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Population Distribution of Plant-parasitic Nematodes of Bananas in Peninsular Malaysia

(Taburan Populasi Nematod Parasit Tanaman Pisang di Semenanjung Malaysia)

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ABSTRACT

A nematode population distribution survey was conducted in banana plantations/farms in Peninsular Malaysia from June 2004 to January 2006. This study highlights differences obtained from the survey compared with previously published reports in terms of species prevalence in banana plantations. As opposed to the widely reported prevalence of Radopholus similis *(Cobb 1893) Thorne, 1949, on banana plants worldwide,* Rotylenchulus reniformis *Linford and Oliveira, 1940, was found to be the most common nematode species in the isolated soil samples (Prominence Value = 824.28;* n=*63) while* Meloidogyne incognita *(Kofoid & White 1919) Chitwood, 1949, was predominant in the isolated root samples (Prominence Value = 449.77;* n=*57) in Peninsular Malaysia. Besides, contradicting previous reports,* M. incognita *was found to dominate Cavendish plantation areas in this region instead of* R. similis. *Inter-species interaction resulting in species predominance and co-dominance in banana rhizosphere was also observed in this study.*

Keywords: Banana; Meloidogyne incognita; *nematode; prominence value;* Rotylenchulus reniformis

ABSTRAK

Suatu tinjauan taburan populasi nematod telah dijalankan di ladang-ladang pisang di Semenanjung Malaysia dari bulan Jun 2004 hingga ke Januari 2006. Kajian ini menunjukkan dapatan yang berbeza daripada segi corak taburan spesies di ladang pisang, berbanding tinjauan yang pernah dilaporkan. Di Semenanjung Malaysia, spesies nematod yang paling kerap ditemui dalam sampel tanah ialah Rotylenchulus reniformis *Linford dan Oliveira 1940, (Nilai keutamaan = 824.28;* n=*63), manakala* Meloidogyne incognita *(Kofoid & White 1919) Chitwood, 1949, paling kerap ditemui dalam sampel akar (Nilai keutamaan = 449.77;* n=*57). Penemuan ini bercanggah dengan keputusan yang sering dilaporkan dengan* Radopholus similis *(Cobb 1893) Thorne, 1949 adalah spesies utama di kawasan tanaman pisang di seluruh dunia. Selain itu,* M. incognita *juga didapati mendominasi kawasan-kawasan tanaman Cavendish berbanding* Radopholus similis *dan kenyataan ini juga bercanggah dengan yang terdahulu. Kajian ini juga mendapati bahawa terdapat interaksi antara spesies hasil daripada kewujudan spesies dominan dan ko-dominan dalam rizosfera pisang.*

Kata kunci: Meloidogyne incognita; *nematod; nilai keutamaan; pisang;* Rotylenchulus reniformis

Introduction

Bananas (*Musa* AAA) and Plantains (*Musa* AAB) (henceforth referred to as banana) are important components of human diet in almost every country in the world (Marin et al. 1998). Both crops (*Musa* spp.) are grown in tropical regions worldwide, especially in lowland areas where rainfall is in excess of 1250 mm per year (Gowen et al. 2005; Sharma 1997). This perennial plant plays a key role in the economy of many developing countries with a total international trade value ranging between US$4.5 and 5 billion per year (Arias et al. 2003), placing it as the world's fourth most important food crop after rice, wheat and maize.

Worldwide, nematode infestation on bananas causes 20% annual yield loss (De Waele & Elsen 2007; Marin et al. 1998; Sasser & Freckman 1987; Speijer & De Waele 1997) in which *Radopholus similis* (Cobb 1893) Thorne, 1949 (the burrowing nematode) was identified as the most serious nematode pest (Gowen & Quénéhervé 1990; Huang et al. 2006; Sarah et al. 1996). This worldwide spread was believed to be due to the dissemination of infected planting materials that was principally connected to the establishment of plantations for the export trade over the last century. Other plant-parasitic nematodes (PPN) threatening banana crop production includes *Meloidogyne incognita* (Kofoid & White 1919) Chitwood, 1949, *Meloidogyne javanica* (Treub 1885) Chitwood, 1949, *Pratylenchus coffeae* (Zimmermann 1898) Goodey, 1951, *Pratylenchus goodeyi* Sher and Allen 1953, *Helicotylenchus multicinctus* (Cobb 1893) Golden, 1956 and *Rotylenchulus reniformis* Linford and Oliveira, 1940 (Bridge 1993; Gowen & Quénéhervé 1990; Gowen et al. 2005; Sarah 1989).

