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Identification of Plant Parasitic Nematodes Associated with Citrus in Dibrugarh District

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present investigation was carried out to determine the occurrence and distribution of plant parasitic nematodes associated with citrus plants of different citrus orchards in Dibrugarh district. A total of 149 soil and root samples were collected randomly from the rhizosphere of citrus plants from the twenty four different citrus orchards of seven blocks from Dibrugarh district. Eight genera of plant parasitic nematodes were found to be associated with citrus plants of Dibrugarh district. Genera of plant parasitic nematodes recorded were namely *Tylenchulus*, *Hoplolaimus*, *Helicotylenchus*, *Tylenchorhynchus*, *Meloidogyne*, *Xiphinema*, *Paratylenchus* and also nematodes genera found under criconematids. Among them *Hoplolaimus* sp., *Helicotylenchus* sp., *Tylenchorhynchus* sp. and *T. semipenetrans* were found to be present in most of the samples. Community analysis of plant parasitic nematodes revealed that the genus *Hoplolaimus* ranked first

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in relative frequency, absolute frequency, absolute density, relative density and prominence value. Genus *Helicotylenchus* ranked second in absolute density, relative density and prominence value and genus *Tylenchorhynchus* ranked third in case of absolute density absolute frequency and prominence value. Results shown that *Tylenchulus semipenetrans* ranked second in absolute frequency and relative frequency and fourth in prominence value.

Keywords: Survey; community structure; citrus orchard; nematodes.

1. INTRODUCTION

Citrus is the one of most important fruit crops particularly in the tropical and sub tropical areas of the world. It belongs to the family, Rutaceae. It includes different citrus fruit crops like oranges, lemons, grapefruit and limes.

Population densities of plant parasitic nematodes vary greatly in time and space under the influence of a complex of abiotic and biotic factors in their niche. The host growth, temperature, moisture and some physicochemical properties of the soil have been identified as the most important factors responsible for the spatial heterogeneity in nematode population densities both horizontally and vertically in the soil profile as well as with time [1]. The juice contains a high quantity of citric acid giving them their characteristic sharp flavour. The genus is commercially important as many species are cultivated for their fruit, which is eaten as fresh, pressed for juice, or preserved in marmalades and pickles. They are also good sources of organic acid, amino acids, sugars, carotenoids and vitamin C

In Assam lemon, Sweet lemon (Mousambi) and orange occupied an area of 16058, 385 and 12680 ha with the production of 160339, 6056 and 179168 metric tonne respectively [2]. In Dibrugarh district, it covers 468 ha of Assam lemon with production of 4067 metric tonne and oranges 75 ha with the production of 966 metric tonne [3].

Chona et al. (1966) reported that *T. semipenetrans* is widely distributed in India and its association with decline of the tree has been recorded in Punjab, Delhi, Assam, Rajasthan, Orissa, West Bengal, Kerala and Maharastra. Phukan and Sarmah [4] recorded high population of *T. semipenetrans* from Dibrugarh district. The high population of nematode was also recorded from Karbi Anglong district (Anon.,1985). Sinha [5] recorded presence of citrus nematode *T. semipenetrans* in five district of Assam. (Cachar, Kamrup, Jorhat, Sivasagar, Dibrugarh). Sinha [6] reported that the citrus nematode, *T. semipenetrans* was as high as 38,000 per 200g and 1842 per 250g of soil respectively in Tinisukia district. Singh (1997) recorded that maximum population of citrus nematode during the month of January in Vidarbha region of Maharastra. Crozzoli et al. [7] surveyed the main citrus growing areas of Venezuela and collected a total of 1110 soil and root sample and analyzed. They found that thirty four species were associated with citrus and among them *T. semipenetrans* was most economically important and wide spread species. Bark et al. (2005) carried out a survey to know the frequency of occurrence of both root-knot nematodes *Meloidogyne* sp., and citrus nematode *T. semipenetrans* (Cobb,1913) in the new reclaimed lands in three different governorates in Egypt. Results revealed that percentage of occurrence of *Meloidogyne* sp.,was 96.26% in the surveyed fields while *T. semipenetrans* was 85.18%. Nandwana et al. (2005) recorded that five phyto-nematodes were associated with citrus trees in orchards and nurseries in and around Jhalawar district, and among them *T. semipenetrans* was predominantly and most widely prevalent with highest prominence value followed by *Pratylenchus* sp.; *Helicotylenchus indicus*; *Rotylenchulus reniformis* and *Hoplolaimus indicus* respectively.

