# PLANT-PARASITIC NEMATODE DIVERSITY AND ABUNDANCE IN RHIZOSPHERE SOIL SAMPLES OF COMMERCIALLY IMPORTANT CROPS IN MYSURU TALUK, KARNATAKA STATE, INDIA.

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***Abstract:*** Soil samples of nine crop fields having nine different plants, viz., *Moru alba (*Mulberry), *Phaseolus vulgaris* (Beans), *Solanum lycopersicum* (Tomato), *Musa balbiciana* (Banana), *Manikara zapota* (Sapota), *Vaccinium corymbosum (*Blueberry), *Saccharum officinarum* (Sugarcane), *Ricinus communis* (Castor) and *Annona squamosa* (Custard apple) were quantitatively and qualitatively analysed for plant-parasitic nematodes. Modified Cobb's sieving and decanting and Baermann's funnel techniques were employed to efficiently extract the nematodes from rhizosphere soil samples. Soil temperature, soil pH and soil conductivity were also determined from the rhizosphere soil samples collected from each field to elucidate the relationship with nematode abundance. 25 nematode genera were reported in the present study. Out of 34 major phytonematodes reported from India, *Hemicycliophora* spp. was found in high frequency, *Aphelenchoides* spp. was showing the highest prominence value and density, whereas, it is noteworthy to record complete absence of *Belanolaimus, Criconemoides, Paratylenchus, Hoplolaimus, Heterodera, Globodera, Dolichodorus, Naccobus* and *Anguina* were completely absent. Shannon index and Simpson's Diversity index values were found to be highest for the soil sample having Sugarcane crop. In contrast, the soil sample having Castor crop was showing the lowest values. The Bivariate Pearson's correlation revealed that the abundance of nematodes showed positive correlation with the Soil pH and Soil conductivity. The results have been discussed with the present and past literature.

***Keywords:*** Plant-parasitic nematodes, Mysuru, Mulberry, Beans, Tomato, Banana, Sapota, Blueberry, Sugarcane, Castor, Custard apple, Cobb's sieving and decanting, Baermann's funnel method, Soil pH, Soil conductivity, Soil temperature.

## I. INTRODUCTION:

Nematodes are multicellular, triploblastic, bilaterally symmetrical, pseudocoelomate, unsegmented worms having an elongated and cylindrical body, whichare commonly called eelworms or roundworms belonging to the Phylum Nematoda. Nematodes are widespread in distribution and found in soil, fresh water and salt water wherever organic matter exists, from oceans to mountains, from Arcticto tropics and are said to be 'ubiquitous' (Thome, 1961). There are 25,045 species of nematodes described so far (Zhang, 2013). Phylum Nematoda includes threeclasses, viz., Chomadorea, Enoplea and Dorylaimea (Hodda, 2011). The nematodes feed on bacteria, fungi, protozoans and even other nematodes. Some procure food by parasitizing animals or plants.

Nematodes that feed on other organisms are important participants in the cycling of minerals and nutrients in the ecosystem that is fundamental to other biological activity. Some of these nematodes may have major roles in decomposition, including biodegradation of toxic compounds. Some nematode species are used as pollution indicators. Insect-parasitic nematodes can be of importance in regulating insect populations and are being used in the biological control of insect pest. The study of plant-parasitic nematodes and their interaction with plants are called Plant Nematology or Phytonematology. The body of phytonematodes is vermiform with tapered ends. A cuticle protects the body. The anterior mouth has a hard cuticular protrusible needle like structure called Stylet that helps in penetrating plant cell wall forming large lesions. The body parts are highly modified to parasitize plants. Plant-parasitic nematodes are considered as 'unseen enemies' of plants because the symptoms seen in the aerial parts of the plant mimic the nutritional deficiency or abiotic stress. Aside from the lack of specific symptoms, it is difficult to detect phytonematodes, as they are small and soil dwelling organisms. Nematodes can affect crops by directly feeding on plants through the stylet, disrupting plant physiology through the growth of plant-specific structures. It also can result in secondary infection by opportunistic pathogens (bacteria and fungi) or, in some cases, transmitting plant viruses. These creatures are the important limiting factor for crop production. Especially in India, there is an annual crop loss of about 40.3 million dollars due to plant-parasitic nematodes (Singh *et al.,* 2015). For the survival of phytonematodes, soil abiotic factors play a vital role (Norton, 1989). The soil texture, soil temperature, pH, etc. influence the distribution and abundance of diverse nematode species. Jones, Burns, Norton, Jayrajpuri etc. are some of the nematologists who studied the relationship between soil abiotic factors and nematode population.

