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Disease prevalence and severity assessment of *Pratylenchus coffeae* on an infected banana in Peninsular Malaysia

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Abstract

Pratylenchus spp. cause considerable damage to the banana plant (*Musa* spp.) globally and although reported extensively in Malaysia, disease prevalence and infection severities have not been defined. The objective of this research, therefore, was to determine the prevalence of *Pratylenchus coffeae* and the extent of their damage on *Musa* spp. Of the 13 banana fields sampled, *Pratylenchus* nematodes were found in 76% of them. Proportions of root cortexes occupied by reddish brown lesion were significant in all the states. All the sampled areas exceeded the threshold limit, which is a 1% increment above 5% and is considered highly significant damage. The pathological reaction of *P. coffeae* against Pisang Berangan multiplications was observed after 12 weeks of growth in a glasshouse experiment. There were significant differences in vegetative growth within the various pathogen inoculation levels evaluated. Pisang Berangan showed a high level of susceptibility through the activity of polyphenol oxidase and peroxidase-induced resistance at all days after inoculation with *P. coffeae* compared to the control, except at week 12 where it declined or was non-significant from the control. Educating banana growers on the prevalence of this pathogenic parasite is therefore imperative for management decisions.

Keywords – disease evaluation – *Musa* spp. – nematodes – root lesion

Introduction

Banana (*Musa* spp.) originates from Malaysia (Heslop-Harrison & Schwarzacher 2007) and belongs to the family Musaceae. It is one of the world's most prominent fruits and is also among the major staple foods worldwide. About 107 million metric tons of bananas were produced in 2011, across more than 130 countries, covering an area of about 0.1% of the agricultural area utilized in the world (Agritrade 2013). Banana production is an important revenue source for Malaysian farmers and contributes approximately US$24 million annually. It is the second most commonly grown fruit crop after durian, with a cultivated area of about 29,000 ha and a production of 530,000 metric tons (Tengku et al. 2011). Bananas are produced in almost all the states in Malaysia. However, Johor, Sabah and Sarawak are the major producers with orchards covering 27,543 hectares in 2009.

Several factors have threatened banana production in Malaysia and among the major pest and

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diseases are *Fusarium* wilt and bacterial wilt (Jamaluddin 1999, Tengku et al. 2011). Outbreaks of these diseases are often associated with plant parasitic nematodes, as nematode infection predisposes plants to diseases (McKeen & Mountain 1960). These nematode infections often lead to plant damage. Lesion causing nematodes (*Pratylenchus* spp.) and burrowing nematodes (*Radopholus similis* Cobb) are the most prevalent and damaging species to banana plants (Gowen et al. 2005). In Malaysia about 28 species of both migratory and endoparasitic nematodes are found infecting banana (Hassan 2004, Sidam & Bilal Mat 1983, Rahman et al. 2014), of which *Pratylenchus* spp. are significantly reported. *Pratylenchus* spp. are often found in banana fields together with other species like *Radopholus similis*, and the root-knot nematodes; the former is believed to provide a feeding site for penetration by the latter. In *Pratylenchus* spp. the two main components of pathogenicity are virulence and reproductive fitness (Shaner et al. 1992), of which the understanding and assessment of disease reactions of plants to pathogens are based. *Pratylenchus* damage on crops is often assessed on the basis of soil densities at the time of planting and in the roots throughout the growing season. Thus, for economic decisions for nematode control, damage threshold levels are effectively employed (Ferris 1981). However, the possession of thick root epidermis by the perennial banana plant makes the effect of nematode infestation asymptomatic, and easily overlooked by growers. The damage, which is often noticed late, forces banana growers to resort to destroying the plant. However, neither the severity nor damage status of root-lesion nematodes has been recorded. Information regarding the damaging level of *Pratylenchus* spp. on banana plants in Malaysia is required. The objective of this research therefore, is aimed at determining the disease prevalence of *Pratylenchus coffeae* and the status of their damage on *Musa* spp. in Peninsular Malaysia to assist management decisions.

Materials & Methods

Sampling of nematodes

Thirteen banana fields from seven districts in four states i.e. Selangor, Perak, Pahang, and Johor in Peninsular Malaysia were sampled (Fig. 1). Sampling was based on information obtained from the Malaysia Agriculture and Rural Development Institute (Tengku et al. 2011). Roots and soil samples were taken from five banana cultivars namely Pisang Berangan, Pisang Nangka, Pisang Abu, Pisang Tanduk Lang, and Pisang Lemak Manis, between August 2014 and March 2015, following procedural guidelines (Speijer & De Waele 1997). A hole measuring 20 cm^3 was dug adjacent to the corm of each banana plant. For each field and or banana cultivar, 20 samples were taken in a "W" pattern. Field symptoms of nematodes infestation were also recorded. The samples were then placed in a cooler containing dry ice and transported to the Nematology Laboratory of the Plant Protection Department, Faculty of Agriculture, Universiti Putra Malaysia for processing.

Isolation of nematodes

The Whitehead tray method (Whitehead & Hemming 1965) was used for recovering nematodes from soil and roots. From each bulk sample, about 200 cc of soil sub-samples were wrapped in two layers of facial tissue and placed in a tray on top of a mesh. Approximately 200 ml of water was added to the tray, until the mesh was just covered with water. The soil was allowed to sit in the water for 24 hours to allow the nematodes to move out of the soil. To count the nematode number, triplicates of 1 ml from a 100 ml homogenized suspension was placed in a Huxley nematode counting slide and observed using a compound light microscope (10× magnification, Nikon Eclipse 50i microscope, Kent, WA) (Speijer & de Waele 1997). An average of three counts was expressed as the mean population of nematodes per 200 cc or 100 ml.

Morphological and molecular identification of nematodes

To identify the nematode population from the isolated samples, nematodes were individually picked and mounted on slides and observed using a compound light microscope. Characteristics

such as the overall body length, body width, stylet length, percent (%) of shaft to stylet, lip height and width, length and width of median bob, diameter of body at vulva, speckle and gubernaculum length and width of tail were measured with the aid of Dino capture camera, according to synoptic key of Orton-Williams & Siddiqi (1973).

