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Community analysis of nematodes associated with Sapota (*Manilkara zapota*)

Pranaya Pradhan*, Rudra Pratap Subudhi and Niranjan Kumar Sahoo

Department of Nematology, College of Agriculture, Orissa University of Agriculture and Technology, Bhubaneshwar 751 003, Odisha, India

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ABSTRACT

Sapota is a major fruit crop of India widely cultivated in tropical climatic regions of India. Nematodes pose a severe threat to sapota-growing areas throughout the country. Khordha district is one of the major sapota cultivation hotspots situated in Odisha, India. A survey was conducted in Khordha to assess the economic risk imparted by nematodes. Around 120 requisite soil samples were collected from sapota growing areas of the Khordha district. Among different parasitic nematodes of plants, *Tylenchorhyn chusmashoodi* was the highest occurrence (80), along with the absolute frequency (66.67). Moreover, *Hoplolaimus indicus* was found the most prominent species with a higher prominence value (40.40), followed by *Tylenchorhyn chusmashoodi* (16.98), while the lowest prominence value (1.79) was recorded in *Aphelenchu savenae*. Among the free-living nematodes, *Dorylaimid* exhibited the highest frequency of occurrence (120), with absolute frequency (100%), followed by *Mononchid* and *Rhabditid* (93.33% and 46.67%, respectively). *Dorylamide* showed the highest prominence value (54), followed by *Mononchid* (34.22) and *Rhabditid* (16.10). Thus, due to the minimal existence of harmful nematodes in the soil sample, the Khordha district can be recommended for cultivation of sapota successfully.

***Key words**: Community analysis, Nematode association, Sapota, Manilkarazapota*

Introduction

Sapota (*Manilkara zapota*), also known as *Chiku*, is one of the major crops of India. It is cultivated not only for its taste, flavour and nutritive value but also for commercial extraction of Chickle gum in India and Mexico, Guatemala, and other countries (Kulkarni *et al*., 2007; Guillen *et al*., 2012). Due to the prevalence of such popularity, during the year 2018-19, India produced 1.20 million tonnes of sapota from an area of around 0.10 million hectares. In Odisha state, the Khordha district dominates the sapota cultivation with a growing area of 340 hectares (National Horticulture Board, 2018-19). Although the national productivity of sapota is comparatively high, the predominance of several soil-borne pathogens, including nematodes, restrict the sapota genotypes from reaching their true potential (Jain *et al*., 2007; Kumar *et al*., 2020).The extent of damage depends upon the pathogenic potential, and the population growth of nematodes is greatly influenced by their initial population densities (Chandra *et al*., 2010). The abundance and distribution of the plant-parasitic nematodes, in turn, are influenced by the soil textures, crop cycle, and anthropogenic factors (Chirchir *et al*., 2008). Thus, community analysis of plant nematodes is essential to assess the pathogenic potential of the nematodes in a particular region and a necessary criterion for identification of hotspots of nematode attack in a given area.

Several works have been documented for the

plant-parasitic nematodes associated with various crops in the states of Madhya Pradesh (Rathour *et al*., 2010), Manipur (Joymati and Mema, 2007) and Tamilnadu (Senthilkumar and Rajendran, 2005) of India. Similarly, Community analysis of the plant-parasitic nematodes have been studied by Srinivasan *et al*., 2011 (Banana, Thanjavoor, Tamailnadu), Negi *et al*., 2009 (Pine trees, Himachal Pradesh), Roy *et al*., 2007 (leguminous vegetable crops, West Bengal), Patel *et al*., 2007 (Crops, Gujarat and Diu Union territory. However, no work has yet been reported in the community structure of the phytonematodes associated with sapota in Odisha and any other state of India. Hence, the current investigation on the community structures of the plant nematodes associated with the sapota in the Khorda district may be the first recorded documentation of the nematode population of this state.

Materials and Methods

Survey site

The survey was carried out at different places of Khordha district, Odisha (Figure 1), India, during the year 2018-2019, to record the frequency of various parasitic nematodes infesting sapota. The maximum and minimum temperatures of 42.2 °C and 21.1°C prevailed during the survey period across the region, respectively. The site experienced the maximum rainfall of 1408 mm from 2018-19, with a relative humidity of 46% to 89%. The soil texture varied from loamy and clay to sandy loam soil.