So far, only a handful of studies about plant-parasitic nematodeshas been conducted in South India due to the lack of profound taxonomic papers and the advanced identification tools (DNA barcoding, 4D microscopy etc.).Hence, the present study mainly emphasise on the identification of plant- parasitic nematodes from different rhizosphere soil samples having different commercially important crop plants. This study also tries to draw a relation between the soil physio-chemical parameters and nematode abundance.

## II. MATERIALS AND METHODS:

### 2.1 Sampling:

The rhizosphere soil samples were obtained from 9 different crop fields in 5 different places of Mysuru taluk of Karnataka State, India. The 9 different plants are Mulberry, Beans, Tomato, Banana, Sapota, Blueberry, Sugarcane, Castor and Custard apple. Sampling was carried out during January to April 2018. From each crop field, 10 sub-samples were collected randomly to avoid bias (Bezooijen, 2016). About 1000g of rhizosphere soil was collected in morning time when the temperature was relatively low and favourable for the nematodes collection. Samples were collected in a clean & dry polythene bags.

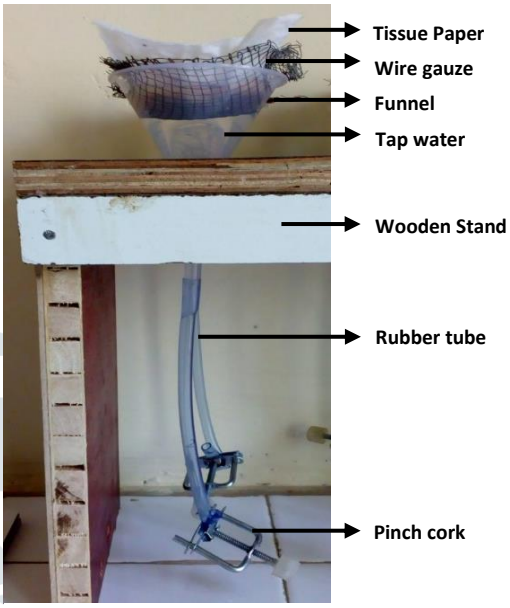


**Fig. 1:** Bearmann's funnel setup.

### 2.2 Physio-chemical analysis:

On- site analysis like Soil type (Hanlon, 2015) and soil temperature were recorded at the depth of 5cm using a hand-held mercury thermometer. Soil pH and soil conductivity were also determined (Rayment *et al.*, 1992).

### 2.3 Processing and extracting nematodes:

The plant-parasitic nematodes were isolated from the rhizosphere soil sample using Cobb's sieving and decanting method (Cobb, 1918) followed by Baermann's funnel extraction technique with some modifications (Christie and Perry, 1951). The nematodes were isolated from 100g of soil using 3 Cobb's sieves (150µm, 63µm and 45µm). The decanted residue from 45µm sieve was collected in a beaker. The residue was poured to the modified Baermann's funnel setup (Fig. 1) and left undisturbed for 48 hrs. Later, the phytonematodes were collected in a petri dish from Baermann's funnel, and were fished using a dropper. The plant-parasitic nematodes were collected in an Eppendorf tube and staining was done using Seinhorst's protocol (Ryss, 2017). This technique was modified by using Eosin as a stain and the fixation was done simultaneously. For this, 4% hot formalin and 1% Eosin stain were used. The nematodes were observed and identified under compound microscope (Olympus CX21). The nematode identification was done using the identification keys given by Mekete *et al.* (2012) and Dasgupta (1998).

