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Spatial and temporal prevalence of plant parasitic nematodes of citrus in Vidarbha region

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Abstract

The present investigation was conducted at Plant Protection Division, ICAR-Central Institute for Cotton Research, Nagpur during the year 2014-2018 respectively. During the present studies 11 genera were identified from 57 samples collected from 19 talukas of 05 districts in Vidarbha region of Maharashtra state. The absolute and relative frequencies along with densities was recorded highest in various genera viz. *Helicotylenchus*, *Pratylenchus*, *Tylenchulus* and *Tylenchus* genera. However, the highest absolute and relative frequency was recorded in *Helicotylenchus* (93.99 & 18.53%) followed by *Pratylenchus* (84.51 & 16.80%) in citrus ecosystem. Whereas, the *Helicotylenchus* and *Pratylenchus* genera noticed the highest absolute and relative density of (2.10 & 17.46%) and (1.89 & 15.76%) respectively. The prominent value indicates the prominent presence of the genera *Helicotylenchus* (7.62), *Tylenchulus* (6.97), *Pratylenchus* (5.77) and *Tylenchus* (2.59) in citrus ecosystem. The high density spots recorded at 19 spots in 16 talukas of 05 districts.

Keywords: *Helicotylenchus*, *pratylenchus*, *Tylenchulus*, *Tylenchus*, *Rotylenchulus*, *Hoplolaimus*, *Tylenchorhynchus*, *Hirschmanniella*, *Meloidogyne*, *Aphelenchoides*, Absolute frequency, relative frequency, Absolute density, relative density, prominence value, etc

Introduction

The survey for Prevalence and distribution of plant parasitic nematodes (PPN) in Vidarbha region was conducted in Amravati and Nagpur division comprising of 119 talukas. The soil samples were collected from each taluka and evaluated for presence of plant parasitic nematodes in citrus ecosystem. In view of the importance of the plant parasitic nematodes causing losses to the different agricultural crops, the occurrence of different plant parasitic nematodes in vidarbha region were assessed by survey and collection of soil samples and analysing it in the laboratory. Citrus nematodes *Tylenchulus semipenitrans* causing slow decline disease is also associated in dieback disease in citrus and reducing 8.7 to 12.2% yields in citrus. Prevalence of plant parasitic nematodes has been reported in cotton, citrus crops under All India Co-ordinated Research project on plant parasitic nematodes at Department of Entomology, Mahatma Phule Krishi Vidyapeeth, Rahuri (Anonymous, 2012) [8].

The 11 genera were subjected to analysis where the mean population was recorded highest for genera *Rotylenchulus* (411.80) followed by *Helicotylenchus* (226.54), *Hoplolaimus* (214.59), *Pratylenchus* (188.98), *Tylenchulus* (154.01), *Meloidogyne* (150.31), *Hirschmanniella* (110.46), *Aphelenchoides* (77.83) and *Tylenchus* (58.36).

During the survey, citrus crop ecosystem were sampled for the presence of the plant parasitic nematodes.

Materials and Methods

The survey was conducted for in 5 districts comprising of 19 talukas. The soil samples were collected from each taluka and evaluated for presence of plant parasitic nematodes in citrus ecosystem.

Experimental material

The material required for conducting the experiment such as sieves 18 mesh, 60mesh, 100mesh, 200mesh, 350mesh, Polythene bags, Tags & Labels etc. Also the facilities for isolation and extraction of nematodes, Stereobionocular microscope, and Compound microscope with image analyzer were utilised.

Experimental details

The soil samples were collected from citrus cropping system at different locations in 19 talukas of 05 districts of the Vidrabha region.

Collection of soil sample for plant parasitic nematodes

1. Samples were collected from citrus agro ecosystem.
2. Representative soil/plant sample was taken from the crop ecosystem.
3. Most intensive cultivated area which represents the cropping plan of the district was considered for sample collection.
4. Approximately 5-6 samples were taken per taluka depending upon the crop ecosystems and area.
5. The sample was taken from the rhizosphere area of the crop. The soil sample were taken from depth of approximately 0-15 or 30 cm using a hand shovel/small kudal.
6. The soil sample was collected in clean polythene bags and the date, specific location, crop type, stage of crop and sample number was marked using a permanent ink pen on adhesive paper label stuck to the bag.
7. The samples were collected when crop was at peak growth phase.
8. These samples were brought to the laboratory and further extraction of nematodes was done in the Nematology Laboratory, Crop Protection Division, CICR, Nagpur.

