International Journal of Bio-resource and Stress Management
https://ojs.pphouse.org/index.php/IJBSM

Crossref

IJBSM July 2025, 16(7): 01-07
Article AR6259

Research Article
Stress Management
DOI: HTTPS://DOI.ORG/10.23910/1.2025.6259

Diversity and Community Structure of Plant Parasitic Nematodes in Khasi Mandarin Orchards of Assam, India

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ABSTRACT

The study was conducted during December, 2017–February, 2018 in the soil rhizosphere of Khasi mandarin orchards across four districts (Kamrup (R), Kamrup (M), Tinsukia and Biswanath) in Assam, India to study the diversity and community structure of plant parasitic nematodes (PPN). Soil samples collected from different orchards revealed the presence of six major PPN genera viz., *Tylenchulus semipenetrans*, *Helicotylenchus* sp., *Tylenchorhynchus* sp., *Hoplolaimus* sp., *Pratylenchus* sp. and *Rotylenchulus* sp. Among the identified PPN, *T. semipenetrans* was the most abundant species observed in all surveyed districts, indicating its widespread distribution and potential as a key threat to Khasi mandarin production. It was followed by *Helicotylenchus* sp. and *Tylenchorhynchus* sp. in terms of abundance. The highest juvenile count of *T. semipenetrans* was recorded in Tinsukia, while the lowest was observed in Kamrup (M). The remaining PPN genera showed variable occurrence depending on the location and orchard condition. The nematode community structure was thoroughly analyzed using various ecological parameters, including frequency, density and prominence value. *T. semipenetrans* had the highest frequency (AF=100%), relative frequency (RF=38.12%), and prominence value (PV=422.25), establishing it as the dominant PPN in Khasi mandarin orchards. This detailed identification and community analysis of PPN genera associated with Khasi mandarin orchards provided fundamental information for designing suitable nematode management strategies to minimize yield losses, thereby enhancing the sustainability and productivity of Khasi mandarin cultivation and contributing to long-term food security in the region.

KEYWORDS: Plant parasitic nematode, citrus, abundance, community analysis

***Citation* (VANCOUVER):** Kumar and Das, Diversity and Community Structure of Plant Parasitic Nematodes in Khasi Mandarin Orchards of Assam, India. *International Journal of Bio-resource and Stress Management,* 2025; 16(7), 01-07. HTTPS://DOI.ORG/10.23910/1.2025.6259.

Data Availability Statement: Legal restrictions are imposed on the public sharing of raw data. However, authors have full right to transfer or share the data in raw form upon request subject to either meeting the conditions of the original consents and the original research study. Further, access of data needs to meet whether the user complies with the ethical and legal obligations as data controllers to allow for secondary use of the data outside of the original study.

Conflict of interests: The authors have declared that no conflict of interest exists.

RECEIVED on 31st March 2025 RECEIVED in revised form on 05th July 2025 ACCEPTED in final form on 18th July 2025 PUBLISHED on 31st July 2025

1. INTRODUCTION

Citrus is one of the major fruit crops cultivated globally and is a significant horticultural commodity in international trade (Matheyambath et al., 2016). India ranks third among the top ten citrus producing countries and produces 14.24 mmt of citrus year^{-1}. Among different citrus species in India, mandarin oranges occupy 43.4% of the total area and account for 43.65% of total citrus production, followed by limes, which occupy 29.8% of the area and contribute 24.9% to production, and sweet oranges, with 19.8% of the area and 28% of production. Other species, such as pummelo, grapefruit, sour and bitter oranges, occupy 7.01% of the area and account for 3.43% of total production (Anonymous, 2021). North East India is regarded as the centre of origin for many citrus species. Assam is one of the major citrus producing states of North East India. In Assam, commercially available species include Khasi mandarin (*Citrus reticulata* B.), Assam lemon (*Citrus limon*), Pummelo (*Citrus grandis*) and Rough lemon (*Citrus jambhiri*), along with several other cultivated species. Among these, Khasi mandarin is the most important citrus fruit in Assam with an area of 13.29 th ha area and 185.02 th mt production (Anonymous, 2021). However, the productivity of Khasi mandarin in Assam is limited by various biotic factors such as plant pathogens (*Phytophthora* spp., *Xanthomonas citri* pv. *citri*, *Candidatus* Liberibacter asiaticus and citrus tristeza virus), insects (*Anoplophora versteegi*, *Inderbela quadrinotata*, *Phyllocnistis citrella* and *Papilio* spp.) and plant parasitic nematodes.