Three *Pratylenchus* specimens from banana were included for amplification and sequencing of small subunit (18S) rDNA. Extraction of DNA was done according to Williams et al. (1994) using Worm lysis buffer. An individual nematode was picked, washed in 0.1 mol NaCIO solution then rinsed in distilled water and put in 0.5 ml PCR tube containing 15 µl lysis buffers. The samples were placed on -80ºC for 10 minutes. Samples were warmed at room temperature, and thereafter mineral oil added. It was then incubated for 1 hour at 60ºC after which it was heated at 95ºC for 15 minutes, cooled at 4ºC and 2.5 µl used as template for PCR amplification. TW81 forward and AB28 reverse primers (Joyce et al. 1994) were used to amplify rDNA- ITS region at 1.5 µl in 25 µl volume. The amplified DNA was run in agarose gel electrophoresis and visualized using gel doc XR system (Bio-Rad, UK). Purified PCR product was sent for sequencing by First Based Sdn. Bhd. Malaysia. The ITS sequences from respective samples were aligned using BioEdit softwere version 7. The partial length nucleotides sequences of the *Pratylenchus* isolates were searched for sequences similarities to other sequences which are available in the NCBI database by using BLAST tool.

Fig. 1 – Areas surveyed for root-lesion nematode *Pratylenchus coffeae* in Peninsular Malaysia.

Root damage assessment

For root damage assessment, about 100 roots measuring 10 cm in length were sliced lengthwise for scoring diseases caused by lesion nematodes (Speijer & De Waele 1997). Approximately 20 g of root pieces from each collected sample were blended to extract the nematodes. To measure disease incidence (%) and severity (%), two key factors were measured in accordance with the procedure set by the National Institute of Plant Protection (1997).

$$\text{Disease incidence (\%)} = \frac{\text{Number of necrotic roots}}{\text{Total number of roots evaluated}} \times 100$$

$$\text{Disease severity (\%)} = \frac{\sum (\text{ab})}{\text{N.K}} \times 100$$

Where,

DSI = Disease Severity Index

∑ab = Sum of the product of assessed plants with their corresponding score scale

N = Total number of assessed plants

K = Highest score scale

Root damage was grouped into five classes according to the percentage of root cortex covered by lesions, expressed as followed:

Score 0: No lesions on the root cortex

Score 1: 1–25% root cortex covered by lesions

Score 3: 26–50% root cortex covered by lesions

Score 5: 51–75% root cortex covered by lesions

Score 7: 76–100% root cortex covered by lesions

From the remaining bulk soil samples, 300 g subsamples were taken from each sample, air dried and ground to a fine powder. The electric conductivity, organic carbon, pH, moisture content, organic matter and texture of the soil were measured and assessed.

Glasshouse trials

Tissue-cultured plants of the cultivar 'Pisang Berangan' ***(***A.A, syn. Lakatan*)* was used as a source of nematode-free planting stock. Plant material was transferred to 2 kg plastic pots measuring 25 × 15 cm and filled with autoclaved soil (3:2:1 sand, pit, clay). Mobile stages (a mixture of juvenile and adult stages) of *P. coffeae* were inoculated in three holes (3 × 4 cm) with 10 ml water at densities of 0, 100, 250, 500, 1000, 2000, 3000 and 5000 nematodes/cm^3 soil. After inoculation, the holes were covered with soil. Each nematode inoculum density was repeated five times. The pots were arranged in a complete randomized design (CRD), consisting of seven treatments viz: 0, 100 nematodes inoculated (ni), 250 ni, 500 ni, 1000 ni, 2000 ni, 3000 ni and 5000 ni, for each of *P. coffeae* and a negative control. The potted plants were fertilized on a monthly basis, with Peter's 20:20:20 general purpose N: P: K plant food at a concentration of 0.25 g per liter of water. This trial was repeated twice.

Assessment of plant vigour

The effects of *P. coffeae* on plant vigour were assessed at intervals from two weeks after inoculation till the twelvth-week post inoculation. The increment in plant height, leaf area index, the circumference of the pseudostem girth, and root length and weight indicate improvement in the vigour of the plant and the positive/negative effects of the treatments on plant health. The proportional increment in plant height (cm), leaf area (cm) and circumference of the pseudostem girth and root length were measured using a measuring tape and digital caliper. Height was

measured from the base of the seedling to the top part of the leaves. For the leaf area index, the length and breadth of the selected leaves were also measured and multiplied by a factor (0.752 cm). At the end of the trial, root length was measured using a tape rule while the weight was determined by weighing the total fresh roots of each banana seedling with the aid of a digital balance

Assessment of disease severity

Cortical root necrosis of *P. coffeae* fresh root weight, shoot weight and final nematode densities (P_f) in both roots and soil were determined at 12 weeks after inoculation. The root necrosis severity index was computed following the procedure described by Speijer & De Waele (1997) as described earlier.

Determination of reproductive factor (RF)

Twelve weeks after inoculation, banana roots were carefully excised to remove the adhering soil from the root systems. Five roots with approximately equal lengths were cut into segments, measuring 10 cm from root tip and 10 cm in the middle. The scalpel used to cut the roots was flamed between cuts to avoid transfer of inoculum from one segment to another. Nematodes were recovered from 200 cc of soil subsamples and 10 g of roots using the whitehead tray method. The final nematode population (P_f) was obtained when the nematode suspensions were counted in a Huxley nematode counting slide. The averages of the triplicate counts, representing the nematodes in the pots, were obtained by multiplying the average with the suspension from the 200 ml of soil and roots, and adding up the nematode numbers obtained from both soil and roots. The reproduction factor was determined by using $RF=P_i/ P_f$ (soil and roots). Where RF= reproductive factor, P_i= initial nematode population and P_f= final nematode population.

Determination of peroxidase (PO) and polyphenol oxidase (PPO) activities

Crude extracts of the roots were prepared by taking one gram each of banana roots collected before inoculation, at week 1, 2, 3 and 4 after inoculation, and at harvest. The samples were preserved at -80°C and were later ground to a powder using liquid nitrogen, after which it was kept at -20°C till use. Approximately 1 ml pre-cold 0.05 M sodium acetate buffer (pH 5) was added to each sample in a 2 ml Eppendorf tube followed by the addition of 5 mg of polyvinyl pyrolidone (PVP). The homogenate was centrifuged at 14000 rpm for 20 min at 4˚C. The supernatant was collected into new tubes and was used for PPO and PO enzymes assay.

