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Diversity and community analysis of plant parasitic nematodes associated with citrus at citrus research station, Tinsukia, Assam

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Abstract
A systemic investigation was conducted on February, 2018 to assess the diversity and community structure of plant parasitic nematodes from the soil rhizosphere of ten different citrus species grown at Citrus Research Station, Tinsukia, Assam. Four major plant parasitic nematode species *viz*., *Tylenchulus semipenetrans*, *Helicotylenchus dihystera*, *Hoplolaimus indicus* and *Tylenchorhynchus* spp were found prevalent in the rhizosphere of ten different citrus species. In addition to these, several dorylaimid, rhabditid and predatory nematodes were also encountered. Amongst the plant parasitic nematodes, *T. semipenetrans* was highly abundant (100%) followed by *H. dihystera* (80%), *Tylenchorhynchus* spp. (70%) and *H. indicus* (50%). Among, different citrus species, a higher population of *T. semipenetrans* was encountered on rough lemon and least number was encountered on trifoliate orange.

Keywords: citrus, nematode, diversity, community analysis

1. Introduction
Citrus is the third most important fruit crop grown after mango and banana in India. Among different citrus species, mandarin oranges occupying 40.7% of total area and accounting for 37.3% of total citrus production in India followed by limes (24.6 and 21.9%) and sweet oranges (19.8 and 27.4%). Other species such as pummelo, grapefruit, sour and bitter oranges occupying 14.9% of the area and accounting for 13.4% of total production [1].
North East India is considered to be the centre of origin for many citrus species. Khasi mandarin, Assam lemon and Rough lemon are the commercially available species in Assam. Apart from these, several other species are also cultivated in this region. However, several biotic and abiotic factors which hamper the citrus production in the State. Among the biotic factors, plant parasitic nematodes are one of the major constraints in citrus producing areas causing significant yield losses. Although, several nematode species are reported in the citrus rhizosphere, Citrus nematode, *Tylenchulus semipenetrans* have been shown to be of economically important species in all citrus growing regions [2]. The yield losses caused by this nematode are estimated to be in the range of 10 to 30% worldwide where as in India in the range of 6.8-17.5% annually [3]. In India it was first reported from Aligarh (UP) by Siddiqui in 1961. It is causing a slow decline in citrus and responsible for inducing citrus dieback along with other complexes in the country [4]. In addition, several ectoparasitic, migratory endoparasitic and sedentary endoparasitic nematodes are also reported in citrus. On considering the importance of the crop, the Citrus Research Station (CRS) was established at Tinsukia in 1976 under Assam Agricultural University (AAU) to conduct research and extension activities on citrus. However, the relative abundance and distribution of plant parasitic nematodes on different species grown at the research station is not yet known. Therefore, the present investigation was carried out to assess the diversity and community structure of different plant parasitic nematodes from the soil rhizosphere of different citrus species grown at CRS.

2. Materials and Methods
2.1 Survey and sample collection
A survey was conducted during February, 2018 for the community structure of plant parasitic nematodes at CRS (Latitude- 27°31'N; Longitude- 95°21'E). Soil samples were collected separately at a depth of 15-30 cm around the feeder roots from ten different citrus species *viz*.,

khasi mandarin, sweet orange, trifoliate orange, grape fruit, pummelo, cleopatra mandarin, rough lemon, assam lemon, citrange and troyer citrange. 200cc of composite soil sample from each citrus species was labelled further and transported to laboratory for nematode isolation.

2.2 Isolation of plant parasitic nematodes from soil

200 cc of soil from each composite sample was processed by Cobb's sieving and decanting technique [5] followed by Baermann funnel technique [6]. Nematode suspension was taken in a beaker and dipped in hot water at 60 ºC for 2 minutes. After cooling, the suspension was concentrated to 25 ml and counted different groups of plant parasitic nematodes under a stereoscopic binocular microscope. The counting was repeated three times and average was taken for population count. Temporary mounts were prepared to identify the nematode species under a compound microscope. In addition to plant parasitic nematodes, the data of predatory, rhabditid and dorylaimid group of nematodes also taken.