Plant parasitic nematodes (PPN) pose a significant biotic constraint in citrus producing regions, leading to substantial yield losses. While various nematode species are reported in the citrus rhizosphere, Citrus nematode, *Tylenchulus semipenetrans* is recognized as one of the most important species across all citrus-growing regions (Duncan, 2009; Abd-Elgawad, 2014; Kumar and Arthurs, 2021; Sweelam et al., 2022; Kiptoo et al., 2022; Natesan et al., 2024). Global yield losses caused by this nematode are estimated to range from 10 to 30% (Kumar and Arthurs, 2021; Baniya et al., 2025), while in India losses have been reported to reach up to 27% (Kumar et al., 2020). It causes a slow decline in citrus and contributes to citrus dieback, often in association with other disease complexes. Infested feeder roots often appear dark and are covered with soil particles, with branch rootlets being shorter than those of healthy roots (Duncan, 2009; Abd-Elgawad, 2020). Above-ground symptoms include chlorosis, leaf defoliation, reduced fruit size, premature fruit drop, and twig dieback starting from the upper branches (Duncan, 2009; Kumar and Arthurs, 2021). Additionally, various ectoparasitic (*Helicotylenchus* spp., *Hoplolaimus* spp., *Hemicycliophora* spp.), migratory endoparasitic (*Pratylenchus, Radopholus* spp.), and sedentary endoparasitic (*Meloidogyne* spp.) nematodes have also been reported pathogenic to citrus (Rao, 2008; Patel and Patel, 2013; Ravichandra, 2014; Reddy, 2018; Kumar and Arthurs, 2021; Yangchan et al., 2025; Kumar et al., 2025).

Despite the importance of PPN, information on their relative abundance and distribution in Khasi mandarin orchards in Assam remains limited. Since the distribution and abundance of the nematode species tends to vary with climatic, edaphic, and agronomic factors, a renewed assessment of their abundance and spatial distribution in Khasi mandarin orchards is crucial. This information is vital for developing a targeted, location-specific integrated pest management (IPM) strategy. Hence, a comprehensive survey was conducted to evaluate the diversity and community analysis of PPN in the soil rhizosphere of Khasi mandarin orchards across four districts viz., Kamrup (R), Kamrup (M), Tinsukia, and Biswanath of Assam. Understanding the population density and distribution of these nematodes can aid in assessing ecological disturbances, evaluating potential disease risks, and implementing effective management strategies against nematode infestations.

2. MATERIALS AND METHODS

2.1. *Survey and sample collection*

The study was conducted during December, 2017–February, 2018 in four Khasi mandarin growing districts of Assam viz., Kamrup (R), Kamrup (M), Tinsukia and Biswanath to understand the diversity, distribution and community structure of PPN. Composite samples were collected from ten different geographical locations among four districts (Table 1). Samples were collected from 15–30 cm depth around the feeder roots of mandarin. All the collected soil samples were labelled and transported to laboratory for nematode extraction and identification.

2.2. *Nematode processing and identification*

A 200 cc soil sample from each composite sample was processed using Cobb's sieving and decanting technique along with modified Baermann funnel technique (Southey, 1986). After 48 h of incubation, nematode suspensions were collected and examined under a stereoscopic microscope (Olympus SZX16). Selected nematode specimens extracted from the soil were killed and fixed with hot 4% formaldehyde, then mounted on glass slides for identification under a compound microscope (Olympus BX41) following the procedure as described by Shurtleff and Averre, 2000. Nematode taxa present in the soil samples were subsequently counted under the stereomicroscope. PPN in each sample were identified to genus level based on morphological characteristics, including body shape and size, stylet length and type, lip region, pharyngeal overlap,