Zalpuri et al. [8] surveyed occurrence of important plant-parasitic nematodes associated with citrus crops during 2008-2009 in Jammu Region and they found that *Meloidogyne javanica, Hoplolaimus* sp., *Xiphinema* sp., *Pratylenchus* sp., *T. semipenetrans* were mostly associated with the citrus crop. Anon. (2013) surveyed for the plant parasitic nematode associated with citrus growing areas of Tinisukia district. Soil and root samples were collected randomly from the different khasi mandarin orchards. Results revealed that seven species of plant parasitic nematodes were associated with khasi mandarin plants the Ibrahim Said K. et al. (2016) reported that the root knot nematode (*Meloidogyne* sp.), *Tylenchulus* sp., *Xiphenema*

sp., *Rotylenchus* sp., *Pratylenchus* sp., *Longidorus* sp., *Tylenchulus* sp. and *Radhopholus* sp. were most common on citrus trees in Lebanon.

Very little work has so far been done on citrus crop in Assam, except work done by Phukan and Sarmah (1983); on survey of citrus nematode in Dibrugarh district, Assam. Therefore present investigation is an attempt to study the plant parasitic nematode associated with citrus and to study community structure of soil inhabiting nematodes in citrus orchards of Dibrugarh district of Assam**.**

2. MATERIALS AND METHODS

Roving survey was carried out to know the different plant parasitic nematodes associated with citrus in Dibrugarh district. Soil samples including root were collected from the rhizosphere of various citrus plants. In the present investigation a total of 149 samples were collected from the rhizosphere of citrus plants from twenty four orchards of seven blocks of Dibrugarh district in Assam The names of citrus plants from which samples were collected are listed in Table 1.

Table 1. List of citrus plants from which samples were collected

Common name	Scientific name
Khasi mandarin	*Citrus reticulata*
Rough lemon	*Citrus jambhiri*
Pomelo	*Citrus maxima*
Assam lemon	*Citrus Limon L.*

Each bulk sample was constituted of several sub samples. Samples were collected randomly and all relevant information was recorded at the time of collection of samples. The samples were transferred to laboratory and stored in refrigerator at 4°C till the extraction of nematode was made. The extraction of nematode from soil samples were done by modified Cobb's sieving and decanting technique [9] and extraction of nematode from roots by Baermann funnel technique. The killing and fixing of nematodes were done in 8% hot formalin.

2.1 Community Analysis of Plant Parasitic Nematode

Community analysis of plant parasitic nematode was done by using the methods given by Norton, (1978).

2.2 Absolute Frequency is Expressed as a Percentage

Absolute frequency = (Number of samples containing a species / Number of samples collected) × 100

3. RESULTS AND DISCUSSION

Eight genera of plant parasitic nematode recorded from the seven blocks were *Hoplolaimus* sp., *Helicotylenchus* sp., *Tylenchorhynchus* sp., *Paratylenchus* sp., *Tylenchulus semipenetrans, Meloidogyne* sp., *Xiphinema* sp. and Criconematids. Among them *Hoplolaimus* sp., *Helicotylenchus* sp., *Tylenchorhynchus* sp. and *T. semipenetrans* were found to be present in most of the samples. All the eight genera were recorded from Tinkhong, Barbarua and Lahowal block. Criconematids were found to be associated in all the blocks except Jaipur block, *Paratylenchus* sp. was recorded from all the blocks except Tengakhat. *Meloidogyne* sp. was recorded from all the blocks except Tengakhat and Khowang and *Xiphinema* sp. was recorded from all the blocks except Tengakhat and Panitola.

