

Journal of Botanical Research https://ojs.bilpublishing.com/index.php/jbr



### ARTICLE A Primary Study for Checking the Occurrence of Plant Parasitic Nematodes with the Crop Banana at Agricultural Areas of Palakkad Taluk, India

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### ARTICLE INFO

Article history Received: 29 June 2020 Accepted: 22 July 2020 Published Online: 31 July 2020

Keywords: Analysis Nendran Diversity Pathogen Kerala

#### ABSTRACT

The reviews on production of banana during the recent years were not a satisfying one with respect to fourth position of Kerala in area of cultivation in India. Among so many factors for this declination, plant parasitic nematodes also found as a major negative factor. Thus the present study tried to prove this predict and conducted a survey in the unexplored rhizosphere region of an important crop banana (Nendran) in Palakkad taluk of Kerala, India during the post monsoon season of 2017. A total of twenty seven samples each were collected from banana rhizosphere soil and roots and processed for this study. The analysis revealed that the most abundant nematode population was Radopholus spp. and most frequently occurred genus was Meloidogyne spp. in the studied banana fields. The major diversity showing area were Elappully panchayath for rhizosphere soil samples and Kannadi panchayath for root samples. Different plant parasitic nematodes such as Aphelenchus spp., Criconemoides spp., Dory laimoides spp., Helicotylenchus spp., Hoplolaimus spp., Meloidogyne sp p., Pratylenchus spp., Radopholus spp., Rotylenchulus spp., Tylenchoryn chus spp. and Tylenchus spp. were observed in both soil and root samples examined.

### 1. Introduction

The productivity rate of banana expressed in agricultural statistics reports of 2016 and 2017 was not a satisfying one with respect to the fourth position in area of cultivation in the Indian state Kerala<sup>[3,4]</sup>. Plant parasitic nematodes are reported to cause a yield loss in banana<sup>[21]</sup>. The reviews on the plant disease surveys of Kerala showed that there was no much attention given to the crop loss due to plant parasitic nematodes. Consequently a systematic study on species abundance, distribution and pathogenicity of nematodes are lacking. Any disease management studies should also focus on occurrence and diversity of nematodes on a crop area. Knowledge on diversity and occurrence of nematode, as well as the major environmental and agronomical cues for understanding their distribution in specific areas is of vital importance for designing its control measures

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<sup>[2]</sup>. To make more practical management, such baseline studies provide information to make suitable strategies. Along with identification of plant nematodes of a region its diversity analysis is important to assess the pathogenic potential of that region and became an important criterion for identification of hot spots of nematode attack <sup>[20]</sup>. Nematodes affect crops through feeding plant roots and it also leads to infestation of secondary pathogens such as fungi and bacteria <sup>[13]</sup>.

The major parasitic nematode species of banana such as *Radopholus similis, Helicotylenchus* spp., *Pratylenchus coffeae* and *Meloidogyne* spp. are to be controlled not only because of the damage they cause but also due to their pronounced variability and interactions with different banana cultivars <sup>[1,10]</sup>. In the present study diversity and occurrence of plant parasitic nematodes were assessed by a survey. The sampled region of the present study is a valuable benchmark area where passes the Palghat gap in Nilgiri Biosphere Reserve in Western Ghats. Diversity studies on fauna suggested influence of this gap for diversification of various taxa <sup>[19]</sup>.