Table 1: Field surveyed localities in Assam, India

District	Village/tehsil	Crop/cropping system	Latitude	Longitude
Kamrup (R)	Bhutargari, Chhaygaon	Khasi mandarin	25° 58'	91° 18'
	Kahibama, Chhaygaon	Khasi mandarin	25° 58'	91° 17'
	Laruboma, Santipur (B.O), Bamunigaon (S.O), Boko	Khasi mandarin	25° 59'	91° 15'
Kamrup (M)	Ghaguah, Digaru (P.O.), Sonapur	Khasi mandarin	26° 10'	91° 57'
	Modaikuchi, Khetri (P.O.)	Khasi mandarin	26° 05'	92° 4'
Tinsukia	CRS, Tinsukia	Khasi mandarin	27° 31'	95° 21'
	Wathoigaon	Khasi mandarin-Arecanut	27° 34'	95° 48'
	Kakopathar (Gondoigudi)	Khasi mandarin	27° 38'	95° 39'
	Borgaon	Khasi mandarin-Tea	27° 31'	95° 24'
Biswanath	Japow Bari, Biswanath Chariali	Khasi mandarin	26° 46'	93° 09'

tail type, and vulva position using dichotomous keys (Mai and Lyon, 1975; Mai and Mullin, 1996).

2.3. Community analysis of plant parasitic nematodes

The community structure of the identified nematodes was analyzed using various parameters viz., absolute frequency (AD), relative frequency (RF), absolute density (AD), relative density (RD) and prominence value (PV) (Norton, 1978).

3. RESULTS AND DISCUSSION

3.1. Microscopic identification and community structure of PPN genera

The survey of PPN conducted across ten different sites in four Khasi mandarin growing districts of Assam revealed the presence of six genera of nematodes, namely: *T. semipenetrans*, *Helicotylenchus* sp., *Pratylenchus* sp., *Tylenchorhynchus* sp., *Hoplolaimus* sp. and *Rotylenchulus* sp. (Figure 1). Among these, *T. semipenetrans* was the most abundant nematode species followed by *Helicotylenchus* sp., *Tylenchorhynchus* sp., *Rotylenchulus* sp., *Hoplolaimus* sp. and *Pratylenchus* sp. However, *Hoplolaimus* and *Pratylenchus* were not observed in Kamrup (M), Tinsukia and Biswanath districts. While, *Rotylenchulus* was not observed in Tinsukia and Biswanath districts. In case of *T. semipenentrans*, the highest number of juveniles was recorded in Tinsukia district, while Kamrup (M) reported the lowest nematode counts (Figure 2 and 3).

Morphological identification of the nematodes was based on distinct features: *T. semipenetrans* was confirmed by its long, pointed tail and excretory pore located at 50–60% of the body length. The spiral body shape was characteristic of *Helicotylenchus* sp., while *Tylenchorhynchus* sp. was identified by their rounded lip region, offset by a slight constriction, and conoid tail. *Rotylenchulus* sp. was identified by a high and conoid-rounded lip region, 'C' shape when heat killed and well-developed ventrally curved spicules. *Hoplolaimus*

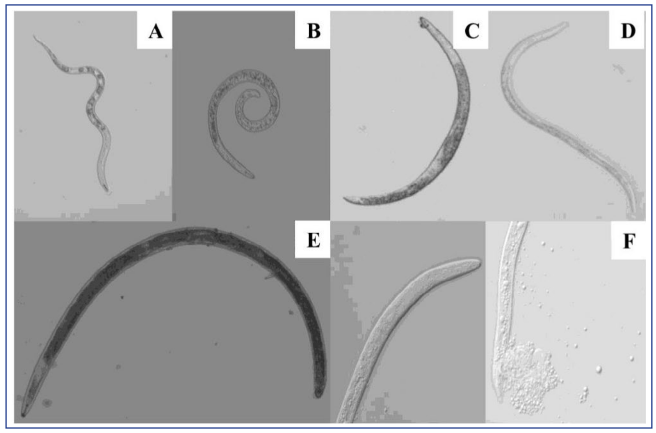

Figure 1: Microscopic images of plant-parasitic nematodes identified in Khasi mandarin. (A) *Tylenchulus semipenetrans*, (B) *Helicotylenchus* sp., (C) *Pratylenchus* sp., (D) *Tylenchorhynchus* sp., (E) *Hoplolaimus* sp., (F) *Rotylenchulus* sp. Images were captured at 40× magnification (Olympus BX41, Model BX41TF, Tokyo, Japan)

sp. was identified by the presence of a well-developed stylet with robust, slightly curved stylet knobs and a bluntly rounded tail. Finally, *Pratylenchus* sp. was confirmed by their low, flattened small body, sclerotized head frame, and distinct tail region.

