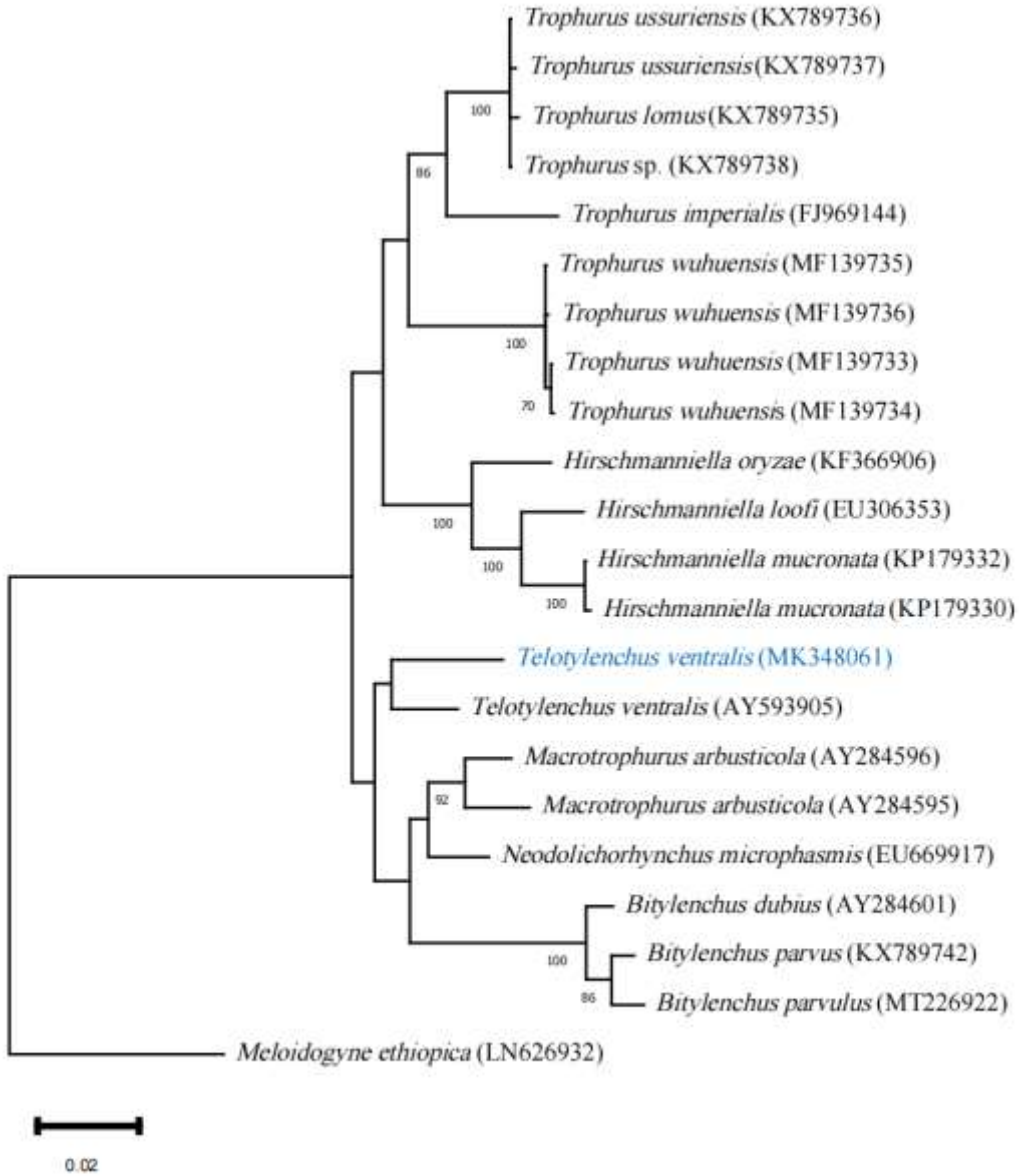

Figure 7. Phylogenetic relationships of *T. ventralis* and other related nematode sequences of 18S rDNA based on Maximum likelihood (ML) phylogenetic tree using MEGA 11. The analysis was performed using 1000 replicates. Posterior probability values exceeding 70% are given on appropriate clade. The sequence from this study is indicated with the accession number MK348061.

