

EFFECT OF INOCULUM DENSITIES ON THE REPRODUCTIVE FITNESS AND PATHOGENICITY OF *PRATYLENCHUS COFFEAEE* AND *MELOIDOGYNE INCOGNITA* ON *MUSA PARADISIACAL* L. IN PENINSULAR MALAYSIA

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ABSTRACT

Three weeks old tissue culture seedlings of *Musa paradisiaca* grown in pots containing autoclaved soil were inoculated with *Pratylenchus coffeae* and *M. incognita* inoculated with 100, 250, 500, 1000, 3000 and 5000 juveniles for each and mixture of the two nematodes at selected inoculum densities of 1000, 3000 and 5000 juveniles, and a negative control. Pathogen multiplications were observed after 12 weeks of growth. There were significant differences in vegetative growth ($p \leq 0.05$) within the pathogens and among the various inoculum densities evaluated. Multiplication factors or $R_f = \text{population final/population initial}$ ranged between 1.6-4 in *P. coffeae* and 0.6-7 in *M. incognita*. Reduction in root, shoot weights and lengths were significant ($p \leq 0.05$). *Musa paradisiaca* L. showed high level of susceptibility to the various inoculum densities evaluated. Despite the variation in reproductive factors between inoculum densities and among the nematodes examined, root lesion indices showed higher disease severity at all inoculum densities evaluated. At both minimum (100) and maximum (5000) inoculum densities studied, damage to the crop was severe.

Key words: *Meloidogyne incognita*, *Musa paradisiaca*, Pathogenicity, *Pratylenchus coffeae*, Reproductive fitness.

INTRODUCTION

Crop production has been threatened by nematode attack in the world; however, nematodes have not been stressed as crop pests of significance in Malaysia. Also, the damaging status of nematodes in agriculture has not received serious attention in the country. The reason for the lingering progress is not unrelated to the agricultural policies which lay preference on traditional perennial crops such as oil palm, cocoa and rubber which are seldom infected with nematodes (AbdulRahman *et al.*, 2014). Damages to horticultural and agricultural plants by nematodes in Malaysia seem to be overwhelming and thus, require urgent attention (AbdulRahman *et al.*, 2014).

Crops like banana (*Musa acuminata* Colla) [Razak, 1994; Razak and Loof, 1998; Hassan, 2004; AbdulRahman *et al.*, 2014], guava (*Psidium guajava* L.) in Perak [Razak and Lim, 1987], chili (*Capsicum frutescens*), black pepper (*Piper nigrum* L.) and even grass (turf grass) on golf courses [Razak and Loof, 1998] have been affected. The available documented reports are mere field surveys, which point to the necessity of more investigations to establish the basics and attempt to discover solutions to this worm's problem.

In Malaysia, two species of migratory plant-parasitic nematodes are important on *Musa* spp., viz

Pratylenchus spp. and *Meloidogyne* spp; as they have replaced *Radopholus similis*, a worldwide nematode of banana (AbdulRahman *et al.*, 2014). About 76 species have so far been recorded in the genus *Pratylenchus* (De Waele and Elsen, 2007). Of these species only few are of agricultural importance and are responsible for significant crops damage and high yield losses. *Pratylenchus coffeae* (Zimmermann) Filipjev and Schuurmans Stekhoven, is one of the root-lesion nematodes that are of pathogenic importance to plants. Besides its wide host range, it also has a worldwide distribution (Castillo and Vovlas, 2007).

Pratylenchus spp. are often found in banana fields together with other nematodes species, like *Radopholus similis*, and the root-knot nematodes, which provide feeding site for their penetration. In nematology, the two main components of pathogenicity are virulence and reproductive fitness (Shaner *et al.*, 1992), of which understanding and assessment of disease reactions of plants to pathogens are based. Pathogenicity is the ability of an organism to infect host plants and cause disease condition (Inomoto *et al.*, 2007), while reproductive fitness is defined as the multiplication ability of a species or population on a specific host plant (Inomoto *et al.*, 2007).