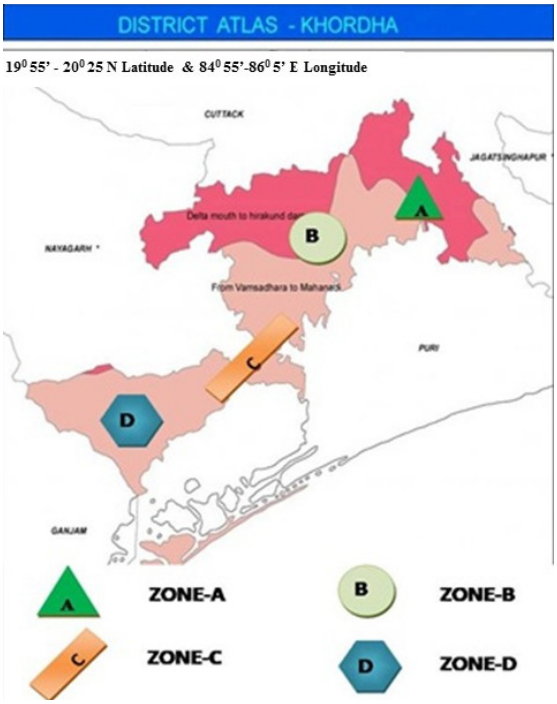

Fig. 1. Illustration showing the experimental site where this experiment was conducted

Collection of soil sample

The survey site was divided into 4 zones *viz*., zone-A, zone-B, zone-C, zone-D. 25-40 soil samples were collected from each zone near the rhizosphere of sapota plants. A total of 120 soil samples were collected.

Extraction of nematodes

For the extraction of nematodes, 250cc of soil was measured per sample. Extracting of nematodes was performed by following Cobb's method of sieving and decanting (Cobb, 1918). Different mesh sieves such as 500-1000 µm, 350-375 µm, 175 µm, 100 µm, 45 µm were used to screen the nematodes. An apparent nematode suspension was then prepared following modified Baermann's funnel technique (Southey, 1986). Out of this apparent nematode suspension, 100 ml working solution was kept for further analysis.

Counting and fixing of nematodes

The counting was done by using a counting disc (syrcase disc) under a dissecting microscope. Care was taken to carry out the counting procedure immediately after nematode extraction in the suspension to visualise live nematodes. For the fixing of the nematodes, nematodes were killed by heating the beaker containing nematode suspension at 65-90 °C in hot water. Fixing nematodes was done immediately using a hot fixative (4% paraformaldehyde at 85 °C orhigher) (Seinhosrst, 1959; Seinhosrst, 1966).

Classical morphological Identification

Identification of nematodes was made based on morphological and anatomical characters using light microscopy, which is considered a fast and reliable method of identification (Oliveira *et al*., 2011; Carnerio, 2017). Identification was made by observing morphometric differences like body length, presence and the shape of the male tail,shape of the head, number of annuals, size of the stylet, a form of stylet knob, the structure of lateral fields, presence/absence and shape of the spermatheca, shape of the female tail terminus, shape and length of spicule

andgubernaculum, etc. (Bogale *et al*., 2020).

Community Analysis

Estimating the existing parasitic nematodes and free living-non parasitic nematodes was done by calculating the community analysis after computing prominence value, relative frequency, absolute frequency, absolute density, and relative density according to the formula given Norton (1978).

Absolute frequency=

$$\frac{\text{No. of samples containing a species}}{\text{.of samples collected}} x100$$

Relative frequency= $\frac{\text{Frequency of species}}{\text{Sum of frequency of all}} x100$

Absolute density = number of individuals per unit of soil.

Relative density = $\frac{\text{No. of individuals of a species in a sample}}{\text{Total of all individuals of a sample}} x100$

Prominence value (PV) = Absolute Density "Absolute Frequency

Results and Discussion

This survey revealed the existence of numerous plant-parasitic as well as free-living nematodes in the nematode suspension (Figure 2). The community structure of nematodes (parasitic and free-living nematodes) associated with sapota fruits in various places of the Khordha district has been presented in Table 1 and Table 2 respectively. Results show that more soil samples contain *Tylenchorhynchus*

Table 1. Occurrence and distribution of nematode species associated with sapota fruit trees of Khordha district of Odisha

Si. No.	Name of the Zones (as divided) in Khordha District	No. of sample collected	Soil sample containing nematode species¥									
			Aa	Ch	Hm	Hi	Pc	Rr	Tm	D	M	R
1	Zone A	29	5	8	8	10	9	7	19	27	29	10
2	Zone B	40	9	12	10	18	8	15	28	35	30	18
3	Zone C	25	4	10	7	13	13	8	13	25	25	12
4	Zone D	36	6	10	7	15	10	10	20	33	28	16
	Total	120	24	40	32	56	40	40	80	120	112	56

¥*Aphelenchus avenae* (Aa), *Caloosia heterocephala* (Ch), *Hemicriconemoides mangiferae* (Hm), *Hoplolaimus indicus* (Hi), *Pratylenchus coffeae* (Pc), *Rotylenchulus reniformis* (Rr), *Tylenchorhynchus mashhoodi* (Tm), *Dorylaimid* (D), *Mononchid* (M), *Rhabditid* (R).