### 2. Materials and Methods

### 2.1 Survey and Sample Collection

The study area Palakkad taluk of India lies at geographic co-ordinates between 10°53'37" N; 76°36'52" E in Northern end, 10°50'39" N; 76°49'37" E in Eastern end, 10°43'43" N; 76°40'22" E in Southern end and 10°44'27" N; 76°33'09" E in Western end. The identification and characterization of nematode fauna was done by following methods. Samples were collected from rhizosphere region of banana (Musa AAB "Nendran") by an intensive survey from different banana fields in Palakkad taluk during August to December of 2017. The collection sites designated as per local self government bodies namely "panchayath"/Municipality. They were included Akathethara panchayath, Elappully panchayath, Kannadi panchayath, Kodumba panchayath, Malampuzha panchayath, Marutha road panchayath, Mundur panchayath, Pudupariyaram panchayath and Pudussery panchayath. A total number of twenty seven samples with three samples each from a panchayath/Municipality were collected from both rhizosphere soil and root samples. At each place a random of rhizosphere soil and root samples were collected from banana plants at 25-30 cm away from the bole of the plant and to a depth of 25-30 cm. Samples were collected in polythene bags, properly labeled and stored at room temperature until it was processed for nematode extraction in Laboratory.

### 2.2 Extraction of Nematodes from Soil Samples

The collected soil samples were processed for nematode assay by Cobb's decanting and sieving followed by the modified Baermann<sup>[6]</sup> funnel technique<sup>[16]</sup>. All collected samples were taken in uniform quantity of 250 g. Then it was transferred to a plastic container and mixed well with tap water. After settlement of large soil particles it was poured into meshes having different mesh size arranged one above the other. The nematodes trapped in the lower most mesh (BSS 400) were gently decanted into a plastic beaker by adding clear water. Then it was poured onto a tissue paper over layered on wire gauge mesh which was placed in a plastic petridish with clear water. This set up was maintained for 12 hours to collect nematodes.

### 2.3 Extraction of Nematodes from Root Samples

The infected root bits were taken from semi hard portion of the main roots. Roots were washed thoroughly to remove adhered soil particles and then cut into 4 cm sized pieces. These pieces having 10g (fresh weight) were taken from each sample and macerated gently using kitchen mixer grinder (Panasonic, Japan). Then it was poured onto a tissue paper over layered on wire gauge mesh which was placed in a plastic petridish with clear water. This set up was maintained for 12 hour to come down nematodes towards clear water stores in plastic petri dish.

## 2.4 Identification and Analysis of Samples for Nematodes

Nematodes collected from soil samples were killed and fixed by using 4% hot formaldehyde solution. Nematode population is estimated by using a stereomicroscope (Magnus MSZ-TR) and images were taken by using camera attached Compound microscope (Olympus CX2*li*). The nematodes present in the suspension were identified up to generic level based on morphology using nematode identification key of Tarjan *et al.*<sup>[19]</sup>. Occurrences of population of each nematode in each sample were recorded. To check the nematode diversity, nematode density and nematode population abundance measures such as Absolute Density (AD), Absolute Frequency (AF) and Prominence value (PV) were calculated by using the formula proposed by Norton<sup>[12]</sup> in which:

Number of samples containing nematodes
Absolute frequency = × 100
Number of samples collected
Number of nematodes in all samples
Absolute density = × 100
Number of sample collected
Prominence value = Absolute density $\times \sqrt{Absolute}$ frequency

Total number of a genus Occurrence (%) of a genus at a study area =  $----- \times 100$ Total number of nematodes at a study area

### 3. Results

### 3.1 Analysis of Soil and Root Samples

From twenty seven soil and root samples collected from banana fields of Palakkad taluk twelve plant parasitic nematodes were obtained. One genus was seeming to be new in morphological features. Even though plant parasitic nematodes were found in all banana fields, they were found below Economic Threshold Level (ETL) only. ie., the maximum nematodes observed from a collection cite was 216 only from 250g soil at Elappully panchayath. The different types of plant parasitic nematodes observed at this study area were *Aphelenchus* spp., *Criconemoides* spp., *Dorylaimoides* spp., *Helicotylenchus* spp., *Hoplolaimus* spp., *Rotylenchulus* spp., *Tylenchorynchus* spp. and *Tylenchus* spp..