3.2. Community analysis of PPN genera

The community structure of the identified PPN genera was analyzed using several parameters viz., Absolute frequency (AF), Relative frequency (AF), Absolute density (AD), Relative density (RD) and Prominence value (PV). The data of four surveyed districts were presented in Table 2.

3.3. Frequency

The consolidated data of four surveyed districts showed that *T. semipenetrans* was the most frequently occurring nematode (AF=100% and RF=38.12%) followed by *Helicotylenchus* (AF=87.5% and RF=31.87%), *Tylenchorhynchus* (AF=54.15%

Table 2: Community analysis of PPN (200cc soil) infecting Khasi mandarin in four districts of Assam

Parameter	*Tylenchulus semipenetrans*	*Helicotylenchus* sp.	*Hoplolaimus* sp.	*Tylenchorhynchus* sp.	*Rotylenchulus* sp.	*Pratylenchus* sp.
Kamrup (Rural)						
AF	100	100	33.3	100	33.3	33.3
RF	25	25	8.32	25	8.32	8.32
AD	32.65	30.16	0.8	5.0	3.5	3
RD	43.35	40.03	1.32	6.64	4.64	3.98
PV	326.5	301.6	4.61	33.2	20.19	17.31
Kamrup (Metro)						
AF	100	50	-	-	50	-
RF	50	25	-	-	25	-
AD	9	5	-	-	4	-
RD	50	27.7	-	-	22.2	-
PV	90	35.35	-	-	28.28	-
Tinsukia						
AF	100	100	-	66.6	-	-
RF	37.5	37.5	-	24.98	-	-
AD	100.75	15.25	-	4.62	-	-
RD	83.5	12.64	-	3.83	-	-
PV	1007.5	152.5	-	37.7	-	-
Biswanath						
AF	100	100	-	50	-	-
RF	40	40	-	20	-	-
AD	26.5	13.5	-	4.5	-	-
RD	59.5	30.3	-	10.11	-	-
PV	265	135	-	31.81	-	-

(AF: Absolute frequency; RF: Relative frequency; AD: Absolute density; RD: Relative density; PV: Prominence value); AF of sp.: No. of samples containing species/No. of samples collected ×100; RF of sp.: Frequency of species/Sum of frequencies of all species present in samples×100; AD of sp.: No. of individuals of a sp. in a sample/Volume or mass or unit of a sample×100; RD of sp.: No. of individuals of a sp. in a sample/Total no. of individuals of all species in a sample×100; PV of sp.: Absolute density √Absolute frequency

and RF=17.49%) and *Rotylenchulus* (AF=20.82% and RF=8.33%). While, *Hoplolaimus* (AF=8.32% and RF=2.08%) and *Pratylenchus* (AF=8.32% and RF=2.08%) were least abundant (Table 3).

3.4. Density

The data showed that *T. semipenetrans* had the highest absolute and relative density (AD=42.22% and RD=59.08%) followed by *Helicotylenchus* (AD=15.97% and RD=27.66%). Whereas, *Tylenchorhynchus* (AD=3.53% and RD=5.14%), *Rotylenchulus* (AD=1.87% and RD=6.71%), *Pratylenchus* (AF=0.75% and RF=0.99%) *Hoplolaimus* (AD=0.2% and RD=0.33%) were having the lowest density and relative density (Table 3).

3.5. Prominence value

The prominence value of nematodes in the soil samples ranged from 1.15 to 422.25. Based on the nematode population and frequency of occurrence, *T. semipenetrans* was the most prominent nematode (PV=422.25) in Khasi mandarin followed by *Helicotylenchus* (PV=156.11). The least prominent nematodes in the community were *Tylenchorhynchus* (PV=25.67), *Rotylenchulus* (PV=12.11), *Pratylenchus* (PV=4.32) and *Hoplolaimus* (PV=1.15) (Table 3).