Table 2. Community analysis of plant-parasitic and free-living nematodes embedded with sapota fruit in different Zones of Khordha District

Sl. No	Nematode species	Total number of samples collected	Number of samples having the species	Absolute frequency	Relative frequency	Absolute density	Relative density	Prominence value
Plant-parasitic nematode								
1	*Aphelenchus avenae*	120	24	20.00	4.00	4.00	1.67	1.79
2	*Caloosiahetero cephala*	120	40	33.33	6.67	10.00	4.17	5.77
3	*Hemicriconemoides mangiferae*	120	32	26.67	5.33	7.50	3.13	3.87
4	*Hoplolaimus indicus*	120	56	46.67	9.33	59.14	24.68	40.40
5	*Pratylenchus coffeae*	120	40	33.33	6.67	7.20	3.00	4.16
6	*Rotylenchulus reniformis*	120	40	33.33	6.67	18.00	7.51	10.39
7	*Tylenchorhynchus mashhoodi*	120	80	66.67	13.33	20.80	8.68	16.98
Free-living non-parasitic nematode								
8	*Dorylaimids*	120	120	100.00	20.00	54.00	22.53	54.00
9	*Mononchids*	120	112	93.33	18.67	35.42	14.78	34.22
10	*Rhabditids*	120	56	46.67	9.33	23.57	9.84	16.10
				500.00	100.00	239.63	100.00	