Peroxidase (PO) assay was carried out by adding 0.67 ml of enzyme extract to 1 ml of reaction substrate (80 ml of 0.1M sodium phosphate buffer (pH 6), 1 ml of 1mM H_2O_2 and 20 µl guaiacol) and the reaction mixture incubated at (25˚C). The changes in absorbance at 470 nm at three second intervals for 1 min were recorded with a spectrophotometer. A blank was prepared from the reaction substrate without any enzyme extracts. The enzyme activity was expressed as changes in the absorbance unit g^{-1} tissue following the formula designated by Kokkinakis & Brook (1979) as follows:

$$\text{Unit g} - 1 \text{ tissue} = \frac{\text{optical density} \times \text{dilution factor}}{\text{g of tissue used in the assay}} \times 100$$

Polyphenol oxidase (PPO) activity was determined by colour changes in intensity of pyrrol oxidation products. The reaction mixture consisted of 100 µl of the enzyme extract of each sample which was added to 1.5 ml of 0.2 M sodium acetate buffer (pH 5) at 4˚C and modified by adding 200 µl of 0.02 M pyrogallol in place of catechol and the activity expressed as changes in absorbance at 410 nm. A blank was prepared from the reaction substrate without enzymes extract.

The enzyme activity was expressed as changes in the absorbance unit g^{-1} tissue following the formula defined by Kokkinakis & Brook (1979).

Statistical analysis

The survey data were first transformed using log transformation and subjected to an analysis of variance (ANOVA) using the SAS software (SAS Institute 2008), while necrosis damage examinations, expressed as percentages of root necrosis, were converted to root necrosis index (RNI %). Data collected from the glass house trials were subjected to a one way ANOVA. The significant differences among the mean at $p<0.05$ was detected with the aid of Duncan's multiple range test (DMRT).

Results

Disease prevalence and severity

The mean population of *Pratylenchus coffeae* was higher in both roots and soil collected from Kluang, Johor (root= 16,800 and soil= 12,322) followed by Jerum Ular, Pahang (root= 10,582 and soil= 6,866) (Table 1).The lowest mean populations were recorded in Kuala Selangor, Selangor and Gunung Semangol, Perak following by Batu Pahat, Johor and Sungai, Pahang. *Pratylenchus coffeae* was abundant when the pH of the soils ranged from 5.50–7.62, with the maximum abundance observed at pH 5.50 and the lowest abundance recorded at pH 4.62. Disease incidence ranged from 25–81% in most of the areas sampled, with Titi Gantung, Perak and Serdang, Selangor having the highest incidences (81 and 80%, respectively) even with moderate mean populations.

Table 1 Incidence and mean populations of root lesion nematodes for 13 fields sampled in Peninsular Malaysia.

Areas surveyed	No. of fields	Population mean per 200 ml soil	Population mean per 20 g roots	Root lesion disease incidence (%)
Serdang, Selangor	3	600	776	80
Kuala Selangor, Selangor	3	297	62	25
Kluang, Johor	1	12,322	16,800	76
Batu Pahat, Johor	2	734	667	62
Gunung Semanggol, Perak	1	126	340	39
Titi Gantung, Perak	1	335	2001	81
Sungai Pahang, Pahang	1	333	733	32
Jeram Ular, Pahang	1	6,866	10,582	39

Above ground symptoms of nematode infection were evident in the fields with stunted growth, toppling and smaller fruits (Fig. 2). Pisang Berangan had the highest populations of *Pratylenchus coffeae* followed by Pisang Tanduk and Pisang Nangk. The other cultivars were either not suitable hosts or susceptible (Table 2). The highest disease severity percentage of cortical root necrosis (49.3%) was also recorded in Pisang Berangan, while Pisang Nangka the lowest root necrosis percentage of 6.2% (Table 3).

Identification of nematodes based on the morphological and molecular characterization

The morphological characteristic of the female *Pratylenchus* populations collected in Peninsular Malaysia are given (Table 4, Fig. 3). The body length of the *Pratylenchus* females ranged from 423–659 μm (mean 541 μm). The female population from Johor had the shortest mean body length (423 μm) while the population from Selangor had the highest mean body length (659 μm). The mean body width of the females ranged from 19–35 μm those from Pahang being the narrowest and those from Selangor the widest. Percentages of shafts in relation to stylets were smaller in Pahang and highest in Selangor, ranging from 6–12 μm. Stylet length ranged from 14–25 μm with the population in Johor having shorter stylets than the Selangor population. Lip heights

ranged from 1–3 µm and width from 6–8 µm. Median bulb height was 1–2 µm and bulb width 6–8 µm. Tails were 33–51 µm long and 14–25 µm wide. The diameter of body at vulva ranged from 19–50 µm.

Fig. 2 – Above ground symptoms of root lesion nematodes on infected banana. A Stunted and toppled plant. B Smaller fruits than normal.

Table 2 Occurrence of *Pratylenchus* spp. on different banana cultivars in different states in Peninsular Malaysia.

Banana cultivars	Selangor	Johor	Perak	Pahang
Pisang Berangan	+	+	+	+
Pisang Tanduk/Lang	+	+	0	0
Pisang Lemak Manis	0	0	0	0
Pisang Nangka	+	0	0	0
Pisang Abu Nipah	0	0	0	0

+: nematodes present, 0: nematodes absent

Table 3 Disease severity indexes and mean population of *Pratylenchus coffeae* infesting banana cultivars in Peninsular Malaysia.

Area	Banana cultivar	Population mean (soil)	Population mean (root)	Disease severity (%)
Serdang/Selangor	P. Berangan	600	776	25
	P. Abu Nipah	-	-	-
	P. Lemak Manis	-	-	-
Kuala Selangor/Selangor	P. Tanduk/Lang	264	-	-
	P. Nangka	33	62	6.2
	P. Berangan	-	-	-

Table 3 Continued.

Area	Banana cultivar	Population mean (soil)	Population mean (root)	Disease severity (%)
Kluang/Johor	P. Berangan	12,322	16,800	49.3
Batu Pahat/Johor	P. Berangan	667	583	31
	P. Tanduk/Lang	67	667	28.7
Gunung Semanggol/Perak	P. Nangka	126	340	20.2
Titi Gantung/Perak	P. Nangka	335	2001	20.3
Sungai Pahang/Pahang	P. Berangan	333	733	16.6
Jeram Ular/Pahang	P. Berangan	6,866	10,582	24.3

The ITS region was amplified for three representative samples and sequenced with a PCR product size of approximately 1000 bp. The sequences were blasted and showed 99–97% identity with other ITS sequences of *Pratylenchus coffeae* deposited in Genbank database. The sequences were deposited in Genbank with accession identities KX011054, KX011055 and KX011056. Phylogenetic analysis showed that *P. coffeae* formed a clade with other *P. coffeae* and distinct from other *Pratylenchus* species, which validated the morphological identification of *P. coffeae* in this study (Fig. 4).

