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Diversity of Plant-Parasitic Nematodes Associated with Assam Lemon

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Authors' contributions

This work was carried out in collaboration among all authors. Author KKK designed the study, performed the analysis, and wrote the first draft of the manuscript. Authors AKD and DPS managed the literature searches and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

A comprehensive study was conducted to assess the diversity and community analysis of plant parasitic nematodes (PPN) in the rhizosphere soil of Assam lemon orchards across four districts in Assam. The study identified six predominant PPN genera: *Tylenchulus semipenetrans*, *Helicotylenchus* sp., *Hoplolaimus* sp., *Tylenchorhynchus* sp., *Pratylenchus* sp. and *Rotylenchulus* sp. The nematode community structure was analyzed using various ecological parameters. Among the identified PPN, *T. semipenetrans* and *Helicotylenchus* sp. were the most abundant (100%), followed by *Hoplolaimus* sp. (80%), *Tylenchorhynchus* sp. (60%) and *Pratylenchus* sp. (60%). This identification of PPN genera associated with Assam lemon orchards provides essential information for developing effective nematode management strategies aimed at reducing yield losses and enhancing food security.

Keywords: Plant parasitic nematode; Assam lemon; community analysis.

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1. INTRODUCTION

Citrus is the third most important fruit crop in India, following mango and banana. Among the various citrus species, mandarin oranges dominate, occupying 43.4% of the total citrus area and contributing 43.65% to production, followed by limes, covering 29.8% of the area and contributing 24.9% to production. Sweet oranges account for 19.8% of the area and 28% of production. Other species, including pummelo, grapefruit, sour and bitter oranges, occupying 7.01% of the area and 3.43% of total production (Department of Agriculture & Farmers Welfare, 2021). North Eastern India is recognized as a centre of origin for many citrus species, with Assam being a major citrus producing state in the region. In Assam, commercially available species include Khasi mandarin (*Citrus reticulata*), Assam lemon (*Citrus limon*), Pummelo (*Citrus grandis*) and Rough lemon (*Citrus jambhiri*), along with several other cultivated species. Among these, Assam lemon has gained prominence as the second most widely cultivated citrus variety in the state, covering over 16,000 hectares and producing more than 160,000 metric tons annually (Directorate of Economics and Statistics, Government of Assam, 2023). Assam lemon has high demand in both domestic and international markets. However, the productivity of Assam lemon in Assam is limited by various biotic factors such as plant pathogens (*Phytophthora* spp., *Xanthomonas citri* pv. *citri*, *Elsinoe fawcettii*), insects (*Papilio* spp., *Phyllocnistis citrella*, *Podagrigomela nigripes* and *Toxoptera citricida*) and plant parasitic nematodes.

Plant-parasitic nematodes (PPN) are a major biotic constraint in citrus-growing regions, causing significant yield losses. Among the various nematode species found in the citrus rhizosphere, the citrus nematode (*Tylenchulus semipenetrans*) is the most prevalent across all citrus-producing areas (Duncan, 2009; Kumar and Arthurs, 2021). This nematode is estimated to cause yield losses ranging from 10 to 30% globally and 6.8 to 17.5% annually in India (Khan et al., 2010). It causes a slow decline in citrus, contributing to citrus dieback and other disease complexes in the country. Infested feeder roots appear dark, encrusted with soil particles, and exhibit shorter branch rootlets than healthy roots. Above-ground symptoms include chlorosis, leaf defoliation, reduced fruit size, premature fruit drop, and twig dieback, typically beginning from the upper branches (Duncan, 2009; Kumar and Arthurs, 2021). Additionally, various ectoparasitic, migratory endoparasitic, and sedentary endoparasitic nematodes have been identified as pathogenic to citrus, further impacting tree health and productivity.

Despite the significance of PPN, information on their relative abundance and distribution in Assam lemon orchards remains limited. To address this, an extensive survey was conducted across four Assam lemon growing districts of Assam *viz*., Kamrup (R), Kamrup (M), Tinsukia and Biswanath to understand the diversity, distribution and community structure of PPN.

2. MATERIALS AND METHODS

2.1 Sample Collection

Composite samples were collected from eight different geographical locations within these districts (Table 1). Samples were collected from 15-30 cm depth around the feeder roots of Assam lemon trees. Each collected sample was labelled and transported to the laboratory for nematode extraction and identification.

2.2 Nematode Isolation and Identification

200 cc soil sample from each composite was processed using Cobb's sieving and decanting technique, followed by the Baermann funnel technique (Southey, 1986). After 48 hours of incubation, the resulting nematode suspensions were placed in beakers and immersed in hot water at 60°C for 2 minutes. Once cooled, the suspensions were concentrated to 25 ml, and different categories of PPN were counted under a stereoscopic binocular microscope. The counting process was repeated three times, and the average population count was recorded. Temporary mounts were prepared for species identification using a compound microscope.

2.3 Community Analysis

The nematode community analysis was carried out by using different formulae (Norton, 1978) *viz*., Absolute frequency (AF), Relative frequency (RF), Absolute density (AD), Relative density (RD) and Prominence Value (PV) as follows.