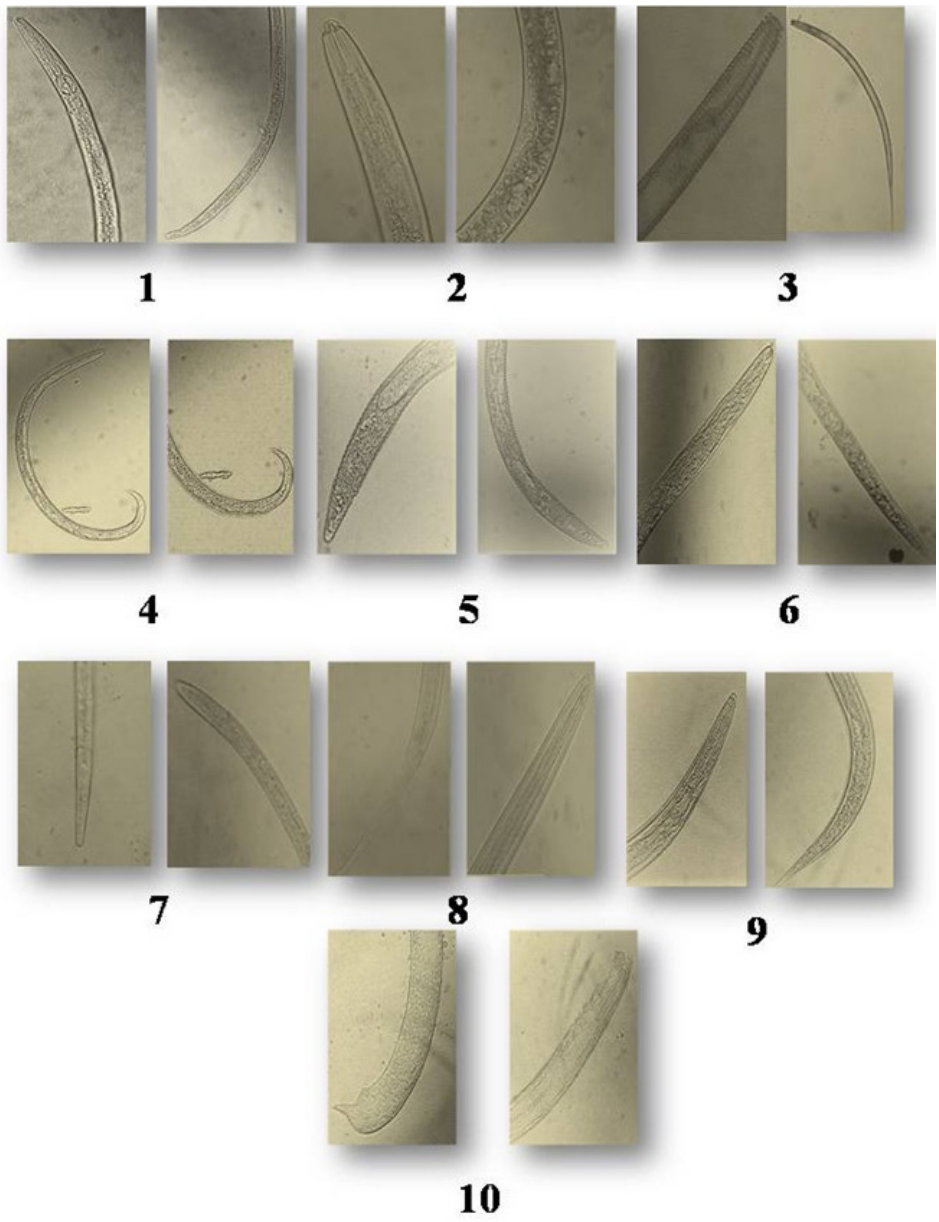

Fig. 2. Nematode specimen collected during survey; **1**-*Aphelenchus avenae*, **2**-*Hoplolaimus indicus*, **3**-*Caloosia heterocephala*, **4**-*Rotylenchulus reniformis*, **5**-*Hemicriconemoides mangiferae*, **6**-*Pratylenchus coffee*, **7**-*Tylenchorhynchus mashhoodi*, **8**-*Dorylaimids spp.*, **9**-*Rhabditids spp.*, **10**-*Mononchidsspp.*

mashhoodi, *Dorylaimids* spp. And *Mononchids* spp. (Figure 3).

Free-living nematodes

So far as the free-living nematodes were concerned, *Dorylaimids* spp. exhibited the highest frequency of occurrence (120), followed by *Mononchids* (112) and the minor frequency of occurrence was found for *Rhabditids* (56). The highest density was observed in the case of *Dorylaimids* (54), followed by *Mononchids* (35.42) and *Rhabditids* (23.57), respectively. The highest relative density was recorded in *Dorylaimids* (22.53%), followed by *Mononchids* (14.78%), and the least density was recorded in the case of *Rhabditids* (9.84%). The highest prominence value was found in the case of *Dorylaimids* (54), followed by *Mononchids* (34.22), and *Rhabditids* exhibited the lowest prominence value (16.10). However, the severity of this

nematode towards sapota is minimum (Sen *et al*., 2014; Lopes-Caitar *et al*., 2019), and they show predatory action towards the parasitic nematodes.

Plant-parasitic nematode population

The analysis of the nematode community pointed towards the association of ten nematode species prevailing in Sapota. Among the different plant-parasitic nematodes, *Tylenchorhynchus mashhoodi* had the highest frequency of occurrence (80) and absolute frequency (66.67 %), followed by *Hoplolaimus indicus* (56) with a fundamental frequency of 46.67%. Caloosia heterocephala, *Pratylenchus coffee* and *Rotylenchulus reniformis* each exhibited the frequency of occurrence of 40 along with the absolute frequency of 33.33 %. The lowest frequency of occurrence was observed in *Aphelenchus avenue* (24), with a fundamental frequency of 20.00 %. The highest density of nematode per unit soil was observed for *Hoplolaimus indicus* (59.14 %), followed by *Tylenchorhynchus mashhoodi* (20.80 %), while the lowest density of nematode was found in *Aphelenchus avenae* (4 %). The highest relative density was recorded in *Hoplolaimus indicus* (24.68%), followed by *Tylenchorhynchus mashhoodi* (8.68%) and the lowest density found in *Aphelenchus avenae* (1.67%).

Considering both frequencies and densities, the highest principal value was observed for *Hoplolaimus indicus* (40.40), followed by *Tylenchorhynchus mashhoodi* (16.98), and the lowest prominent species was *Aphelenchus avenae* (1.79). *Hoplolaimus spp.* and *Tylenchorhynchus* spp. are known to be highly devastating nematodes, severely reducing the yield of several horticultural crops (Skaria *et al*., 2006; Li *et al*., 2017). In our current study, these nematodes were the major parasitic nematode in the survey in the Khrodha district of