### 2.4 Statistical data:

Absolute frequency (AF), relative frequency (RF), mean density (MD), relative density (RD) and prominence value (PV)were applied to the collected data (Norton, 1978).Shannon-Wiener index (H') and Simpson's Diversity index (1-D) were also calculated. To determine the strength and direction of relationship between soil abiotic factors and nematode abundance, Bivariate Pearson's correlation was used (SPSS 14.0). Following are the formulae used for statistical analysis:

$$1.\ AF = \frac{\textit{Number of samples containing a genera}}{\textit{Total number of samples collected}}\ X\ 100$$

$$2.\ RF = \frac{\textit{Frequency of genera}}{\textit{Sum of frequencies of all genera}}\ X\ 100$$

$$3.\ MD = \frac{\textit{No. of nematode specimens of the genus in all samples}}{\textit{Total number of samples collected}}$$

$$4.\ RD = \frac{\textit{MD of the genus}}{\textit{Sum of MD of all nematode species}}\ X\ 100$$

$$5.\ PV = MD\ X\ \sqrt{AF}$$

$$6.\ H' = -\sum p_i\ ln\ p_i$$

$$7.\ D = \sum \frac{n_i(n_i - 1)}{N(N-1)}$$

where $n_i$ is the number of individuals in a genera i, N is the total number of individuals in a community and $p_i = n_i/N$.