### 3.2 Diversity Analysis between Panchayaths

The nematode diversity studies revealed that Elappully panchayath and Kannadi panchayath had maximum diversity for studied soil samples and Kannadi panchayath showed maximum diversity for studied root samples. The maximum number of genus observed per panchayath was seven for soil samples and six for root samples. The root analysis for plant parasitic nematodes showed that Kodumba panchayath and Pudupariyaram panchayath were represented by two genera only. *Meloidogyne* spp. occurred in both panchayaths and other genus was *Tylenchus* spp. for Kodumba panchayath and *Hoplolaimus* spp. at Pudupariyaram panchayath. None of the genera was found in cent percentage in both soil and root samples collected from the study area. All twelve nematodes spp. were not observed in a single panchayath under study.

On analysing the soil samples, major contribution of 21.16% nematodes were found in Elappully panchavath followed by Kannadi panchayath, Mundur panchayath, Marutha road panchayath, Malampuzha panchayath, Pudupariyaram panchayath, Pudusery panchayath and Kodumba panchayath with a shares of 16.55%, 14.0%, 13.32%, 13.12%, 9.01%, 7.54% and 3.43% respectively and Akathethara panchayath with the least share of 1.86%. Kannadi panchayath (32.31%) showed a remarkable higher variables for percentage of occurrence of nematodes on analysis of root samples and least was observed at Marutha road panchayath (1.46%) and others are observed in the order of Malampuzha panchayath (19.94%), Elappully panchayath (16.74%), Pudussery panchayath (10.19%), Mundur panchayath (9.33%), Akathethara panchayath (4.66%), Pudupariyaram panchayath (3.2%) and Kodumba panchayath (2.18%). For understanding the distribution, patterns of nematodes population and population abundance of each nematode in each panchayath were clearly given in the Tables 1, 2 & 3 for both soil and root samples.

Panchayaths	media	Aph	Cri	Dor	Hel	Нор	Mel	Pra	Rad	Rot	Tyl	Tyr	Unk
Akathethara	Soil	-	-	++	-	+++	+	-	-	-	++	-	-
	Root	-	-	-	-	+++	+	+++	-	-	-	-	-
<b>F</b> 1 11	Soil	-	-	+	-	+++	+++	+++	+++	+++	+++	-	-
Elapully	Root	-	++	-	-	+++	++	+++	+++	-	-	-	-
	Soil	-	-	+	+++	+++	++	-	+++	+++	+++	-	-
Kannadi	Root	-	-	++	+++	++	++	-	+++	+++	-	-	-
Kodumba -	Soil	-	-	-	+++	-	+	-	+++	-	-	-	-
	Root	-	-	-	-	-	++	-	-	-	+++	-	-
Malampuzha	Soil	-	-	++	+++	-	+++	-	-	+++	-	+++	-
	Root	-	-	++	+++	-	+++	-	-	-	-	+++	-
Mall	Soil	+++	-	+	++	-	+++	-	+++	+++	-	-	-
Marutha road	Root	-	-	+	-	-	+	-	-	-	+++	-	-
N 1	Soil	-	-	++	+++	-	+++	-	+++	+++	-	-	-
Mundur	Root	-	-	++	-	+++	+++	-	+++	++ -	-	-	-
Pudupariyaram -	Soil	-	-	+	-	-	+++	-	+++	-	-	-	-
	Root	-	-	-	-	+++	++	-	-	-	-	-	-
D 1	Soil	-	++	-	+++	+++	+++	-	+++	-	-	-	+
Pudussery	Root	+	-	-	+++	-	+++	-	+++	-	-	-	-

Table 1. Nematode distribution in rhizosphere soil and root of banana "Nendran" (AAB) samples at Attappady hill area

Notes:

Aph - Aphelencus spp.; Cri - Criconemoid Spp.; Dor - Dorylaimoides spp.; Hel - Helicotylenchus spp.; Hop -Hoplolaimus spp.; Mel - Meloidogyne spp.; Pra - Pratylenchus spp.; Rad -Radopholus spp.; Rot - Rotylenchulus spp.; Tyl - Tylenchus spp.; Tyr - Tylenchorynchus spp.; Unk -Unknown spp.