Out of twenty four citrus orchards, *T. semipenetrans* was recorded from seventeen orchards, *Tylenchorhynchus* sp. from twelve orchards, *Hoplolaimus*sp. from 16 orchards, Criconematids from 11 orchards, *Helicotylenchus* from 15 orchards, *Meloidogyne* sp. from 11 orchards, *Paratylenchus* sp. from 12 orchards and *Xiphinema* sp. from 10 orchards.

The maximum nematode population recorded in eight genera of plant parasitic nematodes were *Tylenchorhynchus* sp. (46.25), *Hoplolaimus* sp. (46), *Helicotylenchus* sp. (45.71), *Paratylenchus* sp. (45.00), *T. semipenetrans* (30.00), *Meloidogyne* sp. (22.50), *Xiphinema* sp. (22.00), Criconematids (16.66)/250cc soil and minimum population recorded *Tylenchorhynchus* sp. (12.50), *Hoplolaimus* sp. (15.71), *Helicotylenchus* sp. (14.28), *Paratylenchus* sp. (12), *T. semipenetrans* (11.42), *Meloidogyne* sp. (16.00), *Xiphinema* sp. (8.57) and Criconematids (6.25)/250cc soil.

Among the eight genera of phytonematodes 100 per cent frequency observed in *Tylenchorhynchus* sp., *Hoplolaimus* sp., *Helicotylenchus* sp., *Paratylenchus* sp. and *Tylenchulus semipenetrans*, *Xiphinema* sp. and Criconematids and lowest frequency recorded in

Paratylenchus sp. (60), *Tylenchorhynchus* sp. (50), *Meloidogyne* sp. (50) *T. semipenetrans* (50), *Hoplolaimus* sp. (57.14), *Helicotylenchus* sp. (57.14), *Xiphinema* sp. (42.85) and Criconematids (40).

The highest frequency of occurance of *Hoplolaimus* sp.(100%) were recorded from one orchard of Tengakhat, two orchards of Tingkhong, Lahowal, Khowang, Barbaruah block and three orchards of Jaipur block where as lowest frequency of occurance (57.14%) was observed in one orchard of Tengakhat. Further it was observed that the highest frequency of occurance of spiral nematode *Helicotylenchus* sp. in one orchard of Khowang, Lahowal, Tengakhat and Jaipur and two orchards of Barbaruah and lowest frequency was observed (57.14%) in one orchards of Panitola and one orchard of Jaipur. Highest frequency of occurance of *Tylenchorhynchus* sp. was recorded in one orchard of Barbaruah, Tengakhat and Lahowal where as lowest frequency (50%) recorded in one orchards of Khowang. Similarly the citrus nematode *T. semipenetrans* , dagger nematode *Xiphinema* sp. and Criconematids were recorded 100% frequency in one orchard of Lahowal and Jaipur.

High population of *T. semipenetrans* was recorded from citrus growing areas of Dibrugarh district (Phukon and Sarmah; 1983) and from Karbi Anglong district. (Anon 1985). Sinha [6] recorded presence of citrus nematode, *T. semipenetrans* in five district of Assam. Anon. (2013) recorded seven genera of plant parasitic nematodes from the rhizosphere of khasi mandarin in Tinsukia district. Crozzoli et al. [7] surveyed the main citrus growing areas of Venezuela and reported that 34 species were associated with citrus including *Hoplolaimus* sp., *Helicotylenchus* sp., *Tylenchorhynchus* sp., *Paratylenchus* sp., *Tylenchulus semipenetrans, Meloidogyne* sp., *Xiphinema* sp. and Criconematids.