Root damage assessment

All four Malaysian states showed high *P. coffeae* disease severity in the Pisang Berangan cultivar, with Johor having the highest disease severity (36.3%) and Pahang the lowest (20.3%), even though all states/banana cultivars? have attained the threshold levels that could cause a bunch lost with the increase of any percentage above 5% (Figs 5, 6). The initial effects of nematode population density on plant growth parameters of the Pisang Berangan cultivar at the two and 12 weeks after inoculation were measured (Table 5). The Berangan banana cultivar showed growth reductions at all inoculum densities. Plant height decrease ranged from 11.1 to 61.3%, leaf area index decreased from 0.8–80.3% and pseudostem girth from 11.1–52.9%. The significance reduction was mostly between 100ni to 500ni and 1000ni to 3000ni. In a few cases, it was at 5000ni. In general, the 5000ni *P. coffeae* inoculation level caused a higher reduction in plant heights, leaf area index and pseudostem girths. The reduction increased with increase in nematode inoculation densities.

P. coffeae densities affected fresh root weight, length and shoot weight of the Pisang Berangan cultivar. Plants observed at 120 dai showed significant effects from various inoculum levels (Table 6). The percentage reductions were in the range of 17.9–80% for root length, 21–99.8% for root weight, 14.7–45.8% for shoot length and 9.3–61.8% for shoot weight respectively compared to the un-inoculated control plant. Significant reductions were seen from the various levels examined. However, the significance was mostly in the range of between 100ni to 500ni and 1000ni to 3000ni as well as with 5000ni in some cases. In general, the 5000ni *P. coffeae* inoculation level caused the highest reduction in plant height, leaf area index and pseudostem girths. Similarly, root length, weight and shoot length and weight showed reductions at 5000 *P. coffeae* inoculation levels.

Assessment of nematode population and root necrosis

The lowest reproductive factor (RF) was recorded at 100ni and the highest at 5000ni (Table 7). The root necrotic index was proportional to the RF, increasing with the increase of inoculation levels. Root necrotic percentage due to *P. coffeae* was greater than 5% in all inoculum levels, although they increased with increased inoculation levels. This implies that the damage was high across the treatments.

Table 4 Morphometric of females of *Pratylenchus coffeae* populations in Peninsular Malaysia (n = 7).

State	L	W	%SHFT	STYLET	LH	LW	MBH	MBW	TL	TW	DV
Johor	467.64±20.9	21.76±0.3	7.20±0.4	15.5±0.5	1.6±0.1	6.5±0.1	20.8±2.1	9.8±0.1	49.2±0.3	14.9±0.1	21.6±0.2
	(423.2-516.6)	(20.9-22.6)	(6.3-8.2)	(14.6-16.8)	(1.4-1.8)	(6.3-6.7)	(18.1-22.4)	(9.5-10.0)	(48.4-50.0)	(14.75.2)	(20.9-21.8)
Perak	548.68±9.3	24.9±0.9	7.5±0.6	17.6±0.1	2.4±0.2	8.2±0.1	13.3±0.2	10.9±0.1	45.9±2.3	21.1±1.1	23.1±1.7
	(525.7-580.7)	(23.2-28.0)	(5.7-8.6)	(17.3-17.9)	(1.9-3.2)	(7.9-8.4)	(12.9-13.7)	(10.611.3)	(44.8-51.2)	(18.9-25.1)	(19.2-28.1)
Selangor	580.6±79.3	30.3±4.8	12.0±0.8	19.0±6.1	2.5±0.5	7.1±0.1	21.6±2.3	8.0±0.7	51.3±3.36	17.3±1.1	34.2±4.9
	(501.2-659.9)	(25.5-35.1)	(11.3-12.8)	(12.9-25.1)	(2.0-2.9)	(6.9-7.3)	(19.3-23.9)	(8.0-9.3)	(42.7-50.1)	(16.2-17.9)	(20.2-50)
Pahang	481.9±7.6	19.8±0.7	6.9±0.3	15.2±0.2	2.0±0.2	7.4±0,5	11.4±0.4	10.3±0.4	39.2±2.8	22.1±1.2	20.8±3.5
	(472-492.7)	(19.2-21.3)	(6.5-7.4)	(14.9-15.8)	(1.5-2.3)	(7.2-7.9)	(11.0-13.3)	(9.8-11.2)	(33.2-45.7)	(19.3-22.4)	(20.2-23.6)

Measurements in µm. Mean ± standard error; (range)
L: body length, W: body width, SHFT: % shaft from stylet, LH: lip height, LW: lip width, MBH: medium bulb height, MBW: median bulb width, tail length, TW: tail width, DV: diameter of body at vulva.