<b>Table 2.</b> Percentage of occurrence and population density of each plant parasitic nematode species in rhizosphere soil
samples from banana var. "Nendran" (AAB) at different panchayaths in Palakkad taluk, India

Panchayaths	Total per panchayath	% of occurrence		AF	AD	PV
			Dor	66.67	166.67	1360.86
Akathethara	19	1.86	Нор	100	333.33	3333.33
	19	1.00	Mel	33.33	66.67	384.88
			Tyl	66.67	66.67	544.35
			Dor	33.33	66.67	384.88
			Нор	100	533.33	5333.33
			Mel	100	3133.33	31333.3
Elapully	216	21.16	Pra	100	966.67	9666.67
			Rad	100	1766.67	17666.7
			Rot	100	600	6000
			Tyl	100	133.33	1333.33
			Dor	33.33	33.333	192.44
			Hel	100	833.33	8333.33
			Нор	100	333.33	3333.33
Kannadi	169	16.55	Mel	66.67	66.67	544.35
			Rad	100	766.67	7666.67
			Rot	100	2800	28000
			Tyl	100	800	8000
	35	3.43	Hel	100	366.67	3666.67
Kodumba			Mel	33.33	66.67	384.88
			Rad	100	733.33	7333.33
Malampuzha	136	13.32	Dor	66.67	166.67	1360.86
			Hel	100	2133.33	21333.3
			Mel	100	166.67	1666.67
			Rot	100	1500	15000
			Tyr	100	566.67	5666.67
		13.12	Aph	100	566.67	5666.67
			Dor	33.33	66.67	384.88
	134		Hel	66.67	233.33	1905.21
Marutha road			Mel	100	1200	12000
			Rad	100	1300	13000
			Rot	100	1100	11000
			Dor	66.67	66.67	544.35
	143	14	Hel	100	500	5000
Mundur			Mel	100	833.33	8333.33
			Rad	100	3033.3	30333.3
			Rot	100	333.33	3333.33
			Dor	33.33	133.33	769.76
Pudupariyaram	92	9.01	Mel	100	733.33	7333.33
			Rad	100	2200	22000
			Cri	66.67	66.67	544.35
	77	7.54 -	Hel	100	666.67	6666.67
			Нор	100	433.33	4333.33
Pudussery			Mel	100	500	5000
			Rad	100	833.33	8333.33
			Unk	33.33	66.67	384.88

Notes:

Aph - Aphelencus spp.; Cri - Criconemoid Spp.; Dor - Dorylaimoides spp.; Hel - Helicotylenchus spp.; Hop -Hoplolaimus spp.; Mel - Meloidogyne spp.; Pra - Pratylenchus spp.; Rad -Radopholus spp.; Rot - Rotylenchulus spp.; Tyl - Tylenchus spp.; Tyr - Tylenchorynchus spp.; Unk -Unknown spp.

Table 3. Percentage of occurrence and population density of each plant parasitic nematode species in root samples from
banana var. "Nendran" (AAB) at different panchayaths in Palakkad taluk, India

Panchayaths	Total per panchayath	% of occurrence	Nematode genera	AF	AD	PV
			Нор	100	233.33	2333.33
Akathethara	32	4.66	Mel	33.33	100	577.32
			Pra	100	733.33	7333.33
		16.74	Dor	66.67	133.33	1088.69
			Нор	100	133.33	1333.33
Elapully	115		Mel	66.67	133.33	1088.69
			Pra	100	966.67	9666.67
			Rad	100	2466.67	24666.67
			Dor	66.67	133.33	1088.69
			Hel	100	1033.33	10333.33
Kannadi	222	32.31	Нор	66.67	100	816.52
Kannadi			Mel	66.67	166.67	1360.86
			Rad	100	466.67	4666.67
			Rot	100	5500	55000
Kodumba	15	2.18	Mel	66.67	66.67	544.34
Kodumba			Tyl	100	433.33	4333.33
	137	19.94	Dor	66.67	100	816.52
Malampuzha			Hel	100	2500	25000
Malampuzna			Mel	100	1433.33	14333.33
			Tyr	100	533.33	5333.33
	10	1.46	Dor	33.33	33.33	192.44
Marutha road			Mel	33.33	66.67	384.88
			Tyl	100	233.33	2333.33
	64	9.32	Dor	66.67	266.67	2177.38
			Нор	100	233.33	2333.33
Mundur			Mel	100	633.33	6333.33
			Rad	100	833.33	8333.33
			Rot	66.67	166.67	1360.86
Budupariyayam	22	2.2	Нор	100	600	6000
Pudupariyaram	22	3.2	Mel	66.67	133.33	1088.69
			Aph	33.33	66.67	384.88
Duduceere	70	10.19	Hel	100	300	3000
Pudussery			Mel	100	866.67	8666.67
			Rad	100	1100	11000