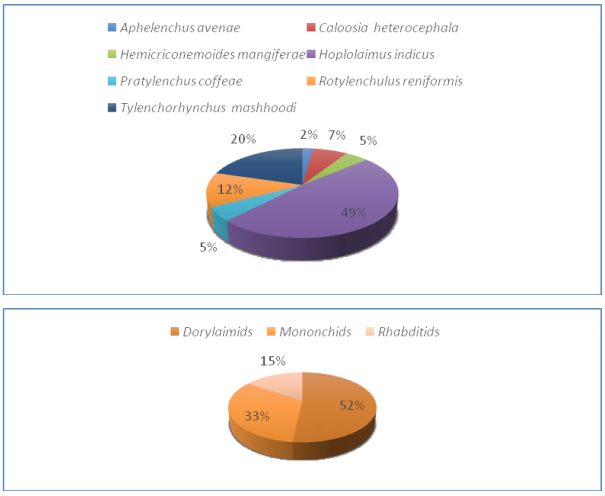

Fig. 4. Percentage comparison of prominence value between plant-parasitic nematodes and free-living nematodes showing higher value of *Hoplolaimus indicus and Tylenchorhynchus mashhoodi.*

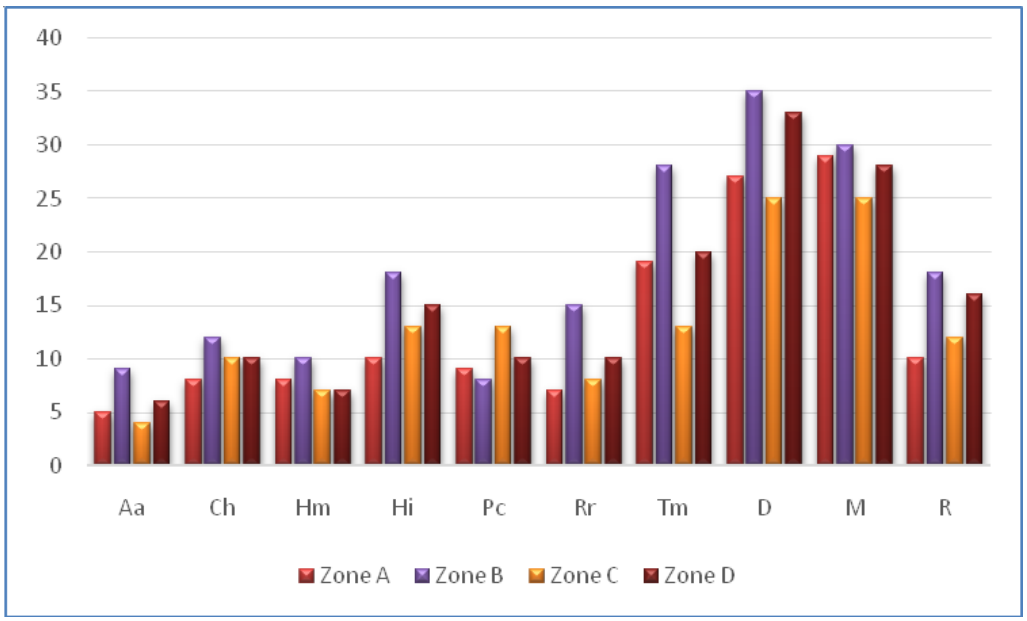

Fig. 3. Illustration showing that more number of soil samples contains Tm, D and M; Aa: *Aphelenchus avena*, Hi: *Hoplolaimu sindicus,* Ch*: Caloosia heterocephala*, Rr: *Rotylenchulus reniformis,* Hm: *Hemicriconemoides mangiferae,*Pc: *Pratylenchus coffee,* Tm: *Tylenchorhynchus mashhoodi*, D: *Dorylaimids spp.*, R: *Rhabditids spp.*, M: *Mononchids spp.*

Odisha. However, the population of these nematodes were deficient, while free-living nematode population was very high. This observation contradicts the result obtained by Bohra (2008) in sapota growing regions of Alwar district of Rajasthan, Nath *et al.*, 2009 in litchi growing areas of Tripura and Devi (2007) in pineapple cultivated areas of Meghalaya. They recorded several highly harmful nematodes such as *Meloidogyne spp*., *Pratylenchus spp*., *Rotylenchulus spp*., etc. Hence, the Khordha district can be recommended for the successful cultivation of sapota due to minimal plant-parasitic nematodes.

Conclusion

In this experiment, a total of 10 nematode species were found affecting sapota, out of which three were found to free-living nematodes, while seven were plant-parasitic nematodes. Out of the numerous plant-parasitic nematodes reported, *Hoplolaimus indicus* and *Tylenchorhynchus mashhoodi* were found to be the most prominent. The data of the low occurrence of these nematodes should facilitate the region's farmers to grow sapota crops without any hindrances involving diseases causing nematodes.

Conflict of interest

The authors declare that they have no conflict of interest.

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