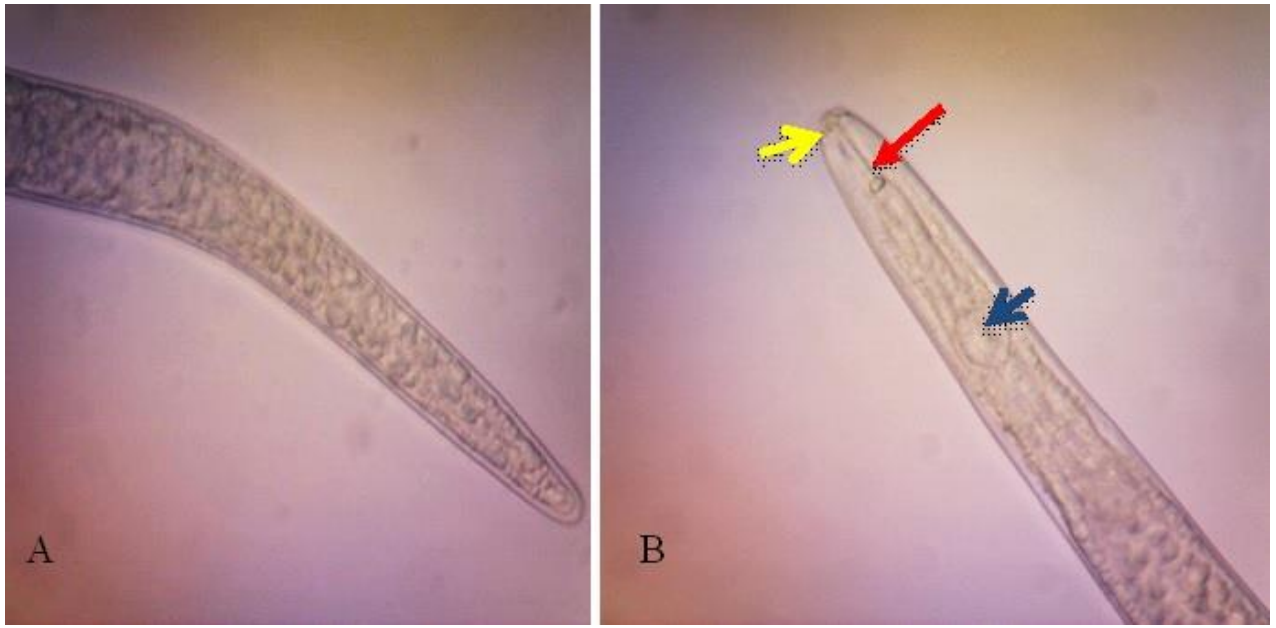

Fig. 3 – Morphological features of female head and tail *Pratylenchus coffeae* nematodes. A) Tail section. B) Head seaction; lip region (yellow arrow), stylet (red arrow) and median bulb (blue arrow).

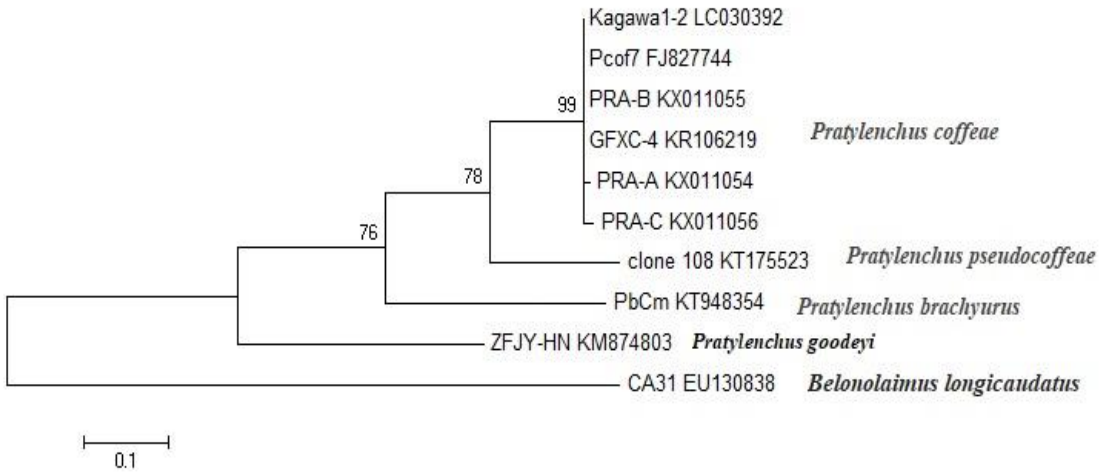

Fig. 4 – Phylogenetic analysis of ITS region sequencing of local *Pratylenchus coffeae* isolates (KX011054, KX011055 and KX011056) with other representative isolates sequences from Genbank database.

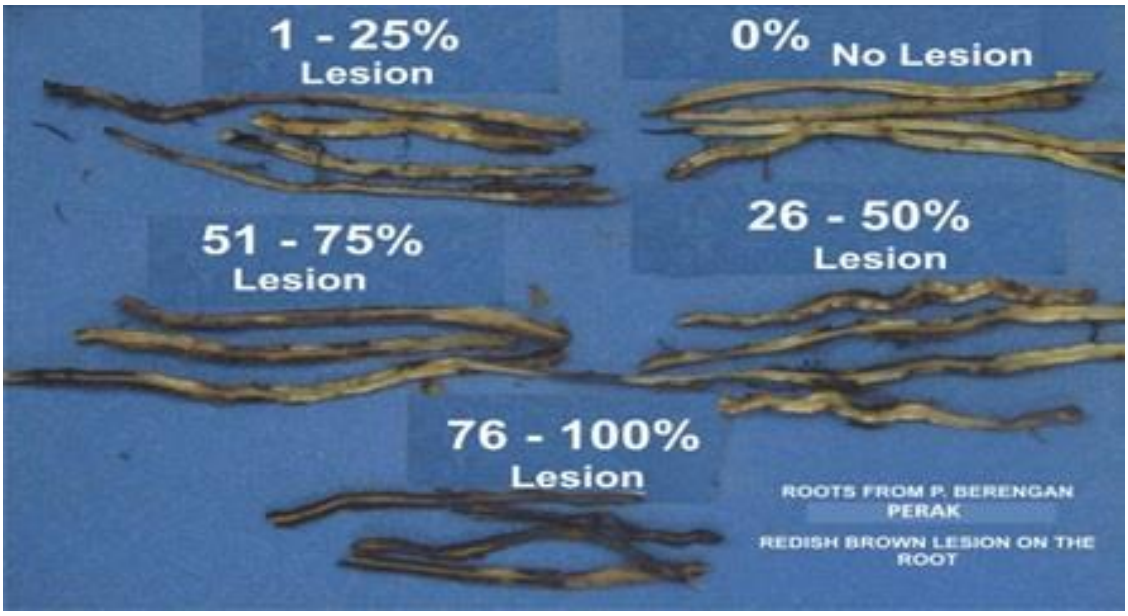

Fig. 5 – Cortical lesion scores of *Pratylenchus* spp. on infected banana from states in Peninsular Malaysia.

Peroxidase (PO) and polyphenol oxidase (PPO) activity in inoculated Pisang Berangan

The activities of peroxidase and polyphenol oxidase in roots of Pisang Berangan infected by *P. coffeae* were analyzed, as these enzymes are involved in the defense responses of plants against infection (Figs 7, 8). There was an increase in PO and PPO activity from 2 weeks after inoculation, but this had declined by 12 weeks after inoculation.