Notes:

*Aph - Aphelencus spp.; Dor - Dorylaimoides spp.; Hel - Helicotylenchus spp.; Hop -Hoplolaimus spp.;* 

Mel - Meloidogyne spp.; Pra - Pratylenchus spp.; Rad - Radopholus spp.; Rot - Rotylenchulus spp.; Tyl - Tylenchus spp.; Tyr - Tylenchorynchus spp.

# **3.3 Distribution Analysis on Nematode Genera Observed**

The analysed data on percentage of occurrence of different nematode genera at Palakkad taluk revealed that the mostly observed genus in soil samples was *Radopholus* spp. with a share of 31.24% and least observed genus *Criconemoides* spp. and unknown nematode were at 0.2%. But in root samples the most observed genus was *Rotylenchulus* spp. with a share of 24.75% and least observed one was *Aphelenchus* spp. at 0.29%. AD (%) of *Radopholus* spp. was notably higher than other genera in rhizosphere soil samples. But the measure of frequency of observation [Absolute Frequency (AF) distribution] was highest for the genus Analysis in root samples also showed

higher AF distribution (%) for the genera *Meloidogyne* spp. and AD (%) for the genus *Rotylenchulus* spp. The most frequently observed genus *Meloidogyne* spp. had an absolute frequency of 81.48% in rhizosphere soil samples and 66.67% in root samples.

For analysing the population abundance of an organism a summative figure of AD and AF known as prominence value was checked. Even though the value of AF (%) and AD (%) for Radopholus spp. were lower than other genera, while considering the prominence value as a measure of population abundance highest value (10419.69) was showed by Radopholus spp. in both rhizosphere soil samples and root samples. Among the soil samples least prominence value (14.26) was observed by new variant spp. Dealing with the root samples lowest value (20.16)was seen for Aphelenchus spp.. The highest prominence value, showing genus Radopholus spp. was not observed in two panchayaths such as Akathethara panchayath and Malampuzha panchayath of this studied area. The survey for plant parasitic nematodes in banana var. Nendran (AAB) showed that five genera were prevalent in Palakkad taluk, Kerala on rhizosphere soil and root. The plant parasitic nematode such as Helicotylenchus spp., Hoplolaimus spp., Meloidogyne spp., Rotylenchulus spp. and Radopholus spp. were those genera with respect to absolute frequency and absolute density. The percentage of occurrence, frequency of distribution and population abundance of different types of nematodes observed in soil and root samples were given in the Tables 4& 5.