The colonisation of more host tissue by *Pratylenchus* spp. and root-knot nematodes due to

their higher reproductive fitness makes it possible for them to cause severe damage on susceptible plants. Damages caused by nematodes to crops are often assessed on the basis of densities of the nematodes in the soil at planting and in the roots throughout the growing season. Thus, for economic decisions for nematodes management, damage threshold levels are effectively employed (Ferris, 1981). *Pratylenchus coffeae* and root-knot nematodes have been reported in banana fields in Malaysia since the early eighties, however, their damaging status hitherto are not yet defined.

Knowledge regarding the injury population level of *Pratylenchus* spp. and root-knot nematodes on *paradisiaca* in Malaysia is highly required. It is necessary to conduct a study on damaging levels and potentials of *Pratylenchus* spp alone or with other phytonematodes for management decisions. The aim of this investigation was, therefore, to determine the population at which damage on banana can occur due to *P. coffeae* infection alone or in combination with *M. incognita*.

METHODOLOGY

Glasshouse experimental layout: Tissue-culture plants of the cultivar *M. paradisiaca* were used as a source of nematode-free planting stock. This plant material was transferred to 2 kg plastic pots of 25 x 15 cm dimension, filled with autoclaved soil in 3:2:1 sand, pit, clay. For each nematode species, mobile stages or mixture of juvenile and adult stages were inoculated in three holes of 3 cm x 4 cm with 10 ml water at densities of 0, 100, 250, 500, 1000, 2000, 3000 and 5000, nematodes/cm³ soil. After inoculation the holes were covered with soil. Each combination of nematode species-density was repeated five times. The pots were arranged in complete randomized design (CRD), consisting of 18 treatments viz: 0 negative control, 100 nematodes inoculum (ni), 250 ni, 500 ni, 1000 ni, 2000 ni, 3000 ni and 5000 ni, for each of *P. coffeae* and *M. incognita* and the mixture of the two nematodes at selected populations of 1000 ni, 3000 ni and 5000 ni. The potted plants were fertilized on monthly basis, with Peter's 20:20:20 general purpose N: P: K plant food at 0.25 g per litre of water. The plants were watered upon requirements and measurement done un-destructively.

Assessment of plant vigour: At 2, 4, 6, 8, 10 and 12 weeks after inoculation (WAI) plant heights were measured every two weeks till the twelfth week. Measurement was conducted from the base of the seedling to the top part of the plant using a ruler tape. For leaf area, length and breadth of the selected leaves, were also measured using ruler tape. The circumference of the *pseudo stem* was taken using a ruler for the same period. At harvest or

12 WAI, shoot and root lengths were measured using measuring tape, while fresh shoot and root weight were measured with the aid of weighing balance SP (1-4 kg) by Zhongshan Camry Electronic Co. Ltd. China. The percent increase and reduction in the growth parameters over the control were calculated by using the formula:

$$\% \text{ reduction} = \frac{(\text{Uninoculated} - \text{Inoculated})}{\text{Uninoculated}} \times 100$$

(Ansari *et al.*, 2018)

Assessment of disease severity: Cortical root necrosis of *P. coffeae* and galling index of *M. incognita*, fresh root weight, shoot weight and final nematode densities (Pf) in both roots and soil were determined at 12 WAI.

For the root damage assessment, about 100 root pieces of 10 cm length were sliced lengthwise for scoring the diseases caused by lesion nematodes as described by Speijer and De Waele, (1997).

$$(\%) = \frac{\sum(N_1 \times 1) + (N_2 \times 2) + \dots + (N_7 \times 7)}{N \times \text{highest rating scale}} \times 100$$

where:

- N1: The number of roots with necrosis at score 1
- N3: The number of roots with necrosis at score 3
- N5: The number of roots with necrosis at score 5
- N7: The number of roots with necrosis at score 7
- N: Total number of roots evaluated

The damage of roots was grouped into five classes according to the percentage of root cortex covered by lesions as follows as described by (Speijer and De Waele, 1997):

- 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

Root galling was assessed using 0-5 scale, according to Taylor and Sasser, (1978), where 0 = no galling, 1 = 1-2 galls, 2 = 3-10 galls, 3 = 11-30 galls, 4 = 31-100 galls and 5 = >100 galls.