Extraction of plant parasitic nematodes**Isolation from soil**

For extraction of nematodes from soil sieving and decanting technique developed by Dr. NA Cobb (1933) ^[9] was followed.

Materials Required

Two plastic pan, 5 sieves (18,60,100,200,350 mesh), 250ml beakers, aluminium wire mesh melded in a shape which can be placed on Petri-plate, facial tissue paper, petriplates.

Method: Extraction of Plant parasitic nematodes (Cobb's Sieving and Decanting Technique)

- The collected moist soil sample were mixed thoroughly by coning and quartering.
- 250 cc soil was taken and mixed with 500cc water and big stones etc. were removed.
- For clayey cotton soil, soil was allowed to stand for about 10 min. so as to dissolve lumps.
- Before use, the sieves were cleaned so that no soil from previous washings clings to the sieve.
- Sieves were wetted with water before use so that a thin water film covers the sieve pores. Thus, the surface tension due to water film prevents loss of nematodes through sieve pores and thus the efficiency of nematode extraction is improved.
- Soil sample that was mixed with water was passed through 18 BSS sieve to remove stones etc.
- The soil suspension was thoroughly shaken and allowed to settle for 1min
- Then soil suspension was sieved through 60 BSS sieve.
- The debris trapped on sieve was collected and directly examined under stereo binocular microscope for cysts if any.
- The soil suspension was again shaken and allowed to settled down for 1 min and then sieved through 100 BSS sieve

- The same procedure was repeated for 200 BSS sieve and also for 350 BSS sieve.
- Material collected on sieves (100, 200 & 350) were collected in a beaker and placed on a tissue paper supported on a wire guage mesh placed in a Petridishes containing water. The water should just wet the bottom of tissue paper supported wire mesh. After 24 hrs. The nematodes that come out in water were collected and examined under stereobionocular microscope.
- The number of nematodes observed per ml were counted.
- Nematodes were identified based on morphological characters visible under stereobionocular microscope. Identification was confirmed under compound microscope.

Statistical analysis

Shannon Evenness Index (E) = $H/Ln S$

$$S$$

Shannon Diversity Index (H) = $-\sum_{i=1} p_i \ln p_i$

$$i=1$$

Where,

P- The proportion (n/N) of individuals of one particular species found (n) divided by the total number of individuals found (N).

Ln- Natural log.

\sum - Sum of the calculations

s- Number of species

The population densities of nematode species in the samples were calculated using the formulae. (Norton, 1978) ^[6]

$$\text{Absolute frequency} = \frac{\text{No. of samples containing a species}}{\text{No. of samples collected}} \times 100$$

$$\text{Relative frequency} = \frac{\text{Frequency of a species}}{\text{Sum of frequencies of all spp.}} \times 100$$

$$\text{Relative Density} = \frac{\text{No. of individuals of a species in a sample}}{\text{Total of all individuals in a sample}} \times 100$$

$$\text{Absolute Density} = \frac{\text{No. of individuals of a species in a sample}}{\text{Volume or mass or units of the sample}} \times 100$$

$$\text{Prominence value} = \frac{\text{Absolute density} \times \sqrt{\text{Absolute frequency}}}{100}$$

Results and Discussion**Absolute & Relative frequency of plant parasitic nematodes.**

The highest values recorded for absolute frequencies and relative frequencies for citrus crop is for genera *Helicotylenchus* (93.99 & 18.52), *Pratylenchus* (84.51 & 16.80), *Tylenchulus* (72.05 & 15.05) and *Tylenchus* (35.94 & 7.35) percent. Whereas the medium values recorded for genera *Rotylenchulu* (32.64 & 5.84), *Hoplolaimus* (24.62 & 4.36), *Tylenchorhynchus* (23.96 & 6.94). However, the low population noticed in *Hirschmanniella* (8.89 & 1.42), *Meloidogyne* (6.60 & 1.09) and *Aphelenchoides* (4.44 & 0.71) genera respectively.