Plant parasitic nematodes are major problem in the cultivation of citrus crops. Major nematodes pests infecting citrus crops are Tylenchulus semipenetrans, Xiphinema index, Meloidogyne spp., Radopholus similis and Pratylenchus coffeae. Among these Tylenchulus semipemetrans is dominating [10].

3.1 Community Analysis of Plant Parasitic Nematodes

In the present investigation, Out of eight genera recorded, *Hoplolaimus* sp was found to be the most frequently occurred plant parasitic nematode with an absolute frequency of 59.73 percent, the next most frequently occurring nematode was *Tylenchulus semipenetrans*(51.00%), followed by *Helicotylenchus* sp. (48.32) *Tylenchorhynchus* sp. (42.95%) *Paratylenchus* sp.(40.26%) *Meloidogyne* sp.(28.85%), *Xiphinema* sp. (26.17%) and Criconematids (21.47%) (Table).

In relative frequency *Hoplolaimus* sp. ranked first with 18.73 percent followed by *Tylenchulus semipenetrans* (16.00%), *Helicotylenchus* sp. (15.15%), *Tylenchorhynchus* sp. (13.47%), *Paratylenchus* sp. (12.63%). Three nematode species *viz*. *Meloidogyne* sp., (9.05) *Xiphinema* sp. (8.21) and Criconematids (6.73) occupied the last position in respect of relative frequency. (Table 2).

Table 2. Plant parasitic nematodes associated with citrus orchards in Dibrugarh district

Name of district	No. sample Collected	Nematode species	Soil		Root	
			Nematode population range	Frequency range	Nematode population range	Frequency range
Dibrugarh	149	*Hoplolaimus* sp	0-80	59.73		
		Helicotylenchus sp	0-70	48.32		
		Tylenchorhynchus sp	0-80	42.95		
		Paratylenchus sp	0-70	40.26		
		Tylenchulus semipenetrans	0-50	51.00	0-50	28.57-66.66
		Meloidogyne sp	0-50	28.85		
		Xiphinema sp	0-50	26.17		
		Criconematids	0-40	21.47		

Table 3. Population of different plant parasitic nematodes associated with citrus in Dibrugarh district

Sl. No	Block	Total no of sample collected	Citrus orchards	Nematode	Population range in 250 cc soil	Average population	Frequency (%)
1	Tingkhong	8	Korangani	*Tylenchulus semipenetrans*	0-40	17.5	62.5
				Tylenchorhynchus sp.	0-80	46.25	75
				*Hoplolaimus*sp.	20-60	36.25	100
				Criconematids	0-30	11.25	50
		8	Nemupathar	*Tylenchulus semipenetrans*	0-50	25	62.5
				Tylenchorhynchus sp.	20-70	28.75	75
				Hopolaimus sp.	20-70	42.5	100
				Helicotylenchus sp.	0-40	15	62.5
				Meloidogyne sp.	0-50	22.5	62.5
		6	Tingkhong chariali	*Tylenchulus semipenetrans*	0-30	18.33	66.66
				Paratylenchus sp.	20-70	45	100
				Xiphinema sp.	0-40	21.66	66.66
				Meloidogyne sp.	0-30	16.66	66.66
2	Jaipur	8	Powali pathar(1)	*Tylenchulus semipenetrans*	0-50	30	75
				Tylenchorhynchus sp.	30-70	42.5	100
				Xiphinema sp.	0-50	21.25	62.55
				Helicotylenchus sp.	0-60	36.25	75
				*Meloidogyne*sp.	0-50	20	62.5
		7	Powali pathar(2)	*Tylenchulussemipenetrans*	0-50	24.28	71.42
				Paratylenchus sp.	0-30	14.28	71.42
				Hoplolaimus sp.	30-60	41.42	100
				Helicotylenchus sp.	0-40	18.57	57.14
		5	Asabam	*Helicotylenchus* sp.	20-60	42	100
				Hoplolaimus sp.	20-50	38	100
				Meloidogyne sp.	0-30	22	80
				Paratylenchus sp.	0-30	12	60
		3	Tanti pathar	*Tylenchorhynchus* sp.	20-40	30	100
				Helicotylenchus sp.	0-30	16.66	66.66
				Xiphinema sp.	0-50	33.33	100
				Hoplolaimus sp.	0-40	22.5	100
3	Barboruah	5	Dulia	*Hoplolaimus* sp.	20-80	46	100
				Paratylenchus sp.	20-50	36	100