# Table 4. Percentage of occurrence, frequency ofdistribution and population abundance of differentnematodes in rhizosphere soil of banana var. Nendran(AAB) in Palakkad taluk, India

Nematode genus	Occurrence (%)	Absolute frequency	Absolute density	Prominence value
Aphelenchus spp.	1.67	11.11	62.96	209.88
Criconemoides spp.	00.2	7.41	7.41	20.16
Dorylaimoides spp.	12.06	37.04	77.78	473.34
Helicotylenchus spp.	13.91	62.96	525.93	4173.18
Hoplolaimus spp.	4.8	33.33	181.48	1047.78
Meloidogyne spp.	19.88	81.48	751.85	6786.75
Pratylenchus spp.	2.84	11.11	107.41	358.02
Radopholus spp.	31.24	77.78	1181.48	10419.69
Rotylenchulus spp.	18.61	55.56	703.7	5245.1
Tylenchorynchus spp.	1.67	11.11	62.96	209.88
Tylenchus spp.	2.94	29.63	111.11	604.81
Unknown spp.	0.2	3.7	7.41	14.26

Table 5. Percentage of occurrence, frequency ofdistribution and population abundance of differentnematodes in rhizosphere soil of banana var. Nendran(AAB) in Palakkad taluk, India

Nematode genus	Occurrence (%)	Absolute frequency	Absolute density	Prominence value
Aphelenchus spp.	0.29	3.7	7.41	20.16
Dorylaimoides spp.	2.91	33.33	74.07	427.67
Helicotylenchus spp.	16.74	33.33	425.93	2495.08
Hoplolaimus spp.	5.68	51.85	144.44	1040.12
Meloidogyne spp.	15.72	66.67	400	3265.99
Pratylenchus spp.	7.42	22.22	188.89	890.43
Radopholus spp.	21.25	44.44	540.74	3604.94
Rotylenchulus spp.	24.75	18.51	629.63	2709.49
Tylenchorynchus spp.	2.91	11.11	59.26	197.53
Tylenchus spp.	2.33	22.22	74.07	349.19

The nematodes seem to be new with morphologically distinguishable feature were observed in rhizosphere soil collected from Pudussery panchayath with a share of 0.2%. It had stylet to show plant parasitic mode of nutrition. Tylenchorynchus spp. was observed only in Malampuzha panchayath for both rhizosphere soil and root samples. Criconemoides spp. found only in soil samples collected from Pudussery panchayath. The nematode genera Aphelenchus spp. occurred only at Marutha road panchayath for soil sample analysis and at Pudussery panchayath for root samples. The Pratylenchus spp. was observed only in both rhizosphere soil and root samples of Elappully panchayath and root samples collected from Akathethara panchayath. Dorylaimoides spp. and Tylenchus spp. were observed only in Marutha road panchayath for root samples. On considering the species diversity and more number of nematodes per panchayath the Kannadi panchayath had more diversity showing region in Palakkad taluk for soil samples.

### 4. Discussion

After a nationwide survey in Palakkad district, a widespread occurrence of *Radopholus similis*, *Meloidogyne incognita*, *Helicotylenchus multicinctus*, *Heterodera oryzicola* and *Pratylenchus coffeae* in banana was reported<sup>[8]</sup>. In this study, these results were also in accordance with the results except none of the surveyed banana growing areas of Palakkad taluk showed the presence of *Heterodera oryzicola*. Plant parasitic nematodes of banana from vellayani, Kerala documented<sup>[15]</sup>. It was found that seven phytonematodes were associated with the banana crop, of which *R. similis*, *H. multicinctus* and *P. coffeae* were recorded as abundant nematodes. This result was in confirmation with the findings of present study. Pratylenchus, Meloidogyne, Helicotylenchus, Tylenchorhynchus, Hoplolaimus, Rotylenchulus, Hirschmanniella, Criconemoides were observed in West Bengal in banana<sup>[7]</sup>. In all soil samples analysed, M. incognita was found to occur at the highest frequency in banana fields of Malaysia<sup>[14]</sup>. But the present study contradicted that result. While many of results for nematodes associated with banana showed the widespread presence of *R. similis* and the present study report also was satisfying with that mainstream view <sup>[5,17]</sup>. The number of Radopholus spp. found high in both soil and root samples. But it was very much higher in soil samples than root samples. In India, the first occurrence of Radopholus spp. ie, R. similis was reported on banana from Palakkad District <sup>[11]</sup>. Subsequently this nematode was reported from banana in South India<sup>[9]</sup>. Both Pratylenchus spp. and Radopholus spp. were co-exist with Meloidogyne spp. in both types of samples in the present study. Almost all nematodes showed in Palakkad taluk were reported from the Tanjavur district of Tamilnadu, India also<sup>[18]</sup>.