Conclusion and Recommendation

This finding highlights the significance of plant parasitic nematodes on ornamental plants in Jimma, Ethiopia. *Helicotylenchus*, *Meloidogyne* and *Scutellonema* are identified as potential problems in establishing most ornamental plants in the study area. These findings provide a valuable foundation for developing a more robust survey, damage potential and population dynamic studies that will eventually help develop integrated nematode management strategies. Meanwhile, employing some of the known strategies, such as applying organic manure, could reduce the impact of these nematodes on the current landscape studied.

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References

Aasia R, Shamim AA, Umar F. 2014. Community analysis of plant parasitic nematodes associated with ornamental plants in Rajouri District (J&K), India. Int. J. Curr. Microbiol. App. Sci., 1: 194-197.

Abebe E, Mekete T, Seid A, Meressa BH, Wondafrash M, Addis T, Getaneh G, Abate A. 2015. Research on plant-parasitic and entomopathogenic nematodes in Ethiopia: a review of current state and future direction. Nematology, 17: 741-759. DOI: 10.1163/15685411-00002919.

Aseffa AW, Addisu FF, Roge GN, Hadis LT, Abera TB, Gero MG, Meressa BH. 2018. Community analysis of phytoparasitic nematodes associated with ornamental plants at Jimma University Agriculture Campus, Ethiopia. Pakistan Journal of Nematology, 1:111-115. DOI: 10.18681/pjn.v36.i01.p111-115.

Asmaa AM. 2014. Nematodes and their associated host plants cultivated in Jazan province, Southwest Saudi Arabia. Egypt. J. Exp. Biol. (Zool.), 1: 35 – 39.

Bala G, Hosein F. 1996. Plant-parasitic nematodes associated with anthrium and other tropical ornamentals. Nematropica, 26: 9-14.

Benson DM and Barker KR. 1985. Nematode - a threat to ornamental plants in the nursery and landscape. Plant disease, 69: 97-100.

Borgohain N. 2016. Plant parasitic nematodes associated with some important commercial flowers. Journal of Global Biosciences, 5:4541–4549

Chandel YS, Kumar S, Jain RK, Vashisth S. 2010. An analysis of nematode problems in green house cultivation in Himachal Pradesh and avoidable losses due to *Meloidogyne incognita* in tomato. Indian Journal of Nematology, 40:198–203.

De Waele D, Mcdonald AH, Jordaan EM, Orion D, Van Den Berg E, Loots GC. 1998. Plant-parasitic nematodes associated with maize and pearl millet in Namibia. African Plant Protection, 4:113-117.

El-Sherbiny AA. 2011. phytoparasitic nematodes associated with ornamental shrubs, trees and palms in Saudi Arabia, including new host records. Pak. J. Nematol, 2: 147-164.

FloralDaily. 2016. Global floricultural News. https://www.floraldaily.com/arti-

cle/9006296/ethiopia-exports-225-millionusd-worth-of-cut-flowers. Accessed on 14 October 2022

Gimenes R, Batista GS, Pivetta KFL, Santos JM, Soares PLM, Martins TA. 2010. Occurrence of plant-parasitic nematodes in ornamental and flowering plants at UNESP/FCAV, Campus of Jaboticabal, São Paulo State, Brazil. Acta Horticulturae, 881: 607-610.

Hagan A. 2005. Nematode pests of annual and perennial flowers, herbs, woody shrubs, and trees. Online at httP://www.aces.edu/pubs/docs/A/ANR-0689/.

Holterman M, van der Wurff A, van den Elsen S, van Megen H, Bongers T, Holovachov O, Bakker J, Helder J. 2006. Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown Clades. Mol Biol Evol, 23(9):1792-800

Hooper D, Hallmann J, Subbotin S. 2005. Extraction, processing and detection of plant and soil nematodes. pp. 53-86. In: Luc M, Sikora RA and Bridge J (eds.). Plant parasitic nematodes in subtropical and tropical agriculture, CABI Publishing, Wallingford pp 53-86.

Johnson SBN, Cannayane I, Rajendran G. 2003. studies on the pathogenic level of *Meloidogyne incognita* on gladiolus and carnation. Current Nematology, 14: 75-78.

Manzanilla-Lopez RH, Marban-Mendoza N. 2012. Practical plant nematology. Biblioteca Basica de Agricultura, Montecillo.

McSorley R, Frederick JJ. 1994. Response of some common annual bedding plants to three species of *Meloidogyne*. Supplement to Journal of Nematology, 26: 773-777.