## III. RESULTS AND DISCUSSION:

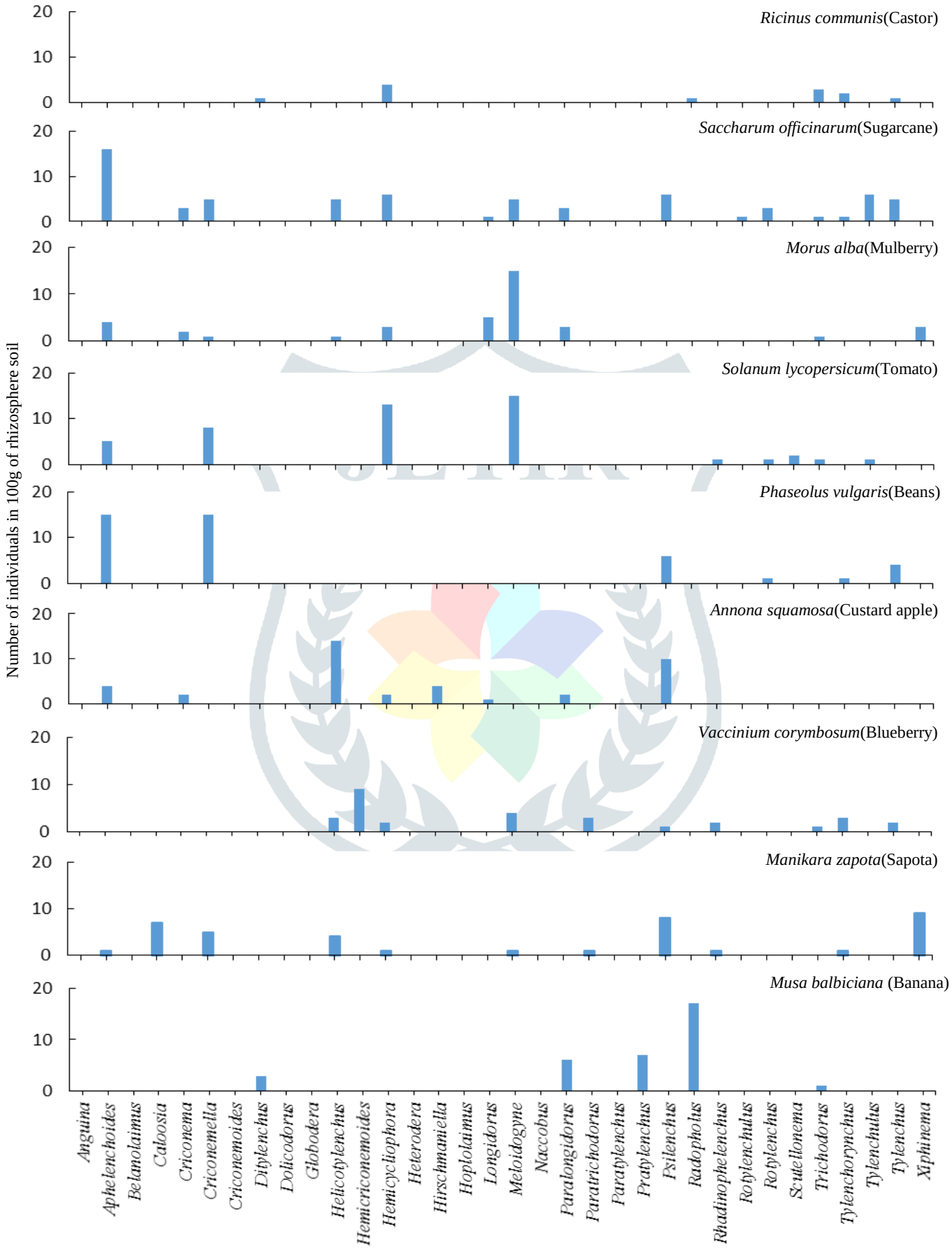


**Fig. 2:** Different genera and number abundance of plant-parasitic nematodes recorded in the rhizosphere soil of 9 commercially important plants in Mysuru taluk, Karnataka State, India.