Table 1: Absolute and relative frequency percent of plant parasitic nematodes in citrus crop ecosystem in Vidarbha region

	Genera Crop	Rotylenchulus	Hoplolaimus	Pratylenchus	Aphelenchoides	Helicotylenchus	Meloidogyne
Absolute frequency	Citrus*	32.64	24.62	84.51	4.44	93.99	6.60
Relative frequency	**	5.84	4.36	16.80	0.71	18.52	1.09
	Genera Crop	Paratylenchus	Tylenchulus	Hirschmanniella	Tylenchorhynchus	Tylenchus	Free Living
Absolute frequency	Citrus*	29.31	72.05	8.89	23.96	35.94	100.00
Relative frequency	**	2.39	15.05	1.42	6.94	7.35	19.53

Absolute & Relative density for plant parasitic nematodes
Absolute and relative density values were recorded highest for genera *Helicotylenchus* (2.10 & 17.46), *pratylenchus* (1.89 & 15.76), *Tylenchulus* (0.74 & 5.63) and *Tylenchus* (0.33 & 2.81) percent respectively. Whereas the medium values

recorded for genera *Rotylenchulus* (0.74 & 5.76), *Hoplolaimus* (0.52 & 4.01), *Tylenchorhynchus* (0.74 & 5.76). However, the low population was observed in *Hirschmanniella* (00), *Meloidogyne* (0.15 & 1.14) and *Aphelenchoides* (0.09 & 0.62) genera respectively.

Table 2: Absolute and Relative density percent of plant parasitic nematodes in citrus crop ecosystem in Vidarbha region.

	Genera Crop	Rotylenchulus	Hoplolaimus	Pratylenchus	Aphelenchoides	Helicotylenchus	Meloidogyne
Absolute density	Citrus	0.74	0.52	1.89	0.09	2.10	0.15
Relative density		5.76	4.01	15.76	0.62	17.46	1.14
	Genera Crop	Paratylenchus	Tylenchulus	Hirschmanniella	Tylenchorhynchus	Tylenchus	Free Living
Absolute density	Citrus	0.12	0.74	0.00	0.20	0.33	2.66
Relative density		1.03	5.63	0.00	1.47	2.81	20.82

The prominence value indicates the prominent presence of the genera viz., *Helicotylenchus*, *Pratylenchus*, *Tylenchulus*, *Tylenchus*, *Rotylenchulus*, *Hoplolaimus*, *Tylenchorhynchus*,

Hirschmanniella, *Meloidogyne* and *Aphelenchoides* in citrus ecosystem.

Table 3: Prominence value of plant parasitic nematodes in citrus crop ecosystem of Vidarbha region

	Genera Crop	Rotylenchulus	Hoplolaimus	Pratylenchus	Aphelenchoides	Helicotylenchus	Meloidogyne
Prominence value	Citrus	0.86	0.68	5.77	0.00	7.62	0.01
	Genera Crop	Paratylenchus	Tylenchulus	Hirschmanniella	Tylenchorhynchus	Tylenchus	Free Living
Prominence value	Citrus	0.01	6.97	0.01	1.91	2.59	8.92

Population indices

The Shannon diversity Index and Shannon evenness index for plant parasitic nematodes in citrus ecosystem was worked out as 1.868 and 0.794, respectively.

Table 4: Population indices of plant parasitic nematodes in citrus crop ecosystem of Vidarbha region

S. No.	Crop	Shannon Diversity Index	Shannon Evenness Index
1	Citrus	1.868	0.794

Discussion

The genera *Helicotylenchus*, *Pratylenchus*, *Tylenchulus*, *Tylenchus*, *Rotylenchulus*, *Hoplolaimus*, *Tylenchorhynchus*, *Hirschmanniella*, *Meloidogyne* and *Aphelenchoides* in citrus ecosystem were recorded in citrus crop ecosystem.

Relative & Absolute frequency were recorded highest for *Helicotylenchus*, *Pratylenchus*, *Tylenchulus* and *Tylenchus* species. Absolute and relative density values were noticed highest in *Helicotylenchus*, *Pratylenchus*, *Tylenchulus* and *Tylenchus* genera, respectively.