As the centre of origin and diversity of this giant herb (Heslop-Harrison & Schwarzacher 2007), banana production in Malaysia is an important economic revenue to the country and was ranked as the fifth major fruit export after melon, papaya, durian and star fruit.

Banana contributed to a total value of US$5.5 million in foreign exchange in 1997 and was forecast to increase by 33.3% in 2010 (Rohizad 1999). However, the 25% decreases of banana acreage to about 30000 ha in 1996 (Jamaluddin 1999) indicated a glitch in the production of the crop. The outbreak of diseases such as *Fusarium* wilt and sigatoka leaf spot were among the identified causal factors (Jamaluddin 1999). Often, such disease outbreaks in bananas were associated with the presence of PPN in the cultivation areas, as nematode infection in turn predisposes plants to fungal pathogen infections (Goswami et al. 1970; McKeen & Mountain 1960; Ross 1965). Such infection results in a dire plant damage. However, since these perennial plants possess thick root epidermis, the effect of nematode infestation in banana plants were often asymptomatic, hence overlooked. The problem will only be realised too late, that the farmer will have no other choice but to opt for gross removal of the plant.

Reports on nematode infestation in Malaysia demonstrate that PPN exist in a polyspecific manner in banana plantations in this region (Sidam & Bilal Mat 1983; Winoto & Sauer 1982). Ideally nematode management programme is species-specific and such identification of nematode species present in any given plantation is important. Therefore, it is crucial to obtain an up to date status on nematode population dynamics and their infestation levels for an effective banana nematode management in any given banana field/plantation area to be designed. In this paper, we report a survey of nematode distribution on *Musa* plantations/farms in Peninsular Malaysia. To the authors' best knowledge, this is the first accessible report on PPN infesting bananas to be published for Malaysia. An observation was also made on whether or not nematode species occurrences have significantly changed since the last reported survey (Sidam & Bilal Mat 1983).

Materials and Methods

Sampling Sites

The distribution pattern of nematode species in Peninsular Malaysia was assessed from data collected from sampling activities conducted on household gardens, individual and commercial farms in June 2004 - January 2006. Nematode isolates were obtained from untreated soil of banana plantations/farms in fourteen localities of six different states in Peninsular Malaysia (Table 1). Four main geographical regions in Peninsular Malaysia were selected, namely Johor (South denoted as S), Selangor and Perak (West-coast denoted as W), Pulau Pinang (North denoted as N), while Pahang and Terengganu represent the East-coast (denoted as E) of Peninsular Malaysia. The sampling regions were denoted as S1, W1, W2, N1, E1 and E2, respectively (Figure 1). For the ease of analysis the results obtained from geographical regions E1 and E2 were combined, representing the distribution for the East-coast region, thus, making five sampling areas.

Soil and Root Sampling

Soil surrounding banana mat was excavated to a depth of 20-30 cm and placed into plastic bags. Three to twelve samples were collected from each farm; depending on the size of the farm. If more than one banana variety were planted in a farm, three to four soil samples of the same banana variety were taken from different mats. These samples were individually analysed, forming replicates to each variety/farm. Roots were sampled in the same manner.

Nematode Extraction from Soil

Nematode extraction was carried out using the Oostenbrink Floatation Method (Oostenbrink 1960). Two hundred milliliter soil sub-sample were immersed with water, topped-up to 1 L and subjected to extraction. The extracted nematode suspension was further sieved using the modified Baermann dish method with 10-mesh sieve (2000 µM; diam=20 cm) and left to stand overnight (Speijer & De Waele 1997). Next, nematode suspension was collected and the volume was adjusted to 100 mL.

Nematode Extraction from Root

Nematodes inhabiting banana roots were extracted using the maceration-sieving method as described by Speijer and De Waele (1997). The root samples were separated from the soil, washed, cut into 2 cm pieces and air-dried. A sub-sample of 5 g (fresh weight) roots were macerated twice (with ten-second intervals) using a kitchen blender and poured into the modified Baermann dish. Subsequent steps were as described earlier.