Mekuria TM, Meressa BH, Hundesa WB. 2023. Prevalence of major parasitic nematodes associated with tomatoes (*Solanum lycopersicum* L.) in two districts of Jimma, Ethiopia. Archives of Phytopathology and Plant Protection, 56(2):158-174. DOI:10.1080/03235408.2023.2178062

Meressa BH, Beyene ZH, Abebe WA, Essubalew GS. 2018. Prevalence of plant parasitic nematodes vary with crop cultivars/clones. Ethiop. J.Appl.Sci. Technol., 1: 31-45.

Meressa BH, Dehne H, Hallmann J. 2012. Distribution of plant-parasitic nematodes associated with cut flowers in Ethiopia. 31st International European Society of Nematologists. September 23-27, Adana, Turkey (abstract).

Meressa BH, Dehne H, Hallmann J. 2014. Host suitability of cut-flowers to *Meloidogyne* spp. and population dynamics of *M. hapla* on the rootstock *Rosa corymbifera* 'Laxa'. American Journal of Experimental Agriculture, 4: 1397-1409. DOI: 10.9734/AJEA/2014/10884.

Meressa BH, Heuer H, Dehne H, Hallmann J. 2015. Molecular and morphological characterization of *Meloidogyne hapla* populations from rose greenhouses in Ethiopia. Russian journal of Nematology, 23:1-20.

Moens M, Perry RN, Starr JL. 2009. *Meloidogyne* species - A diverse group of novel and important plant parasites. *Root-Knot Nematodes*, 1:483

Mohammad AD, Chitambar JJ, Maafi ZT. 2008. Nematodes associated with flowering ornamental plants in Mahallat, Iran. Nematol. Medit., 36: 115-123.

Mostafa FAM, Refaei AR, Khalil AE, Deriny El, Marwa M. 2014. Host suitability of certain ornamental plants to the root-knot nematode, *Meloidogyne incognita* and reniform nematode, *Rotylenchulus reniformis* under greenhouse conditions. International Journal of Advanced Research, 2: 33-42.

Nibedita B. 2016. Plant parasitic nematodes associated with some important commercial flowers. Journal of Global Biosciences, 8: 4541-4549. https://www. mutagens.co.in/jgb/ vol.05/8/050814.pdf.

Norton DC. 1978. Ecology of plant parasitic nematodes. John Willey and Sons. *New York*.

Saeedizadeh A. 2016. Identification and distribution of plant-parasitic nematodes in landscape of Tehran city, Iran. Iranian J. of Pl. Pro. Sci, 47: 43-49.

Salawu EO, Darabidan IA. 2010. Screening of selected ornamental plants to *Meloidogyne incognita* in Nigeria. Pak. J. Nematol, 28: 353-358.

Siddiqi MR. 2000. Tylenchida parasites of plants and insects, 2nd edn. Wallingford, UK, CABI Publishing.

Sigariova DD, Karpliyk VG. 2015. Parasitic nematodes of flowering and ornamental plants: effect of parasite on the plants and response of the plants to the presence of nematodes. Vestnik Zoology, 49: 427-432.

Tamura K, Stecher G, Kumar S. 2021. MEGA 11: Molecular evolutionary genetics analysis Version 11. Mol. Biol. Evol, 38: 3022–3027.

Vasanthakumar K, Bulti M. 2017. A handbook on floriculture and landscaping. https://www.slideshare.net/BultiEtefa/ handbook on floriculture and landsca ing 167436023.Accessed on 18 September 2022.

Waeyenberge L, Ryss A, Moens M, Pinochet J, Vrain TC. 2000. Molecular characterisation of 18 *Pratylenchus* species using rDNA Restriction Fragment Length Polymorphism. *Nematology*, 2:135-142.

Walker JT, Melin JB, Davis J. 1994. Sensitivity of Bedding plants to Southern Root-Knot Nematode, *Meloidogyne incognita* race 3. Journal of Nematology, 26: 778-781.

Wheeler TA, Woodward JE, Walker NR. 2018. Plant parasitic nematodes of economic importance in Texas and Oklahoma. *In: S. A. Subbotin and J. J. Chitambar, eds. Plant parasitic nematodes in sustainable agriculture of North America.* Cham; Switzerland: Springer: pp. 433–451