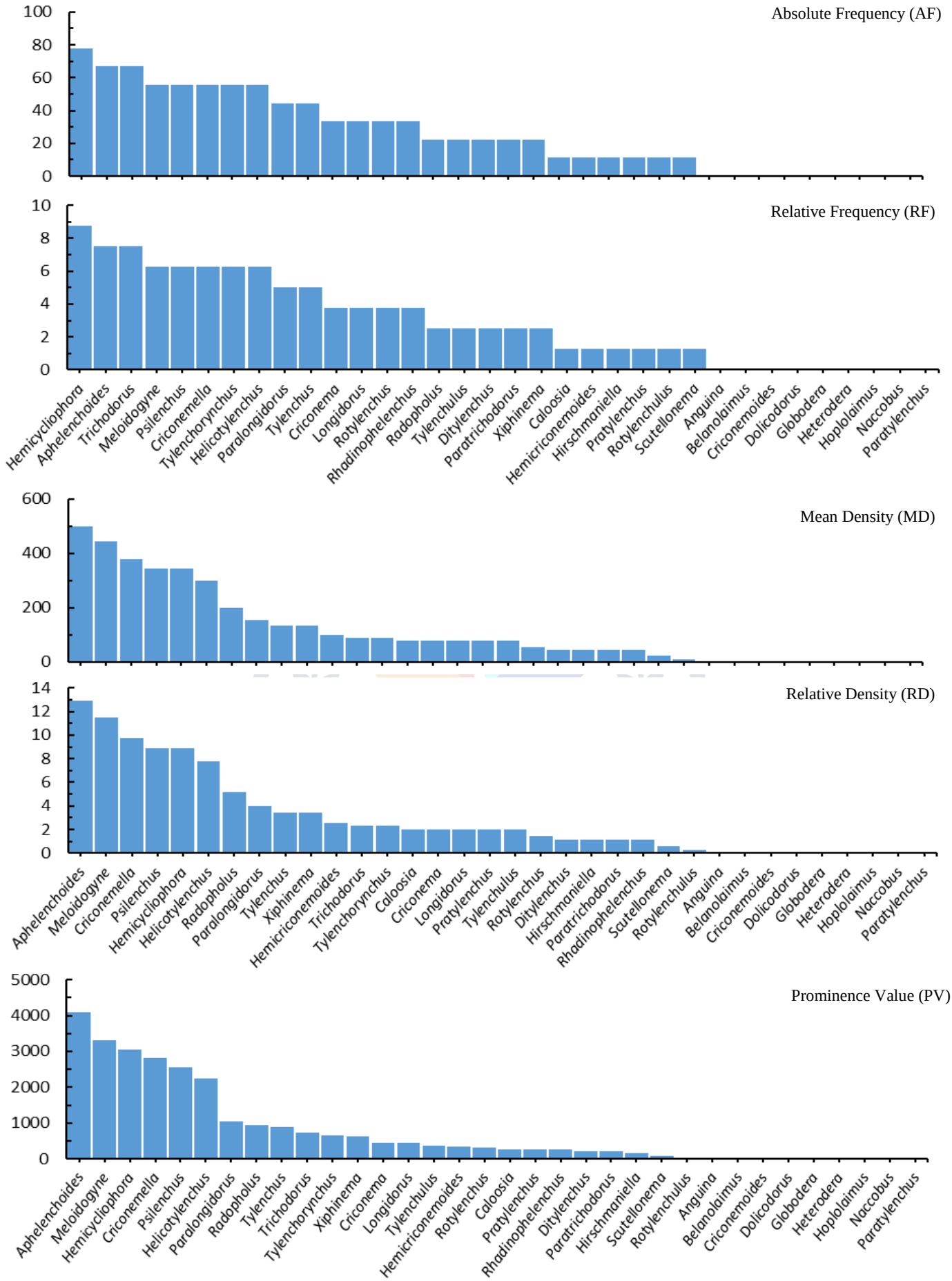


**Fig. 3:** Absolute frequency, Relative frequency, Mean density, Relative density and Prominence value for the 34 plant-parasitic nematode genera.

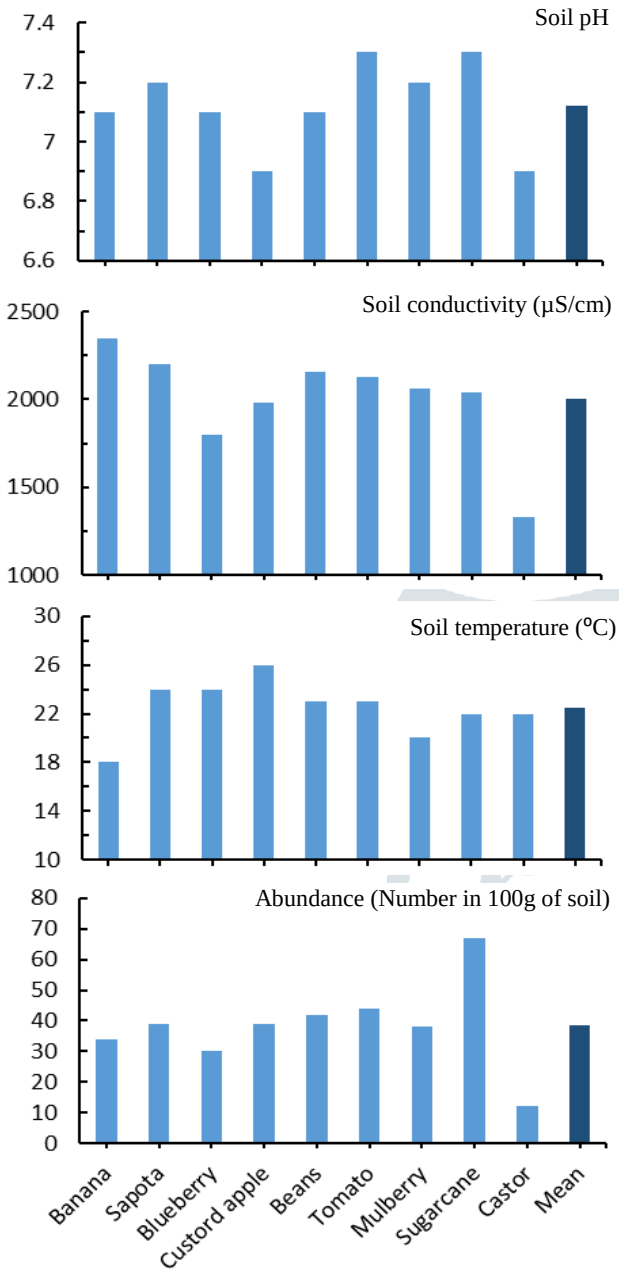


**Fig. 4:** Comparison of nematode abundance, pH, conductivity and temperature recorded from 9 crop field rhizosphere soil samples (Jan- Apr 2018)

**Table 1:** Bivariate Pearson's Correlation table showing Correlation co-efficient (r) for Nematode abundance, Soil pH, Soil conductivity and Soil temperature

| | Nematode Abundance | Soil pH | Soil Cond. | Soil Temp. |
|---|---|---|---|---|
| **Nematode Abundance** | 1 | | | |
| **Soil pH** | 0.707 * | 1 | | |
| **Soil Conductivity** | 0.600 NS | 0.562 NS | 1 | |
| **Soil Temperature** | 0.054 NS | -0.247 NS | -0.240 NS | 1 |