Species Identification

Nematodes were identified to the generic and species levels using key morphological characteristics described in Orton-Williams and Siddiqi (1973), Siddiqi (1973, 1986) and Eisenback et al. (1981). Nematode density of each sampling site was determined under an Olympus stereo microscope with 80× magnification level. A 100 mL nematode suspension was first homogenised and 1 mL of the suspension was pipetted out into a counting dish. To avoid reading error, triplicate of 1 mL suspension were taken and the mean of the three readings was used as the final nematode number, expressed as the population density. PCR-based identification technique was also used for species confirmation. For this purpose, Internal Transcribed Spacer (ITS) 1- *5.8S* - ITS2 region of nematode ribosomal DNA (rDNA) was isolated from five most prevalent species found in banana rhizosphere. Detailed isolation procedure is described elsewhere (manuscript in preparation).

Data Analyses

Analyses were conducted on both soil and root samples. The population density of each nematode genus or species per 200 mL soil or 5 g root sample was estimated by

TABLE 1. Localities visited for sampling activities with *n* = the number of samples taken

States	Sampling sites	Coordinates	Soil types	Banana varieties (genotype)	*n*
Pulau Pinang (North, N1)	Ara Kuda	N 5° 20' 53.4" E100° 12' 15.6"	Clay	Cavendish (AAA)	12
Perak (West Coast, W2)	Batu 6, Tapah	N 04° 21' 04.4" E 101° 20' 24.5"	Sandy Loam	Nipah (ABB)	3
	Batu 7, Tapah	N 04° 14' 43.4" E 101° 18' 50.4"	Sandy Loam	Nipah (ABB)	4
	Batu 17, Tapah	N 04° 21' 04.6" E 101° 20' 24.8"	Sandy Loam	Nipah (ABB)	4
Selangor (West Coast,W1)	Pasir Penambang	N 3° 40' 31.2" E 101° 36' 8.4"	Sandy Loam	Tanduk (AAB)	4
	Batu 28	N 3° 35' 0" E 101° 25' 0"	Sandy Loam	Abu (ABB)	3
	Sg. Terap	N 3° 35' 0" E 101° 25' 0"	Sandy Loam	Berangan (AAA)	3
Pahang (East Coast, E1)	Kg. Sg. Sumsum	N 04° 14' 49.1" E 101° 18' 32.5"	Sandy Loam Clay	Mas (AA) Raja (AAB)	3
	Kg. Janda Baik	N 04° 14' 49.1" E 101° 18' 32.5"	Clay Loam	Berangan (AAA) Mas (AA)	3
	Kg. Cherating Lama	N 03° 18' 41.8" E 101° 52' 27.4"	Sandy Loam	Awak (ABB)	3
Terengganu (East Coast, E2)	Kg. Labohan, Kemaman	N 04° 32' 28.7" E 103° 07' 32.5"	Sandy Sandy Loam	Awak (ABB)	3
	Kg. Batu 7, Paka	N 04° 42' 45.5" E 103° 24' 16.0"	Sandy Clay	Kapas (AAA) Nangka (AAA)	3
	Kg. Gelulur, Marang	N 05° 09' 12.8" E 103° 14' 19.4"	Clay Sandy Loam	Kelat Siam (AAB) Nangka (AAA)	3
Johor (South, S1)	Parit Sulong	N 1° 58' 37.8" E 102° 52' 51"	Clay	Kapas (AAA) Lemak Manis (AA) Mas (AA) Nipah (ABB) Tanduk (AAB)	6

multiplying the mean number of nematodes obtained for 1 mL suspension with 100 (as nematodes were collected in 100 mL suspension after extraction). The maximum number of nematodes counted for each sample was also taken. Nematode prevalence was calculated as a percentage ((Number of samples containing a species / number of samples collected) * 100). The absolute frequency of nematode occurrence were counted as ((the number of samples in which each species was detected / the total number of samples)* 100) while the Prominence value (PV) was calculated as (population density X (√(frequency of occurrence / 10)) as described by De Waele et al. (1998).

RESULTS

Ten nematode genera namely *Helicotylenchus, Hoplolaimus, Macroposthonia, Meloidogyne*, *Radopholus*, *Rotylenchulus*, *Paratylenchus*, *Pratylenchus*, *Tylenchorynchus* and *Tylenchus* were isolated from both soil and roots of banana plants from five sampling areas in Peninsular Malaysia. Nematodes were identified based on morphology and species occurrences were recorded. The species identities of the nematodes were corroborated by PCR-based identification assays. Cloned DNA sequence variants of the isolated region for the five most frequently found species namely *Helicotylenchus dihystera*, *Helicotylenchus multicintus*, *Meloidogyne incognita*, *Radopholus similis* and *Rotylenchulus reniformis* were deposited into the GenBank (Table 2).