Discussion

The abundance of *P. coffeae* as influenced by soil pH is in agreement with Koen (1967) who observed that soil pH values of 5.0–7.3 were not harmful to *P. brachyurus* but pH 1.0 was deadly. Morgan & MacLean (1968) on alfalfa reported that a pH range of 5.2–6.4 supported higher reproduction of *P. penetrants*. Salahi Ardakani et al. (2014) also found that pH 7 supported an abundance of *Tylenchulus semipenetrans* on citrus. The severity of lesions caused by *Pratylenchus* was obviously alarming, which corroborates Khan & Hasan (2010) who reported that even in low

densities, cortical lesions were still evident on banana roots from lesion nematode infection. De Waele & Davide (1998) also reported that in Malaysia most local banana cultivars, such as Pisang Berangan (A.A, syn. Lakatan), Pisang Nangka (AAB) and Pisang Tanduk (AAB), are good hosts to *P. coffeae* either singly or in a mixed population with *M. incognita.* In the current study, root necrosis percentages caused by lesion nematodes varied among banana cultivars and sampled areas. The results are agreed with the observation made by Speijer et al. (1994) that general damage levels by nematodes exhibit marked variability among diverse banana fields in Uganda, although Kamira et al. (2013) observed no significant difference in root tissue necrotic percentage among banana cultivars.

Our morphological identification corresponds with earlier reports on *P. coffeae* by Castillo & Vovlas (2007). Molecular identification using ITS region confirmed the morphological identification of *P. coffeae.* Kim et al. (2016) identified two species, *P. kumamot*oensis and *P. pseudocoffea*e, in Korea based on morphometric and molecular diagnosis. *Pratylenchus scribneri* was detected and discriminated from *P. penetrans* and *P. neglectus* on the basis of conventional and real-time PCR, which implies that molecular tools are adequate for nematodes detection and diagnosis (Huang & Yan 2017).

Toppling of nematode infested banana plants is due to destruction of the root system (Barekye et al. 2000). Therefore, resistance and/or tolerance levels in varietal screenings of *Musa* could be accessed through damage inflicted on the root by plant parasitic nematodes. Higher necrotic percentage and non-reduction in yields implies a level of tolerance of cultivars. Damage levels could be related to yield loss (Peregrine & Bridge 1992, Coyne et al. 2007). Consequently, healthy root enhances productivity, as it supports the plant till harvest, while destroyed root could result in topplings, which affects the yield.

Higher percentage of necrosis recorded on the Pisang Berangan cultivar points to the level of losses incurred by small holders due to this pathogen, which certainly emanates from poor management practices and use of infested planting material (Kamira et al. 2013). This study reveals the level of sensitivity and susceptibility of different banana cultivars to plant parasitic nematodes in general and *Pratylenchus* in particular, in Peninsular Malaysia. A paucity of knowledge by growers about plant parasitic nematodes is one of the major constraint to banana production (Brooks 2004).

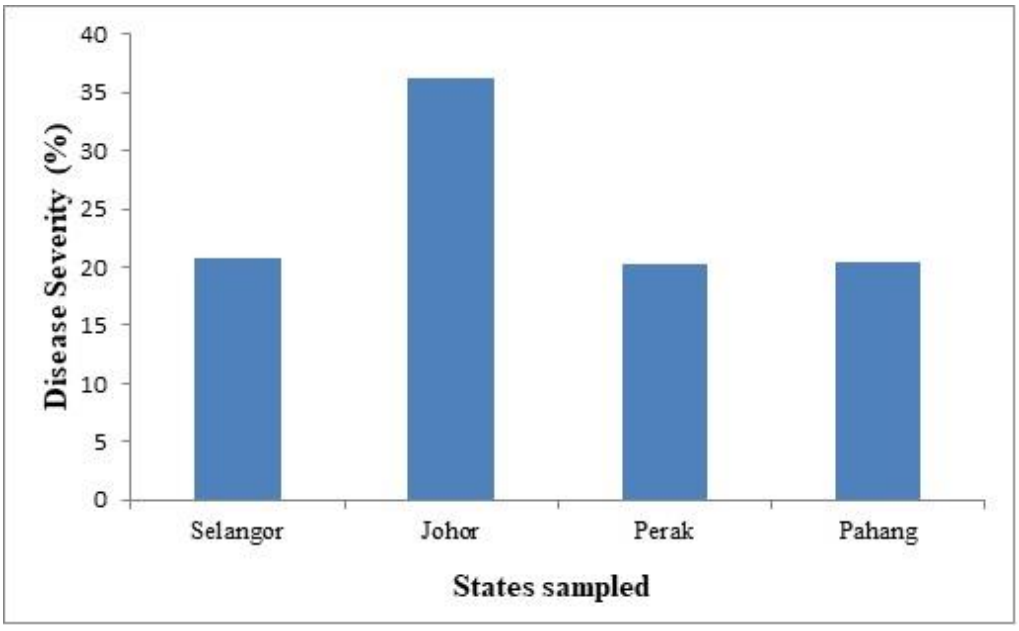

Fig. 6 – Average percentage root necrotic lesion on infected banana cultivars root cortex in different states of Peninsular Malaysia.

Table 5 Effect of different inoculation levels of *Pratylenchus coffeae* on plant heights (cm) leaf area and pseudostem circumference of Pisang Berangan, 12 weeks after inoculation.

WK	2			4			6			8			10			12		
GP	PH	LAI	PC	PH	LAI	PC	PH	LAI	PC	PH	LAI	PC	PH	LAI	PC	PH	LAI	PC
0	24.7a	473.2a	6.4a	27.0a	479.9a	6.9a	30.6a	484.5a	7.2a	34.3a	502.5a	7.6a	39.3a	557.1a	8.6a	44.3a	601.7a	8.8a
1	20.0b	279.6b	5.2b	22.5b	390.6ab	5.9ab	24.8b	421.6ab	6.1b	29.0ab	482.5ab	6.6ab	37.4ab	540.2ab	7.3ab	39.3ab	596.8ab	7.8ab
2	15.2c	255.8b	5.2b	17.4c	319.4bc	5.8b	22.0bc	361.8bc	5.8bc	27.0bc	413.5bc	6.0bc	31.7bc	450.3bc	6.5b	36.1bc	495.4bc	6.8bc
3	13.2cd	277.6b	4.8bc	17.3c	300.1bcd	5.4bc	18.9cd	333.1bcd	5.5bc	20.8cd	393.7cd	5.6bcd	29.8c	440.6cd	5.9bc	31.7c	491.9cd	6.00cd
4	11.9de	159.2c	4.1cd	12.5d	230.1cd	4.9cd	14.2d	298.9cde	5.1c	19.2de	324.7de	5.5bcd	22.2d	350.3de	5.5bc	24.5d	390.5de	5.7cde
5	9.4ef	145.4c	3.7de	11.8d	158.9d	4.4de	13.7d	238.1de	4.9d	18.1de	304.6ef	5.0cd	21.1de	339.7e	5.3bc	23.6d	380.9e	5.4cde
6	8.1f	140.0c	3.7de	11.2d	154.6b	3.8ef	12.8d	217.8de	4.2de	14.6de	230.9fg	4.3de	19.6de	301.2ef	4.4c	22.1d	319.8ef	4.6de
7	8.0f	129.9c	3.3e	10.1d	137.9b	3.4f	11.2d	190.4e	3.9e	13.2e	210.8g	3.9e	14.8e	245.2f	3.9c	17.1b	284.6f	4.2e