***Note:*** * = Significant ($p<0.05$) (2-tailed);
NS= Non-significant ($p>0.05$)

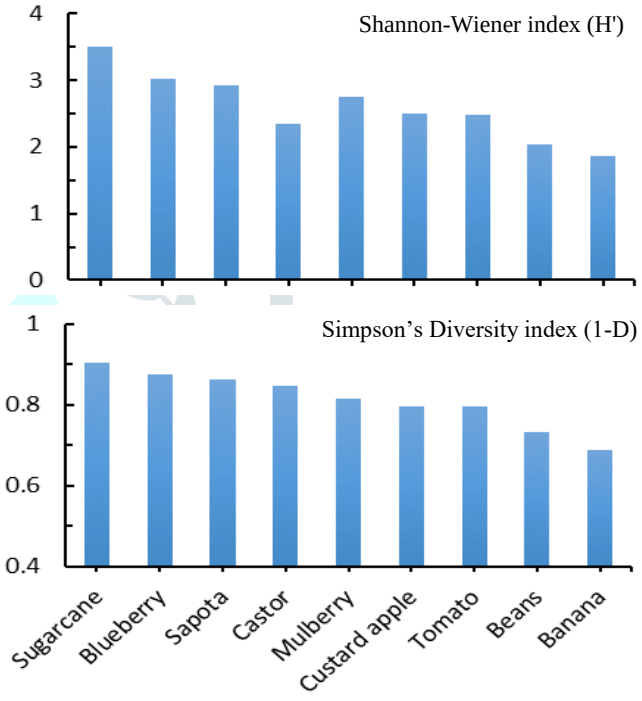


**Fig. 5:** Comparison of Shannon-Wiener indices (H') and Simpson's Diversity indices (1-D) for the 9 crop fields (Jan- Apr 2018).

Twenty-five genera of plant parasitic nematodes were obtained from the rhizosphere soil samples of 9 different fields of Mysuru taluk. Fig. 2 shows the number of individuals in 100g of rhizosphere soil vs. 34 major nematode genera for all the 9 crop fields. Only 5 genera were isolated from Banana crop field soil sample.11 genera were present in Sapota, 10 in Blueberry, 8 in Custard apple, 6 in Beans, 9 in Tomato, 9 in Mulberry, 15 in Sugarcane and 6 in Castor field soil samples. The dominant genera found in Banana crop field was *Radopholus* spp. Cobb (1918) also reported the same result in his study. *Xiphenema* spp. was the dominant genera found in Sapota crop rhizosphere soil. Timm (1965) also reported *Xiphenema* spp. as dominant genera affecting Sapota plants in Thailand and Philippines. *Caloosia* spp. was found in the rhizosphere soil of Sapota field among all the nine soil samples collected. In Blueberry rhizosphere soil, *Hemicriconemoides* spp. was found in highest number and was restricted only to this crop among the 9 commercial crops. Meanwhile, *Trichodorus* and *Psilenchus* spp. were obtained in least number. *Helicotylenchus* spp. was the dominant genera in rhizosphere soil sample from custard apple field. In Beans rhizosphere soil sample, *Criconemodes* and *Criconemella* spp. were highest in number and *Meloidogyne* spp. was absent in this sample. However, Al-Hazmi and Al-Nadary (2015) reported that the infestation of *Meloidogyne incognita* was very high in *Phaseolus vulgaris* in Saudi Arabia. This contradicted result might be because of the geographical difference and variation in the type of pest management practice. In Tomato crop field, *Meloidogyne* spp. was the dominant genera. Yang *et al.* (2016); Corbett *et al.* (2011)

also reported the same dominant genera in Tomato. In this study, *Helicotylenchus, Rotylenchus* and *Radinophelenchus* spp. were found in minimum number. *Scutellonema* was found only in this rhizosphere soil among all the 9 crop fields.In Mulberry rhizosphere soil sample, *Meloidogyne* spp. was the dominant genera, which was also reported by nematologists like Banu *et al.* (2012) and Saha *et al.* (1983). *Aphelenchoides* were dominant among other genera in Sugarcane field rhizosphere soil. *Radopholus* spp. was absent in this sample and the same result was shown by Stirling (2011). In castor rhizosphere soil sample, *Hemicycliophora* spp. was found in more number.