Sl. No	Block	Total no of sample collected	Citrus orchards	Nematode	Population range in 250 cc soil	Average population	Frequency (%)
				Tylenchorhynchus sp.	0-60	24	80
				Meloidogyne sp.	0-40	16	60
		7	Dibuwal(1)	*Tylenchulus semipenetrans*	0-50	27.14	71.42
				Helicotylenchus sp.	30-70	42.85	100
				Hoplolaimus sp.	0-60	22.85	71.42
				Criconematids	0-30	11.42	42.85
				*Xiphinema*sp.	0-30	8.57	42.85
		7	Dibuwal(2)	*Tylenchulus semipenetrans*	0-40	15.71	57.14
				Tylenchorhynchus sp.	20-80	42.85	100
				Hoplolaimus sp.	20-70	40	100
				Xiphinema sp.	0-40	14.28	57.14
		4	Changmaigohaingaon	*Helicotylenchus* sp.	30-70	45	100
				*Paratylenchus*sp.	20-40	27.5	100
				Criconematids	0-30	15	75
				*Meloidogyne*sp.	0-40	17.5	50
4	Tengakhat	7	Abhaypuria(1)	*Tylenchulus semipenetrans*	0-50	21.42	71.42
				*Tylenchorhynchus*sp.	0-60	35.71	85.71
				*Hoplolaimus*sp.	20-80	41.42	100
				*Meloidogyne*sp.	0-50	20	57.14
		7	Abhaypuriya(2)	*Tylenchulussemipenetrans*	0-40	11.42	57.14
				*Tylenchorhynchus*sp.	Oct-70	40	100

Table 4. Community analysis of different plant parasitic nematodes associated with citrus in Dibrugarh district

Nematodes species	Absolute density	Relative density(%)	Absolute frequency(%)	Relative frequency(%)	Prominence value
*Tylenchulus*semipenetrans	15.36	14.16	51.00	16	109.69
Hoplolaimus sp.	22.59	20.82	59.73	18.73	174.58
*Helicotylenchus*sp.	18.64	17.18	48.32	15.15	119.57
Tylenchorhynchus sp.	17.45	16.09	42.95	13.47	114.36
*Paratylenchus*sp.	14.20	13.09	40.26	12.63	90.10
*Meloidogyne*sp.	8.52	7.85	28.85	9.05	45.76
*Xiphinema*sp.	7.00	6.45	26.17	8.21	35.80
Criconematids	4.69	4.32	21.47	6.73	21.73
Total	108.45		318.75		

The result revealed that *Hoplolaimus* spp. had the highest absolute density and relative density (22.59 and 20.82% respectively) followed by *Helicotylenchus* sp. (18.64 and 17.18%), *Tylenchorhynchus* sp. (17.45 and 16.09%), *Tylenchulus semipenetrans* (15.36 and 14.16%), *Paratylenchus* sp. (14.20 and 13.09%), *Meloidogyne* sp. (8.52 and 7.85%), *Xiphinema* sp. (7.00 and 6.45%) and Criconematids (4.69 and 4.32%). Considering both frequencies and densities, prominence values for all the nematodes were calculated. *Hoplolaimus* sp. was found to be the most prominent with a prominence value of (174.58) followed by *Helicotylenchus* sp. (119.57), *Tylenchorhynchus* sp. (114.36), *Tylenchulus semipenetrans*(109.69), *Paratylenchus* sp. (90.10), *Meloidogyne* sp. (45.76), *Xiphinema* sp. (35.80) and Criconematids (21.73).