Of the ten genera obtained, the reniform nematodes (*Rotylenchulus reniformis*) were found to be predominant in the soil samples as they accounted for up to 31.23% of the overall nematode population with the PV of 824.28. This was followed by *Meloidogyne incognita* (21.22%), *Helicotylenchus dihystera* (Cobb 1893) Sher, 1961 (15.76%), *Helicotylenchus multicinctus* (12.47%),

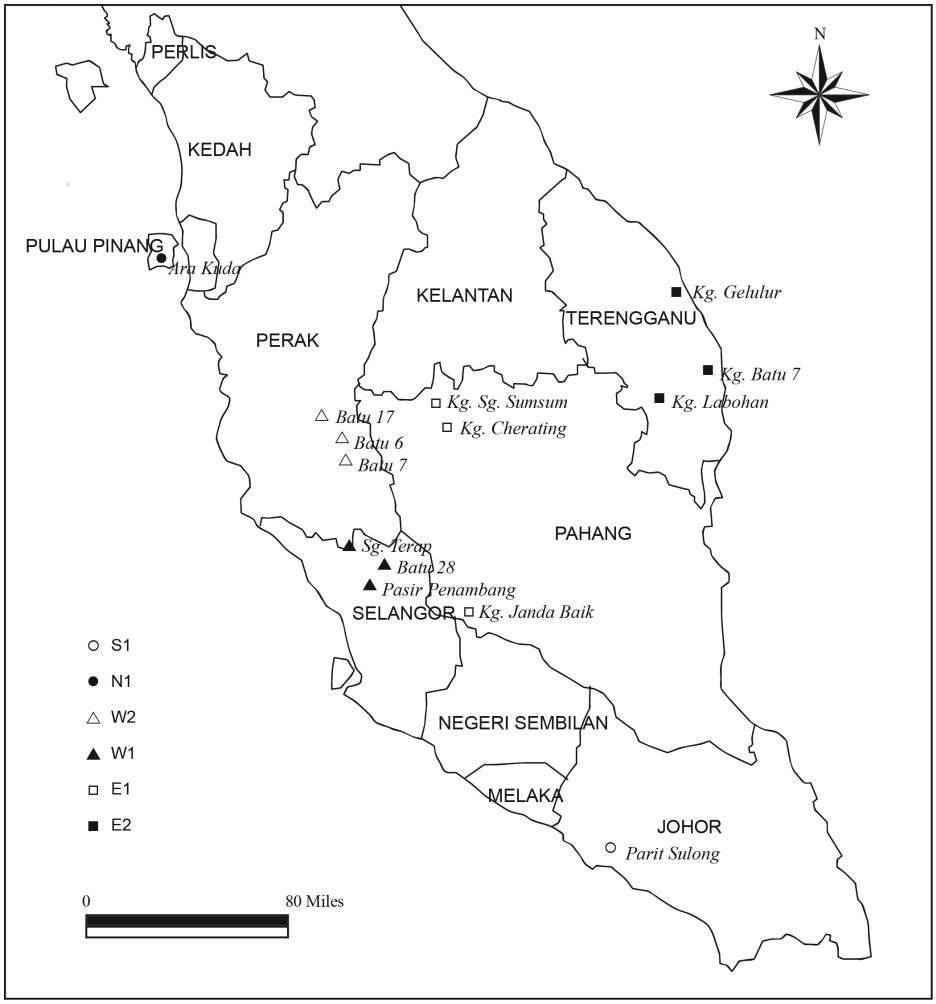

FIGURE 1. Nematode sampling sites representing four main geographical regions in Peninsular Malaysia. Six states were visited namely Johor, Selangor, Perak, Pahang, Pulau Pinang and Terengganu denoted as S1, W1, W2, N1, E1 and E2, respectively

Pratylenchus coffeae (10.19%) and *Radopholus similis* (2.28%) with the PV of 598.6, 385.21, 186.58, 20.44 and 27.88, respectively (Table 2). In all soil samples analysed, *M. incognita* was found to occur at the highest frequency, 50.79% (262.43 nematodes/200 mL soil) compared to *Rotylenchulus reniformis* that occurred 1.14 times less frequent. The least occurring nematode in the soil was *Tylenchus* spp. with the frequency of 1.59% (0.53 nematodes/200 mL soil) (Table 2).