### 5. Conclusion

With respect to the soil and root samples, Kannadi panchayath found as more diverse region in Palakkad taluk. The genera which has been seriously affecting on banana plants ie, Radopholus spp. was observed as the most abundant one and most frequency of distribution was showed by Meloidogyne spp. in this studied area. The plant parasitic nematode such as Helicotylenchus spp., Hoplolaimus spp., Meloidogyne spp., Rotylenchulus spp. and Radopholus spp. showed prominent occurrence in the present study. These results demonstrate the importance of these five nematode genera in banana production as an inverse relationship occurred between the nematodes and growth of banana. The presence five identified population of nematodes in higher density were seems to be hazardous for the better growth of banana. The lost caused by this pathogen should be addressed. So the care should be taken in an economical and eco-friendly manner.

### Acknowledgments

We are grateful to the authorities of KAHM Unity Women's College, Manjeri for their sincere support throughout the study and we also appreciate the assistance of the farmers in the region. We also thankful to Dr. Giribabu P., National Research Centre for Banana, Tiruchirapally, Tamilnadu, India and Dr. Swarnakuamari N., Tamilnadu Agriculture University, Coimbatore, India for their immense help for the identification of nematodes.

### **Importance of the Study**

The productivity rate of an important crop banana expressed in agricultural statistics reports in recent years was not a satisfying one with respect to the fourth position in area of cultivation in the Indian state Kerala. To analyse the reasons behind it, one of the important pathogen such as plant parasitic nematodes were taken under consideration. The severity of its attack can be revealed only by an intensive survey. The reviews on the plant disease surveys for Kerala showed that there was no much attention given to the effects of plant parasitic nematodes. There are only less than two works were reported in this agriculturally important state. The study are included in Palakkad district of the state Kerala which ranks first in an important food crop Banana production and cultivation. Consequently species abundance, distribution and pathogenicity in Kerala were unidentified. Knowledge on diversity distribution in specific areas is of vital importance for designing its control measures to make more practical management. This study also important to assess the pathogenic potential of the study area and became an important criterion for identification of hot spots of nematode attack along with identification of plant nematodes of a region. Another importance comes under he point that the life cycle of this pathogen have an effect by the study area. The sampled region of the present study is valuable benchmark area where passes the Palghat gap in Nilgiri Biosphere Reserve in Western Ghats. Diversity studies on fauna suggested influence of this gap for diversification of various taxa.

### **Conflict Of Interest**

This research work was carried out without any research grants or horarium. I do not have any potential conflict of interest (financial or other).

### **Informed Consent**

I hereby confirmed that written consent was obtained from all participants prior to the study.

### **Ethical Approval**

It is not applicable for this study. I hereby assure that no any animal used or like research work carried out in this study to take ethical approval from the Ethical committee.

### **Trial Registration**

Eg. Name of Trial Registry: Trial Registration Number

### Contributorship

The research work jointly conceived and designed by all three authors (Ashfak Ahammed O., Usman A. and Rasmi A.R). The work carried out and manuscript preparation was done by the first author (Ashfak Ahammed O.). The data analysis work was done by the second author (Usman A.) and preliminary and final proof reading of the manuscript was done by the third author (Rasmi A.R). In each step of the research, strict supervision was handled by both second and third authors.

### Declaration

I hereby state that the manuscript manuscript entitled A Primary study at Agricultural areas of Palakkad taluk of India for checking the occurrence of a negative growth factor Plant parasitic Nematodes with the crop Banana *var*: "Nendran" has not been submitted to any other journal for consideration and has not been published or presented previously (partly or in full). I also states that no data has been fabricated including images to support conclusions. Proper acknowledgements of works are given.

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