From the graph (Fig. 3), it is clear that AF and RF of *Hemicycliophora* spp. was showing the highest value of about 77.78 and 8.74 respectively indicating that it is found most often in the 9 rhizosphere soil samples of Mysuru taluk. *Aphelencoides* and *Helicotylenchus* spp. were showing the second highest values of AF and RF of about 66.67 and 7.49 respectively. *Caloosia, Hirschmaneilla, Rotylenchulus, Hemicriconemoides, Tylenchulus* and *Ditylenchus* spp. were showing the least value of AF and RF of about 11.11 and 1.25. These values indicate that they were the least frequent nematode genera in all the collected rhizosphere soil samples from Mysuru taluk. *Aphelenchoides* spp. ranked first based on absolute density (500.0), relative density (12.9) and prominence value (4082.5) followed by *Meloidogyne* spp. (MD= 444.4; RD= 11.5; PV= 3312.7). The least dense and least prominent genera was *Rotylenchulus* with MD= 11.1, RD=0.29 and PV= 38.04. Interestingly, *Belanolaimus, Criconemoides, Paratylenchus, Hoplolaimus, Heterodera, Globodera, Dolichodorus, Naccobus and Anguina* spp. were completely absent in all the nine collected soil samples. This might be because of the host specificity and difference in geographical locality.

Out of 9 crop fields, Sugarcane field rhizosphere soil showed the highest nematode abundance (67 individuals per 100g of rhizosphere soil sample) when the soil pH= 7.12, soil conductivity=2005.5µS/cm and soil temperature= 22.4 ºC (Fig. 4). Castor rhizosphere soil sample showed the least nematode abundance (12) when soil pH= 6.9, soil conductivity=1330µS/cm and soil temperature= 22.0 ºC. These results indicated that the nematode abundance was positively correlated with soil abiotic factors. This was statistically proven using Bivariate Pearson's Correlation (Table 1). Nematode abundance and soil pH showed positive and statistically significant correlation ($r = 0.707$; $p<0.05$). The correlation between the abundance and soil conductivity was strong and positive but not significant ($r= 0.60$; $p>0.05$). Meanwhile, no correlation was found between the abundance and soil temperature ($r= 0.054$; $p>0.05$). These results indicated that the soil pH and conductivity affects the nematode abundance. Surprisingly, Custard apple crop field also showed the same pH (6.9) as that of the Castor crop field. However, the nematode abundance was 39, which was a moderate value. This was due to the high conductivity (1980 µS/cm) and the soil temperature was highest (26ºC). This result in turn indicated that the abundance of plant parasitic nematodes was multi factorial. On the other hand, the abundance might also depend on the intrinsic factors of the crop in addition to soil abiotic factors. Castor and Banana rhizosphere soil samples showed least nematode abundance. Joshi *et al.* (2004) reported that a water-soluble toxin called Ricin present in castor plant in lower concentration. Therefore, this might be a reason for the less number of phytonematodes in castor plants. In Ayurveda, castor oil and banana peels are used as herbal remedy for helminthiasis (helminth infestation) due to their anthelmintic properties (Manthri *et al.*, 2011; Danamoni *et al.*, 2016). Still there is need of more research to be conducted with reference to their medicinal importance and active compounds responsible for various activities.

In fig. 5, Sugarcane field was ranked first among the 9 crops with Shannon-Wiener index (H') = 3.51 and Simpson's diversity index (1-D) = 0.9 indicating the soil has high genera richness and diversity of nematodes. Banana field showed the least richness and moderate diversity (H' = 1.87 and 1-D = 0.69). Even though castor rhizosphere soil had the least abundance, it was ranked fourth in Simpson's Diversity index (0.85) and seventh in Shannon index (2.36) due to high genera diversity.

## IV. CONCLUSION:

On extensively analysing the rhizosphere soil samples of nine different crop plants in Mysuru taluk, Sugarcane was highly infested with the plant-parasitic nematodes and the least infestation was shown in Castor plant. Out of 25 genera, pest management practices should be carried by giving prominence to *Aphelenchoides, Meloidogyne* and *Hemicycliophora* spp. in these regions. The present study also revealed that the soil pH and conductivity were positively correlated with the abundance of plant-parasitic nematodes. Therefore, by decreasing the soil pH and conductivity through soil treatment may help reducing the nematode infestation.

## V. ACKNOWLEDGMENT:

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