Different occurrence dynamics were obtained for the root samples (Figure 2; Table 2) of which the root-knot nematodes were found predominant, accounting up to 42.99% of the total number of nematodes isolated with the PV of 449.77. This was followed by *H. multicinctus* (18.93%), *H. dihystera* (10.98%), *P. coffeae* (10.75%) and *R. similis* (9.81%) with the PVs of 111.79, 89.05, 67.79 and 50.59, respectively. *M. incognita* occurred at the highest frequency with the mean population density of 215.2/5 g roots while *Tylenchorynchus* spp. was the least, with the mean population density of 4.68/5 g roots. In addition, *R. reniformis* was observed to be 45.36 times less prominent in the roots compared to its population density in the soil samples. Note that *Hoplolaimus* spp., *Macroposthonia* spp. and *Tylenchus* spp. were not detected in the root samples (Table 3).

While a predominant species can often be observed in root samples, some soil samples showed the occurrence of species co-dominance. Soil samples isolated from Selangor, East-Coast of Peninsular Malaysia and Kedah, showed single species dominance (*R. reniformis*, *H. dihystera* and *M. incognita*, respectively). However, *M. incognita* were found co-dominantly present with *H. dihystera* in soil samples isolated from Johor, while *R. reniformis* were co-dominantly occurring with *H. multicinctus* in soil samples isolated from Perak. Single species predominance was observed in all isolated root samples, in which *M. incognita* was found predominant in samples obtained from Johor, Selangor and Kedah while *H. dihystera* and *H. multicinctus* were predominant in samples collected from East-Coast of Peninsular Malaysia and Perak, respectively (Table 3).

TABLE 2. Overall frequency of occurrence, population density, prominence value and predominance of nematode genera and species collected from the soil (200 mL, *n*=63) and root (5 g, *n*=57) samples of banana plants from localities in Peninsular Malaysia. The final column lists cloned sequence variants isolated from the five most prevalent banana nematode species deposited into the GenBank. Dashes (-) in the table signify no data obtained

Species	Sample type	Frequency of occurrence (%)	Population mean	Population density	Prominence value (PV)	Predominance (%)	Accession numbers of sequences deposited to GenBank
Helicotylenchus dihystera	Soil	38.1	197.35	3800	385.21	15.76	FJ427209
	Root	26.32	54.97	1000	89.05	10.98	FJ440620
Helicotylenchus multicinctus	Soil	14.29	156.08	2700	186.58	12.47	FJ460169 FJ460170
	Root	14.04	94.74	2200	111.79	18.93	FJ460171 FJ460172 FJ460173
Hoplolaimus spp.	Soil	7.94	3.7	200	3.3	0.3	-
	Root	-	-	-	-	-	
Macroposthonia spp.	Soil	9.52	5.82	300	5.68	0.46	-
	Root	-	-	-	-	-	
Meloidogyne incognita	Soil	50.79	262.43	3100	598.6	21.22	FJ534515
	Root	43.86	215.2	4600	449.77	42.99	FJ534516
Paratylenchus spp.	Soil	4.76	29.63	1500	20.44	2.37	-
	Root	5.26	11.7	200	8.54	2.34	
Pratylenchus coffeae	Soil	30.16	127.51	1900	221.44	10.19	-
	Root	15.79	53.8	1800	67.79	10.75	
Radopholus similis	Soil	9.52	28.57	1100	27.88	2.28	FJ455828
	Root	10.53	49.12	2200	50.59	9.81	FJ455829 FJ455830 FJ455831 FJ455832 FJ455834 FJ455833
Rotylenchulus reniformis	Soil	44.44	391.01	4200	824.28	31.23	FJ374686
	Root	12.28	16.37	700	18.17	3.27	
Tylenchorynchus spp.	Soil	11.11	46.03	2500	48.52	3.68	-
	Root	1.75	4.68	300	1.97	0.93	-
Tylenchus spp.	Soil	1.59	0.53	100	0.21	0.04	-
	Root	-	-	-	-	-	-