Hoplolaimus sp. is the most frequently recorded species with relative frequency of 18.73%, absolute density of 22.59 per cent, relative density 20.82 percent. Genus *Helicotylenchus* ranked second in absolute density 18.64 per cent and rlelative density 17.18 per cent and genus *Tylenchorhynchus* ranked third in absolute density 17.45 per cent and relative density 16.09 per cent and *Tylenchulus semipenetrans* ranked forth in absolute density 15.36 per cent and relative density 14.16 per cent. It is revealed that *Hoplolaimus* sp. had the highest prominence value of 174.58 followed by *Helicotylenchus* sp., *Tylenchrhynchus* sp. and *Tylenchulus* sp. which were recorded as 119.57, 114.36, 109.69 respectively. The highest absolute frequency recorded in *Hoplolaimus* sp. was 59.73 per cent followed by *Tylenchulus semipenetrans* 51.00 per cent, *Helicotylenchus* sp. 48.32 per cent and *Tylenchrhynchus* sp. 42.95 per cent.

Nandwana et al. (2005) recorded that five phyto-nematodes were associated with citrus trees in orchards and nurseries in and around Jhalawar district, and among them *Tylenchulus* semipenetrans was predominantly and most widely prevalent with highest prominence value followed by *Pratylenchus* sp.; *Helicotylenchus indicus*; *Rotylenchulus reniformis* and *Hoplolaimus indicus* respectively. Rathour *et al.* (2010) made a study on community structure of plant parasitic and mycetophagus nematodes from different cereals, oilseed, fruit, pulse, cash and medicinal plants in Madhya Pradesh. Among the plant parasitic nematodes, *Meloidogyne incognita* was found to be the most frequently occurring with the highest absolute frequency(50), followed by *Rotylenchulus reniformis*(40.38), *Helicotylenchus dihystera*(23). The maximum absolute density was recorded for *R. reniformis* followed by *H. dihystera* and *Tylenchorhynchus indicus*. The highest prominence value was recorded for *M. incognita* (17.12), followed by *H. dihystera* (13.78) and *Hoplolaimus indicus*. Zalpuri *et al*. (2013) recorded the frequency of plant parasitic nematodes associated with citrus, to be*. Meloidogyne javanica*, *Hoplolaimus* sp., *Xiphenema* sp., *Pratylenchus* sp. and *Tylenchulus semipenetrans* in Jammu Region. Among them *Xiphinema* sp., *Pratylenchus* sp. and *Hoplolaimus* sp. were most abundant and frequently occurring nematodes. They also recorded that *Xiphenema* was predominant and most widely prevalent with highest prominence value of 20, followed by *Hoplolaimus* sp. [11]

Kumar and Das (2019) conducted 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 namely viz. *Tylenchulus semipenetrans*, Helicotylenchus dihystera, *Hoplolaimus indicus* and

Tylenchorynchus spp. were found prevalent in the rhizosphere of ten different citrus species. Among 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 found on rough lemon and least number was found on trifoliate orange [12].

4. CONCLUSION

Eight genera of plant parasitic nematodes recorded from the 24 orchards in seven blocks of Dibrugarh districts were *Tylenchulus*, *Hoplolaimus*, *Helicotylenchus*, *Tylenchorhynchus*, *Meloidogyne*, *Xiphinema*, *Paratylenchus* and *Criconematids.* Community analysis of plant parasitic nematodes revealed that Genus *Hoplolaimus* ranked 1st in relative frequency, absolute frequency, absolute density, relatice density, prominence value. The genus *Helicotylenchus* ranked 2nd in absolute density, relative density and prominence value. Further it was observed *Tylenchulus semipenetrans* ranked 2nd in absolute frequency and relative frequency and 4th in prominence value.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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