1. Absolute frequency of sp. X (AF) =No. of samples containing species/ No. of samples collected ×100
2. Relative frequency of sp. X (RF) = Frequency of sp./ Sum of frequencies of all species present in samples × 100

Table 1. Field surveyed localities in Assam

District	Village/tehsil
Kamrup (R)	Bhutargari, Chhaygaon; Kahibama, Chhaygaon; Laruboma, Boko
Kamrup (M)	Ghaguah, Sonapur; Modaikuchi, Khetri; HRS, AAU, Kahikuchi
Tinsukia	Citrus Research Station, Assam Agricultural University, Tinsukia
Biswanath	Biswanath College of Agriculture, Biswanath Chariali

3. Absolute density of sp. X (AD) =No. of individuals of a sp. in a sample/Volume or mass or unit of a sample × 100
4. Relative density of sp. X (RD) = No. of individuals of a sp. in a sample/Total no. of individuals of all species in a sample × 100
5. Prominence value of sp. X (PV) = Absolute density √ Absolute frequency /100

3. RESULTS AND DISCUSSION

Based on the morphological characteristics, six genera of PPN were identified, *viz*., *T. semipenetrans*, *Helicotylenchus* sp., *Pratylenchus* sp., *Tylenchorhynchus* sp., *Hoplolaimus* sp. and *Rotylenchulus* sp. from the soil samples collected across eight different sites in four Assam lemon growing districts of Assam. Among these, *T. semipenetrans* was the most abundant nematode species in all surveyed districts followed by *Helicotylenchus* sp. and *Hoplolaimus* sp. whereas *Tylenchorhynchus* sp., *Pratylenchus* sp. and *Rotylenchulus* sp. were least abundant (Fig. 1). The community structure of these identified PPN genera showed that *T. semipenetrans* and *Helicotylenchus* sp. were the most frequently occurred PPN (AF=100% and RF=22.9%) followed by *Hoplolaimus* sp. (AF=80% and RF=18.39%) and *Tylenchorhynchus* sp., *Pratylenchus* sp. (AF=60% and RF=13.7%). While, *Rotylenchulus* was least frequently occurred in surveyed orchards. In addition, *T. semipenetrans* had the highest absolute and relative density (AD= 191.8 and RD=80%) followed by *Helicotylenchus* (AD=48.75 and RD=16%). Whereas, *Pratylenchus* sp. (AD= 1 and RD=0.4%) and *Rotylenchulus* sp. (AD= 0.4 and RD=0.16%) were found in least numbers. The prominence value was highest for *T. semipenetrans* (19.18) followed by *Helicotylenchus* sp. (4.87). Whereas, *Pratylenchus* and *Rotylenchulus* spp. were least prominent (0.07 and 0.02) in surveyed orchards (Table 2).

The citrus nematode, *T. semipenetrans* was the most abundant species found in all the surveyed Assam lemon orchards, consistent with reports from other citrus-growing regions globally (Sorribas et al., 2008; Abd-Elgawad et al., 2016; Eisvand et al., 2019; Abu Habib et al., 2020; Zoubi et al., 2022). This species is known to pose a significant threat to citrus trees, causing a condition known as "slow decline" in affected orchards. The wide distribution can be attributed to factors such as infected seedlings, contaminated plant material, irrigation practices, and machinery (Abd-Elgawad et al., 2016). The second most prevalent plant-parasitic nematode in the surveyed districts was *Helicotylenchus* sp., a genus commonly reported in citrus-growing regions worldwide (Sorribas et al., 2008; Kumar and Das, 2019; Abu Habib et al., 2020; Zoubi et al., 2022). Other nematode genera, including *Tylenchorhynchus*, *Hoplolaimus*, and *Pratylenchus* and *Rotylenchulus* were also identified in a few sites within the surveyed districts.

Few studies have documented the presence of PPN in citrus orchards across India. Nandwana et al. (2005) reported that *T. semipenetrans* was the predominant species in citrus orchards and nurseries followed by *Pratylenchus* and *Helicotylenchus* spp, in citrus in Jhalawar district, Rajasthan. Mahanta et al. (2018) identified the presence of *T. semipenetrans* and *Helicotylenchus* in Khasi mandarin orchards in Tinsukia district, Assam. Similarly, Kumar and Das (2019) reported the prevalence of *T. semipenetrans*, followed by *Helicotylenchus*, *Hoplolaimus*, and *Tylenchorhynchus*, in ten different citrus species at the Citrus Research Station (CRS), Tinsukia, Assam. Recently, Borthakur et al. (2024) found *Tylenchulus*, *Hoplolaimus*, *Helicotylenchus*, and *Tylenchorhynchus* in citrus orchards in Dibrugarh district, Assam. In contrast, *Xiphinema* was found to be the dominant nematode species, followed by *Pratylenchus*, *Tylenchulus*, and *Helicotylenchus*, in citrus-growing regions of Jammu and Aurangabad district, Maharashtra (Zalpuri et al., 2013; Deshmukh et al., 2016). Despite the recognized importance of *T. semipenetrans*, no detailed studies have been conducted on the extent of its pathogenicity to Assam lemon in Northeast India. The observed distribution patterns suggest that environmental factors may play a significant role in influencing nematode populations across different regions.