The present study offers valuable insights into the PPN associated with Khasi mandarin in Assam, with *T. semipenetrans* being the most abundant species reported

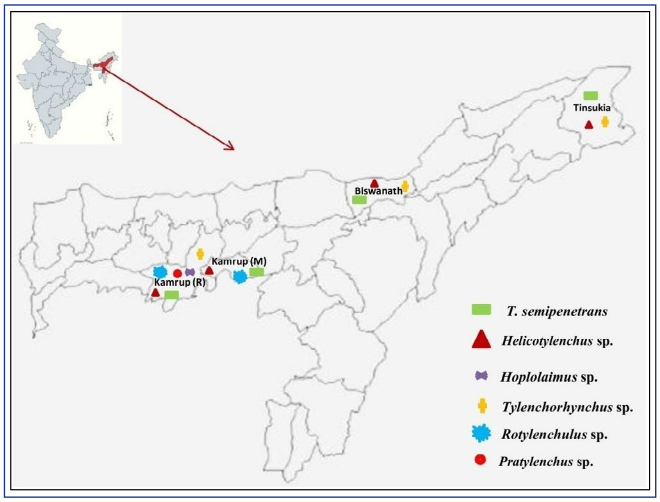

Figure 2: Map displaying the distribution of plant parasitic nematodes in four khasi mandarin growing districts of Assam, India

in all the Khasi mandarin orchards surveyed, as reported in other citrus growing areas in other parts of the world (Sorribas et al., 2008; Abd-Elgawad et al., 2016; Eisvand et al., 2019; Abu Habib et al., 2020; Zoubi et al., 2022; Kiptoo et al., 2022; Sweelam et al., 2022; Natesan et al., 2024). This species has been reported as a threat to citrus as it cause slow decline in citrus. The widespread distribution of *T. semipenetrans* can be attributed to several factors including the use of infected seedlings, contaminated plant materials, irrigation practices, and agricultural machinery (Abd-Elgawad et al., 2016). The second most prevalent plant-parasitic nematode was *Helicotylenchus* in surveyed districts. The prevalence of this genus is reported in other citrus growing regions of the world (Sorribas et al., 2008; Abu Habib et al., 2020; Zoubi et al., 2022). Other nematode genera such as *Tylenchorhynchus*, *Hoplolaimus* and *Pratylenchus* were also reported in few sites in the surveyed districts. Subsequently, one of the most economically important semi endoparasitic nematode, *Rotylenchulus* was detected in Kamrup (R) and Kamrup (M) districts in low density. This genus has been reported as a threat to vegetables, fruit trees and oil seed crops in India (Khan, 2023). However, the occurrence of *T. semipenetrans* in large numbers followed by *Helicotylenchus* sp. and *Tylenchorhynchus* sp. were observed in Khasi mandarin orchards of Tinsukia district.

Few studies have reported the presence of these nematode genera in citrus orchards in India. Nandwana et al. (2005) reported that *T. semipenetrans* was predominant followed by *Pratylenchus* and *Helicotylenchus* spp. in citrus orchards and nurseries in Jhalawar district, Rajasthan. Mahanta et al. (2018) reported the presence of *T. semipenetrans* and *Helicotylenchus* in Khasi mandarin in Tinsukia district of Assam. Similarly, Borthakur et al. (2024) reported the presence of *Tylenchulus*, *Hoplolaimus*, *Helicotylenchus* and *Tylenchorhynchus* in citrus in Dibrugarh district, Assam. Recently, Kumar et al. (2025) reported the prevalence of *T. semipenetrans* and *Helicotylenchus* sp. followed by *Hoplolaimus* sp., *Tylenchorhynchus* sp. and *Pratylenchus* sp. in Assam lemon orchards of Assam. Further, Yangchan et al. (2025) reported the higher abundance of *T. semipenetrans* followed by *Meloidogyne* spp., *Helicotylenchus* spp. and *Pratylenchus* spp. in citrus growing regions of Himachal Pradesh. Our findings further validate these earlier reports. In contrast, *Xiphinema* was identified as the dominant genera, followed by *Pratylenchus*, *Tylenchulus* and *Helicotylenchus* in citrus growing regions of Jammu and Aurangabad district in Maharashtra (Deshmukh et al., 2016). Despite the importance of *T. semipenetrans*, there are no specific reports on the extent of its pathogenicity to Khasi mandarin in North East India. This distribution pattern suggests variations in environmental factors influencing nematode populations.