Khan *et al.* (2010) [5] has reported about the prevalence of nematodes (*Meloidogyne*) on Pomogranate in Solapur, Sangli, Nasik and Ahmednagar and burrowing nematode (*Radopholus similis*) was recorded on banana crop in Jalgaon, Dhule, Nandurbar and Sangli district. However, the incidence of *Heterodera cajani* on Pigeon pea was recorded from Yavatmal and Buldhana districts of Vidarbha region. These reports are in agreement with the present findings which

reports the prevalence and distribution of 11 genera of nematodes from 05 districts of Vidarbha region.

Gokte-Narkhedkar *et al.* (2004) [4] has compiled plant parasitic nematodes of Cotton and their important generic species in Indian context such as *Rotylenchulus reniformis*, *Meloidogyne incognita*, *Hoplolaimus sp.* and *Pratylenchus sp.* in cotton. Present investigations also reported that these genera were associated with cotton and therefore do supports with the present findings.

Similar reports of predominance of nematodes *Meloidogyne*, *Pratylenchus* and *Helicotylenchus* in banana rhizosphere of Andhra Pradesh and Tamilnadu were reported by Sundararaju (2006) [10] and Subramanian (2015) [11] in their respective studies.

Talukawise average population of plant parasitic nematodes (PPN) per 110gm of soil in citrus crop

Amongst genera of plant parasitic nematodes isolated and identified. The genera *Rotylenchulus*, *Hoplolaimus*, *Helicotylenchus*, *Pratylenchus*, *Meloidogyne*, *Tylenchulus*, *Paratylenchus* was prominently recorded in all the districts. According to the damage threshold of the nematodes the high density spots were identified. The damage thresholds for genera *Rotylenchulus* (200/100cc soil), for *Hoplolaimus* (150/100cc soil), for *Pratylenchus* (100/100cc soil), for *Helicotylenchus* (200/100cc soil) and *Meloidogyne* (200/100cc soil). (Nematode guidelines for South Carolina, Jan, 2000. EC 703). (Tylka G, 2009) [12].

Table 5: Talukawise average population of plant parasitic nematode per 100gm soil in citrus crop in Vidarbha region

Genera with Damage threshold S. No.	Rotylenchulus (200/100cc soil)	Hoplolaimus (150/100cc soil)	Pratylenchus (100/100cc soil)	Meloidogyne (100/100cc soil)
1	Ashti (240)	Dhamangaon (Rly) (170)	Nandgaon Khandeshwar(187)	Amravati (250)
2	Kalmeshwar (230)	0	Dhamangaon RLy(170)	Nagpur (136)
3	0	0	Morshi(160)	0
4	0	0	Akola(160)	0
5	0	0	Ashti(140)	0
6	0	0	Darwha(140)	0
7	0	0	Tiwasa(133)	0
8	0	0	Achalpur(127)	0
9	0	0	Kalmeshwar(120)	0
10	0	0	Karanja(120)	0
11	0	0	Anjangaon (Surji)(120)	0
12	0	0	Balapur (Wadegaon)(113)	0
13	0	0	Kalamb(110)	0
14	0	0	Warud(100)	0

** Figures in the paranthesis represent the damage threshold of nematode population (nematode/100gm soil)

High density spots of plant parasitic nematodes in citrus crop ecosystem

High density spots where the population has exceeded the threshold level for citrus crop for genus *Rotylenchulus* were at 2 locations in 2 districts 1 taluka each from Wardha and Nagpur and for genera *Hoplolaimus* 1 spot was identified in Amravati district respectively.

However for genera *Pratylenchus* 14 high density spots were identified from 5 districts with 2 talukas each from Akola, Wardha and Yavatmal, 1 taluka from Nagpur and 7 talukas from Amravati, while 2 high density spots from 2 districts with 1 taluka each from Amravati and Nagpur were identified for genera *Meloidogyne*.

Table 6: High density spots of plant parasitic nematodes in District (No. of talukas) in citrus

Isolate	District	Nagpur	Wardha	Yavatmal	Akola	Amravati	Total
Rotylenchulus		1	1	0	0	0	2
Hoplolaimus		0	0	0	0	1	1
Pratylenchus		1	2	2	2	7	14
Meloidogyne		1	0	0	0	1	2

Conclusion

From the present studies it can be predicted that the population of plant parasitic nematodes in citrus ecosystem is increasing and needs to be focused for the management by strategic management strategies. The increasing population may be a warning for the upcoming problem in citrus orchards of the Vidarbha region of Maharashtra state.

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