2.3 Community analysis of plant parasitic nematodes

In order to study poly specific nematode community with reference to relative frequencies, relative densities, prominence values, the population of nematodes were analysed by different formulae [7] as given below:

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 species/ Sum of frequencies of all species present in samples × 100
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
5. Prominence value of sp. X (PV) = Relative density √ Relative frequency

3. Results and Discussion

It is clear from the data (Table 1) that a total of four major plant parasitic nematode species were encountered on different citrus species at the research station. The identified nematode species were *T. semipenetrans*, *Helicotylenchus dihystera*, *Hoplolaimus indicus* and *Tylenchorhynchus* spp. Among the identified nematodes, *T. semipenetrans* was the most abundant nematode species in all the citrus cultivars and the highest number of *T. semipenetrans* juveniles (5840) were found in the rhizosphere of rough lemon followed by grapefruit and Assam lemon. However, the least number of *T. semipenetrans* were reported in the rhizosphere of trifoliate orange (11) and troyer citrange (42) (Table 1). While, spiral nematode, *H. dihystera* also reported from the soil rhizosphere of all citrus species. The least number were reported in grapefruit and pummelo. Ectoparasitic nematodes, *H. indicus* and *Tylenchorhynchus* spp. were found in some of the citrus species in few numbers.

3.1 Absolute frequency: The citrus nematode, *T. semipenetrans* was identified as the most frequently occurred plant parasitic nematode with an absolute frequency of 100% followed by *H. dihystera* (80%) and *Tylenchorhynchus* spp. (70%). Whereas, *H. indicus* was less frequently occurred with an absolute frequency of 50% on different citrus species. The observation showed the widespread of *T. semipenetrans* on all citrus species at CRS farm (Table 2).

3.2 Absolute density: *T. semipenetrans* was the most abundant nematode with an absolute density of 661.05 followed by *H. dihystera* (22.9). Whereas, *H. indicus* and *Tylenchorhynchus* spp. were found in least numbers (1.25 and 2.35) (Table 2).

3.3 Prominence value: The prominence value was highest for *T. semipenetrans* followed by *H. dihystera*. Whereas *H. indicus* and *Tylenchorhynchus* spp. were least prominent on different citrus species at the farm (Table 2).

The occurrence of *T. semipenetrans* in large numbers followed by *Helicotylenchus* sp., *Tylenchorhynchus* sp., and *Hoplolaimus* sp. has been reported in khasi mandarin growing districts of Assam [8]. Present finding of *T. semipenetrans* followed by *H. dihystera* in large number has also been reported in different citrus species grown at CRS, Tinsukia. Whereas, *Xiphinema* was found to be the dominant species followed by *Pratylenchus*, *Tylenchulus* and *Helicotylenchus* in citrus growing regions of Jammu and Aurangabad district of Maharashtra [9, 10]. The reports suggested that, trifoliate orange and many of the resulting hybrids inherit resistance to *T. semipenetrans* [11, 12, 13]. In this study, lower numbers of *T. semipenetrans* juveniles were reported on trifoliate orange followed by troyer citrange (Table 1).

Table 1: Occurrence (average no.) of plant parasitic nematode species from different citrus cutivars at CRS, Tinsukia (200 cc soil)

Citrus type	*Tylenchulus semipenetrans*	*Helicotylenchus dihystera*	*Hoplolaimus indicus*	*Tylenchorhynchus* sp.
Khasi mandarin	665	84	-	18
Sweet orange	457	43	2	-
Trifoliate orange	11	63	6	7
Grape fruit	2946	32	-	6
Pummelo	369	42	-	3
Cleopatra mandarin	541	75	-	4
Rough lemon	5840	52	4	-
Assam lemon	1692	67	9	7
Citrange	658	-	-	-
Troyer citrange	42	-	4	2

Table 2: Community analysis of plant parasitic nematodes (200cc soil) infecting different citrus cultivars at CRS, Tinsukia

Nematode species	Mean abundance	Absolute Frequency	Relative frequency	Absolute density	Relative Density	Prominence Value
Tylenchulus semipenetrans	1322.1	100	33.3	661.05	96.1	554.55
Helicotylenchus dihystera	45.8	80	26.6	22.9	3.33	17.17
Hoplolaimus indicus	2.5	50	16.6	1.25	0.181	0.73
Tylenchorhynchus sp.	4.7	70	23.3	2.35	0.341	1.64

4. Conclusion

Among the four plant parasitic nematodes associated with citrus, the citrus nematode *T. semipenetrans* was reported as the predominant plant parasitic nematode of different citrus species grown at research farm. Out of ten different citrus species, rough lemon was found to be highly infested by *T. semipenetrans* followed by grape fruit and Assam lemon and least number was encountered in trifoliate orange and troyer citrange. To our knowledge, this is probably the first record to assess the community structure of plant parasitic nematode species on different citrus species at the research station.

5. Acknowledgement

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