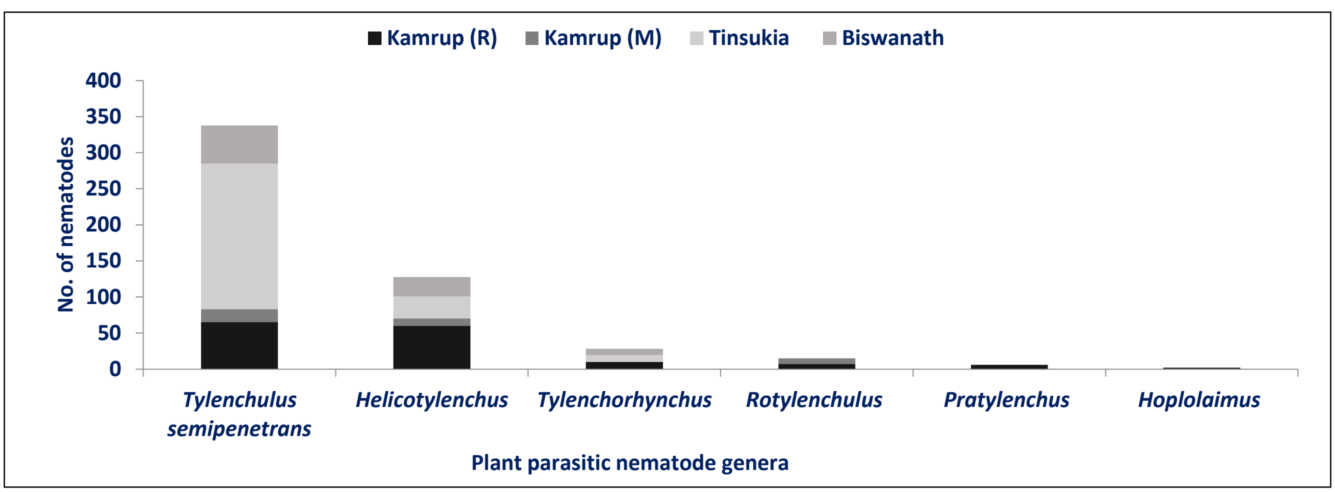

Figure 3: Occurrence (Avg. No. per 200 cc soil) of Plant parasitic nematode genera associated with Khasi mandarin in four surveyed districts of Assam, India

Table 3: Community analysis of PPN (200cc soil) infecting Khasi mandarin in four districts (consolidated) of Assam

Parameter	*Tylenchulus semipenetrans*	*Helicotylenchus* sp.	*Hoplolaimus* sp.	*Tylenchorhynchus* sp.	*Rotylenchulus* sp.	*Pratylenchus* sp.
AF	100	87.5	8.32	54.15	20.82	8.32
RF	38.12	31.875	2.08	17.49	8.33	2.08
AD	42.22	15.97	0.2	3.53	1.87	0.75
RD	59.08	27.66	0.33	5.14	6.71	0.99
PV	422.25	156.11	1.15	25.67	12.11	4.32

AF: Absolute frequency; RF: Relative frequency; AD: Absolute density; RD: Relative density; PV: Prominence value

Enhancing the studies on soil sampling and nematode identification can significantly improve the early detection and management of PPN, including *T. semipenetrans* and other economically important PPN in citrus. However, the accuracy and reliability of PPN identification can be greatly enhanced by combining both morphological and molecular diagnostic approaches. Current findings essence nematode screening in the commercial citrus producing areas in Assam. This study provided valuable insights into the severity of nematode infestation in Khasi mandarin orchards, paving the way for future research initiatives focused on nematode management in these commercial areas.

4. CONCLUSION

Among the six PPN genera identified in Khasi mandarin orchards, the citrus nematode *T. semipenetrans* emerged as the most prevalent across the four surveyed districts of Assam. However, a comprehensive investigation was needed in other Khasi mandarin-growing regions of Assam to better understand the yield losses attributed to PPNs and to develop effective strategies to mitigate these losses.

5. ACKNOWLEDGEMENT

The authors express their gratitude to the Director, ICAR-CCRI for providing necessary facilities to carry out this work.

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