Means within rows that share the same letter are non-significant at p = 0.05.
0=control, 1= 100 nematode inoculum (ni), 2=250 ni, 3= 500 ni, 4=1000 ni, 5=2000 ni, 6=3000 ni, 7= 5000 ni.
WK (Weeks), GP (growth parameters), PH (plant height), LAI (leaf area index), PC (pseudostem circumference)

Table 6 Effects of different inoculation levels of *Pratylenchus coffeae* on fresh root and shoot length (cm) and weight (kg) of Pisang Berangan, 12 weeks after inoculation.

Treatment	Fresh roots and shoots length (cm) and weight (kg)			
	Root length	**Root weight**	**Shoot length**	**Shoot weight**
Control	86.48a	1.042a	102.34a	0.86a
P1	66.0b	0.82b	88.92ab	0.78ab
P2	54.18bc	0.098c	88.1b	0.61bc
P3	39.24cd	0.085c	84.92b	0.59bcd
P4	33.54cde	0.049c	74.18c	0.58bcd
P5	28.02cde	0.005d	70.2cd	0.42de
P6	26.38de	0.003d	59.67de	0.40e
P7	13.01e	0.0015e	55.46de	0.33e
MSD	26.11	0.18	13.51	0.19

Means within rows that share the same letter are non-significant at p = 0.05.
P1= 100 nematode inoculum (ni), P2=250 ni, P3= 500 ni, P4=1000 ni, P5=2000 ni, P6=3000 ni, P7= 5000 ni

Table 7 Reproduction and percentage root necrosis caused by different inoculation levels of *Pratylenchus coffeae* on banana cultivar Berangan.

***Pratylenchus coffeae* population**	**Average number of nematodes Absolute mean population (200 cc soil+10 g root)**	**RF=Pf/Pi**	**Root lesion necrotic index (%)**
control	00	00	00
100	23d	1.7d	5.10
250	102.2d	2cd	5.70
500	228d	2.2c	13.10
1000	484d	2.4c	14.30
2000	1359.6c	3.24b	21.00
3000	2076.8b	3.4b	22.30
5000	4052a	4.1a	45.80

Pi = initial population level, FP = final population, RNI = root necrosis index, RF = reproductive factor.

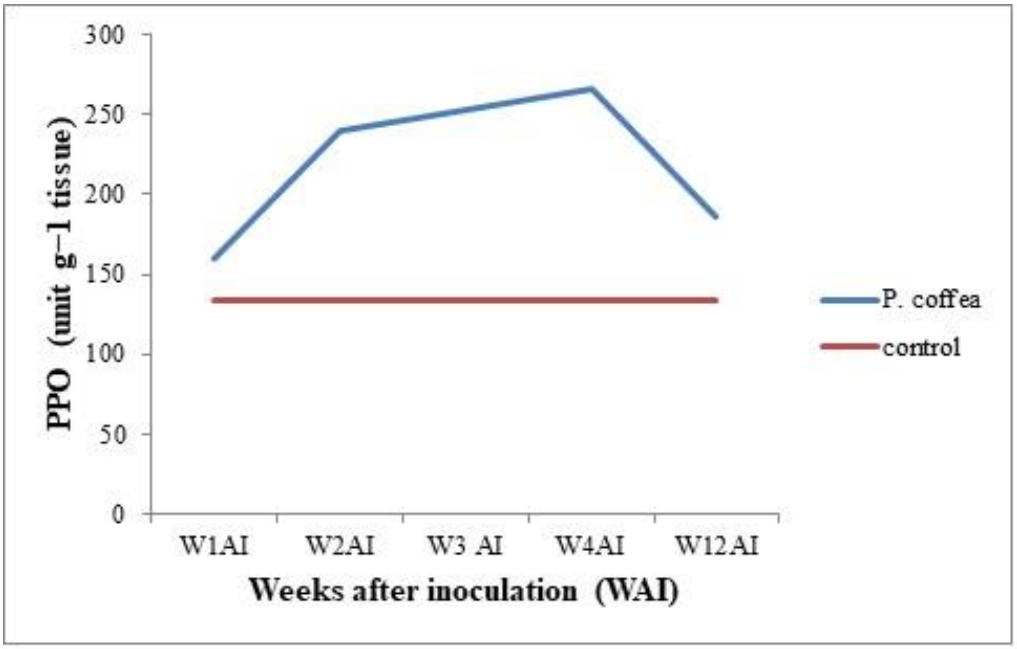

Fig. 7 – Activity of PPO (polyphenol oxidase) of Pisang Berangan roots at W1AI, W2AI, W3AI, W4AI and W12AI weeks after inoculation with *Pratylenchus coffeae* and un-inoculated (control).