DISCUSSION

The prevalence of the most economically important banana nematode, *Radopholus similis* was documented in this study. Our results contradicted the mainstream viewpoint (Araya et al. 2002; Speijer & De Waele 1997) when we found *R. similis* populations to be rather localised instead of widespread as they were expected to be in banana plantations. *R. similis* was found in only two localities namely Johor and the East Coast in a relatively low PV in both soil and root samples (Table 3). In contrast, the most common and widespread nematode species in Peninsular Malaysia is *M. incognita*, which is incidentally the lesser reported banana nematode species. As a matter of fact, we noted that *M. incognita* was 2.93 times more prominent than *R. similis* in the root samples obtained from Johor. This observation certainly is interesting, as to the authors' best knowledge *R. similis* was frequently reported as the dominant banana nematode species when they co-exist with *M. incognita*. For instance, Santor and Davide (1992) has reported that the presence of *R. similis* negatively influences the development and reproduction of *M. incognita*. This was later echoed by Araya et al. (2002), who associated the low *M. incognita* population density obtained in their study with the feeding behaviour of *R. similis*. In addition, the same authors and Sarah et al. (1996) had claimed that *R. similis* was the main nematode problem in commercial Cavendish-type banana plantations. Our result, however, pointed to the contrary when we determined that *M. incognita* dominated the commercial Cavendish-type banana plantation situated in Ara Kuda,

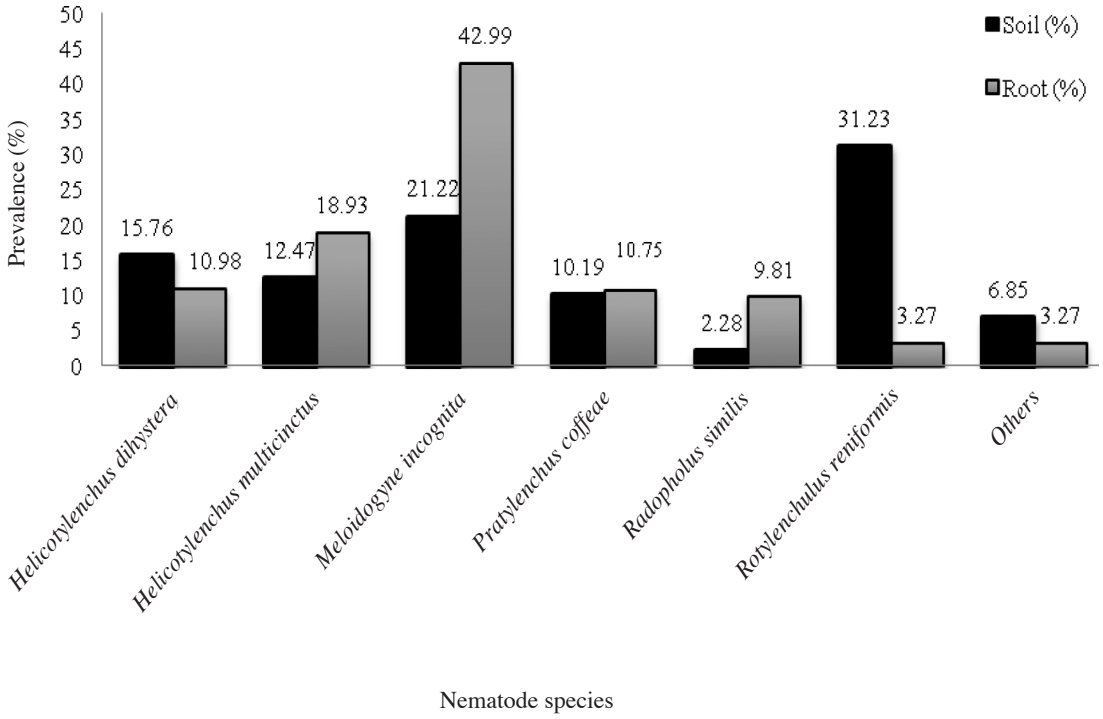

FIGURE 2. Prevalence (%) of nematode species in the soil and roots sampled from the five geographical regions of Peninsular Malaysia

Pulau Pinang. Our findings suggested that pattern of nematode infestation in bananas is localised in nature, as such the occurrence and predominance of a particular species in one country does not necessarily reflect the same in its neighbours. For example, a review by De Waele and Elsen (2007) described a nematological survey of bananas conducted on the north, central and south parts of Vietnam which found that *R. similis* was not a problem in that particular country. In addition, a similar survey on 17 sites in Central Uganda had identified *P. goodeyi*, *R. similis* and *H. multicinctus* as the predominant nematode species. Therefore, the discovery of Pisang Jari Buaya as *R. similis* resistant cultivar is only relevant to plantations facing *R. similis* infestation problem and this solution is impractical to be applied elsewhere. This underlines the needs for regular surveillance as the management strategy very much depends on tackling the nematode at hand.