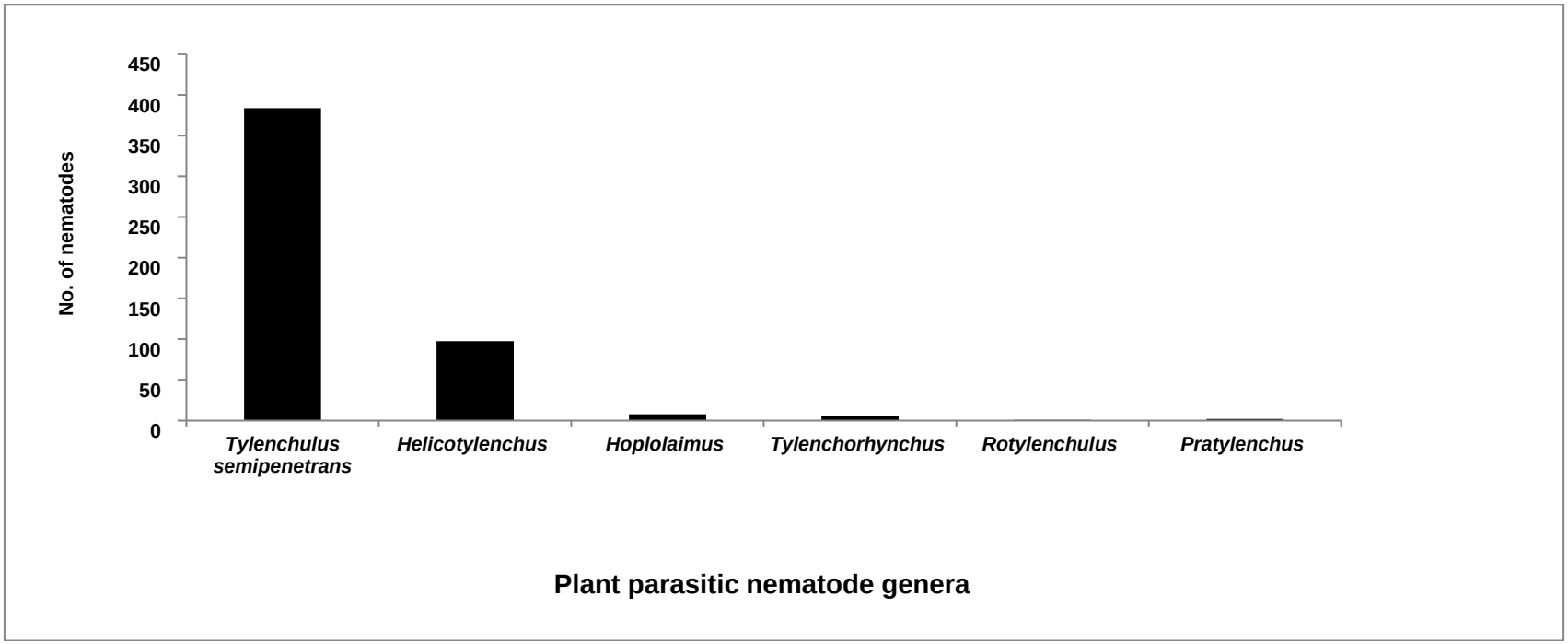

Fig. 1. Plant parasitic nematode genera (Avg. No. per 200 cc soil) associated with Assam lemon in four districts (consolidated) of Assam

Table 2. Community analysis of PPN (200cc soil) infecting Assam lemon in four districts (consolidated) of Assam

Parameter	*Tylenchulus semipenetrans*	*Helicotylenchus* sp.	*Hoplolaimus* sp.	*Tylenchorhynchus* sp.	*Rotylenchulus* sp.	*Pratylenchus* sp.
AF	100	100	80	60	40	60
RF	22.9	22.9	18.39	13.7	9.19	13.7
AD	191.8	48.75	3.9	2.8	0.4	1
RD	80	16	1.6	1.1	0.16	0.4
PV	19.18	4.87	0.34	0.21	0.02	0.07

(AF-Absolute frequency; RF-Relative frequency; AD-Absolute density; RD-Relative density; PV-Prominence value)

Advancing studies on soil sampling and nematode identification can greatly enhance the early detection and management of PPN, particularly *T. semipenetrans* and other economically significant nematode species in citrus. The accuracy and reliability of PPN identification can be significantly improved by integrating both morphological and molecular diagnostic techniques. This study offers valuable insights into the extent of nematode infestations in Assam lemon orchards, setting the stage for future research focused on effective nematode management in these regions.

4. CONCLUSION

Among the six PPN genera identified in Assam lemon orchards, the citrus nematode *T. semipenetrans* was the most prevalent across the four surveyed districts of Assam. A more comprehensive investigation is required in other Assam lemon growing regions to better assess the yield losses caused by PPN and to develop effective strategies to mitigate these losses.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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