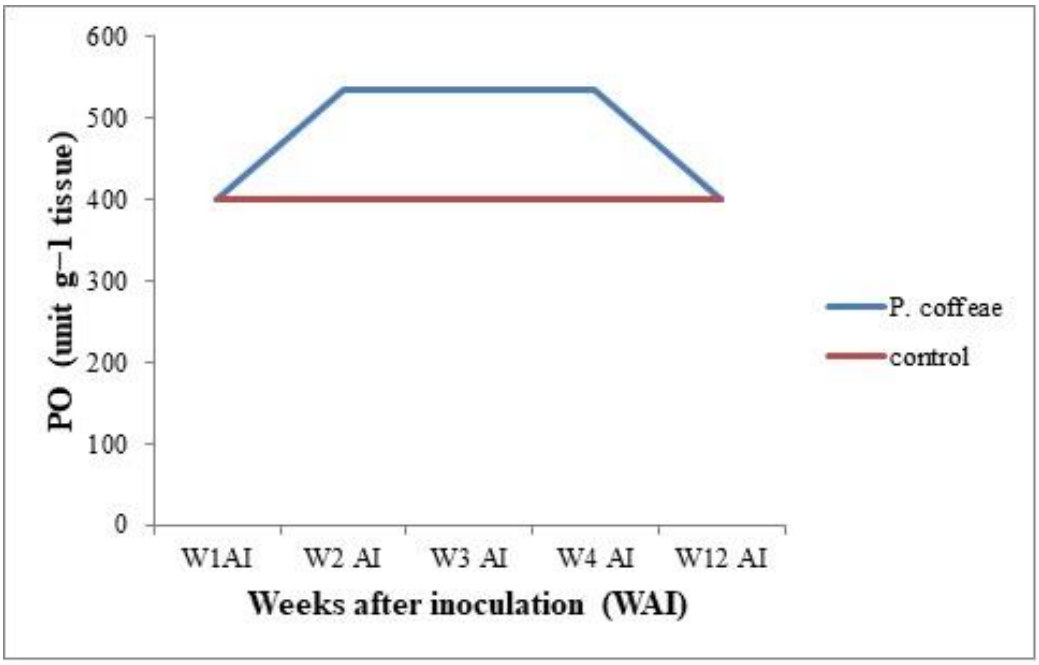

Fig. 8 – Activity of PO (peroxidase) of Pisang Berangan roots at 1 at W1AI, W2AI, W3AI, W4AI weeks after inoculation with *Pratylenchus coffeae* and un-inoculated (control).

Our pathogenicity trial indicated that *P. coffeae* inoculation levels caused substantial damage to banana vegetative parts, thus corroborating the survey results. Our finding therefore aligns with earlier observations by Gowen et al. (2005) who reported that banana plants are suitable hosts for *P. coffeae*. Similarly, Nguyen et al. 2015 describes *P. coffeae* as a pathogenic plant parasitic nematode of banana. As the inoculation densities of *P. coffeae* increased, the RF gradually decreases and the lowest RF was observed at the highest inoculation density. The decrease of the *P. coffeae* population at a higher Pi was very likely to be from a shortage of food supply to the nematodes due to poor plant development (Di Vito et al. 2002). Root mass reduction has been attributed to the *Pratylenchidae* species, which makes plants incapable of tolerating huge nematode numbers (Loof 1991). Castillo et al. (2001), Di Vito et al. (2004) reported similar findings, whereby an increase in nematode reproductive levels goes hand in hand with increasing initial nematode inoculum density. From the seven inoculation levels evaluated in this trial, the reproduction factor of *P.coffeae* was greater than one in all the treatments, thereby corroborating earlier reports by (Gowen et al. 2005) who reported that bananas are suitable hosts for *P. coffeae*. Similarly, Nguyen et al. 2015 describe *P. coffeae* as a pathogenic parasitic plant nematode of banana. The Pisang Berangan banana cultivar used in this trial showed a high level of susceptibility to *P. coffeae*, and this agrees with previous reports of the cultivar being a good host for *P. coffeae* (De Waele & Davide 1998).

Necrotic root percentage due to *Pratylenchus coffeae* was greater than 5% in all the inoculum levels evaluated, even though there was an increase with the increase of inoculation levels. This implies that the damage was high across all treatments. This agrees well with Speijer et al. (1994), who reported that necrosis of the root cortex above 5% is considered high. Coyne et al. (2007) and Peregrine & Bridge (1992) described the extent of root cortical necrosis as a determinant of yield loss. Similarly, toppling was the reported outcome of the destruction of the root system (Barekye et al. 2000). The highest percentage root necrosis score (45%) was from the 5000 inoculum in this trial, indicating what the growers may go through when the nematode population reaches this density in this part of the world

In nematode-plant interactions, PO is considered to be involved in resistance responses with an increase in activity after infection. Greater levels of PO are observed in resistant cultivars in general. Mateille (1994) observed higher rates of PPO activity in cv. Poyo, a susceptible banana

cultivar, compared to the resistant *R. similis* cv. Gross Michel at 8 weeks after inoculation, which may be due to the tissue browning of root damage in the susceptible cultivar. Wuyts et al. (2006) analysed peroxidase (PO), polyphenoloxidase (PPO) and phenylalanine ammonia lyase (PAL) activities in mechanically wounded banana roots compared to the effect of the burrowing nematode *R. similis* at one, three and seven days after inoculation. There was lower enzymatic activities in resistant cv. Yangambi km5 (*Musa acuminata* AAA) compared to the susceptible cv. Grande Naine (*M. acuminata* AAA). Decline of the enzymatic activity at week 12 may possibly be due to the higher reproduction of the nematode at that stage, which might have weakened the plant in producing this enzyme for self-defense.

Our results of PO and PPO activity showed an increase with the increase in the number of days after inoculation, except at 12 weeks when there was a decline in infected banana plants compared to the un-inoculated control. These results are in line with the findings of Aguilar et al. (2000) who observed differences in PO activity in banana against *Fusarium* wilt. In a similar event, tomato cultivars infected with *M. incognita* showed higher PO and PPO activity in susceptible cultivar compared to the resistant ones (Bajaj & Bhatti 1984).

P. coffeae significantly suppresses the vegetative growth of the Berangan banana cultivar. Root lesion indexes showed higher disease severity at all inoculum levels evaluated, indicating that at both minimum and maximum inoculum levels studied, damage to the crop can be severe. *P. coffeae* inoculated plants had a steady RF, with increasing inoculation levels. The activity of PO and PPO increased the longer the inoculation time was and decreased at 12 weeks after inoculation. This event could be attributed to the higher reproductive factor observed at higher inoculation levels of the nematodes.

Based on the observations above, a conclusion may be drawn that banana roots are predisposed to damage from the most virulent nematode species *P. coffeae*. However, its yield reduction potential is hitherto yet to be thoroughly researched either singly or in combination with other phyto nematodes for cost-effective banana production in peninsular Malaysia. The observations generated through this study could be a useful tool for embarking on pest risk assessment. The susceptibility or resistance responses of banana cultivars/types against *P. coffeae* are imperative for management decisions. Predominant nematode species were found, but the biochemical basis for resistance or response is essential for understanding causal effects.

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