It is tempting to speculate that there exist an interaction amongst species in a given banana rizosphere which resulted in species co-dominance or predominance. Although species co-dominance was observed, the majority of our samples showed single species predominance. From our data, we observed that the presence of *M. incognita* in large proportions has affected the presence of other nematode species in banana rhizosphere (Cavendish type) in Pulau Pinang. *Helicotylenchus* spp. and *M. incognita* almost always occur in inverse propotions as observed in samples isolated from Pulau Pinang and Perak. This could be the outcome of a negative interaction taking place between these two species, in that the presence of either species suppresses the presence of another.

It is noteworthy that single species predominance was found in all root samples collected in this study. This observation agrees with reports published by Wallace (1973) and Johnson and Nusbaum (1970) that coincident infection with two or more nematode species will subsequently result in at least one species being suppressed. This observation is preliminary however and interspecific interaction phenomenon warrants further investigation to confirm its effect on nematode community structure.

Our study on PPN distribution in Peninsular Malaysia is in partial agreement with the last reported survey by Sidam and Bilal Mat (1983). In one hand, we can corroborate that *R. reniformis* was the most frequent species found in the soil samples. On the other hand, the present data showed that *M. incognita* was predominant in the root samples compared to *Helicotylenchus* spp. The increased number of *M. incognita* found in the root samples compared to the soil samples was expected as the natural behaviour of these sedentary endoparasites upon reaching the infective J2 stage is to stay immobile at the feeding sites (Williamson & Kumar 2006).

In this study we were able to show the presence of two nematode genera i.e. *Tylenchus* spp. and *Tylenchorynchus* spp., that had not been previously recorded in banana plantation in this country. However, we have failed to detect the occurrences of other PPN species such as *Pratylenchus brachyurus, Hemicriconemoides cocophillus* (Loos 1949), *Meloidogyne javanica* and *Xiphinema* spp. that were reportedly found in banana rhizosphere 29 years ago (Sidam & Bilal Mat 1983). Nevertheless, other PPN species such as *Helicotylenchus* spp., *M. incognita*, *Pratylenchus*

TABLE 3. Prominence values of nematode genera and species collected from the soil and root of banana plants from five sampling areas in Peninsular Malaysia

		Prominence value										
		Helicotylenchus dihystera	*Helicotylenchus multicinctus*	*Hoplolaimus* spp.	*Macroposthonia* spp.	*Meloidogyne incognita*	*Paratylenchus* spp.	*Pratylenchus coffeae*	*Radopholus similis*	*Rotylenchulus reniformis*	*Tylenchorynchus* spp.	*Tylenchus* spp.
Soil (200 mL)	Johor	1703.0	0	0	0	1720.06	161.8	8.51	417.23	562	613.09	0
	Perak	0	2458.34	13.33	26.66	16.67	0	244.99	0	2455.99	0	1.67
	Selangor	46.67	0	0	0	49.83	101.13	123.33	0	1005.17	46	0
	E.Malaysia	644.45	0	3.39	2.26	429.38	0	566.58	6.95	515.77	0	0
	Pulau Pinang	23.31	0	0	1.31	1192.51	0	20.31	0	110.56	6.77	0
Root (5 g)	Johor	242.7	0	0	0	1288.06	0	285.25	439.17	70.77	0	0
	Perak	0	466.67	0	0	0.14	0	0.82	0	0	0	0
	Selangor	80.14	0	0	0	96.74	13.33	23.33	0	36.67	0	0
	E.Malaysia	128.9	0	0	0	0.95	0	74.07	21.74	0	0	0
	Pulau Pinang	0	0	0	0	79.94	0	0	0	0.27	0	0

coffeae, R. similis, R. reniformis, Macroposthonia spp. and *Hoplolaimus* spp. still persist in banana farms/ plantations in Peninsular Malaysia. Our study was conducted almost three decades after the last published report and perhaps variation observed in the pattern of nematode distribution are expected, due to natural changes in the topological and geographical state of a locality over that period of time (Wallace 1973).

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