For the roots, 2g of the galled portions were taken from each of the harvested banana plant and the galls counted, then multiplied by the weight of the root to give the approximate numbers of galls per plant.

Determination of reproductive factor (RF): At 12WAI, banana roots were carefully excised to remove the adhering soil from the root systems. Five roots with approximately equal length were taken and cut into segments, 10 cm from root tip and 10 cm in the middle with flamed scalpel between cuts to avoid transfer of inoculum from one segment to another.

Nematodes were recovered from 200cc soil subsamples and 10 g roots using whitehead tray method reported by Whitehead and Hemming

(1965). The final population (Pf) was obtained when one mL of the nematode suspension used in triplicates was each counted in a Huxley nematode counting slide through the use of the compound microscope. The averages of the triplicate counts represented nematodes in the pots 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 RF was determined from the relation:

$$RF = P_i / P_f \text{ (soil and roots)}$$

Where RF= Reproductive Factor, P_i = initial nematode population and P_f = final nematode population.

Statistical Analysis: Data collected were subjected to one-way analysis of variance (ANOVA) using Statistical Analysis Software (SAS Institute, 2008). The differences among the means were separated using Waller-Duncan k ratio t test at $P \leq 0.05$

RESULTS AND DISCUSSION

Table 1: Effects of different inoculum densities of *Pratylenchus coffeae* on fresh root and shoot lengths (cm) and weights (kg) of *Musa paradisiaca* at 12 weeks after inoculation

Treatment	Fresh roots and shoots lengths (cm) and weights (kg)							
	Root length	% reduction	Root weight	% reduction	Shoot length	% reduction	Shoot weight	% reduction
Control	86.48a		1.042a		102.34a		0.86a	
P1	66.0b	-23.7	0.82b	-21.3	88.92ab	-13.1	0.78ab	-9.3
P2	54.18bc	-37.3	0.098c	-90.6	88.1b	-13.9	0.61bc	-29.1
P3	39.24cd	-54.6	0.085c	-91.8	84.92b	-17	0.59bcd	-31.4
P4	33.54cde	-61.2	0.049c	-95.3	74.18c	-27.5	0.58bcd	-32.6
P5	28.02cde	-67.6	0.005d	-99.5	70.2cd	-31.4	0.42de	-51.2
P6	26.38de	-69.4	0.003d	-99.7	59.67de	-41.7	0.40e	-53.5
P7	13.01e	-84.9	0.0015e	99.9	55.46de	-45.8	0.33e	-61.6
MSD	26.11		0.18		13.51		0.19	

Means within columns that share the same letter (s) are non-significant at $p = 0.05$.

WAI=weeks after inoculation, Cont.=control treatment, P1= 100 nematode inoculum (ni), P2=250 ni, P3= 500 ni, P4=1000 ni, P5=2000 ni, P6=3000 ni, P7= 5000 ni.

Table 2: Effects of different inoculum densities of *Meloidogyne incognita* on fresh root and shoot lengths (cm) and weights (kg) of *Musa paradisiaca* at 12 weeks after inoculation

Treatments	Fresh Root and Shoot Lengths (cm) and Weights (Kg)							
	Root L	% reduction	Root W	% reduction	Shootl	% reduction	Shootw	% reduction
CONT	86.48a		1.04a		44.26a		0.86a	
P8	64.12b	-25.9	0.98b	-5.8	29.66b	-33	0.53b	-38.4
P9	61.21b	-29.2	0.82c	-21	26.82bcd	-39.4	0.52bc	-39.5
P10	58.72b	-32.1	0.80c	-23.1	23.4cde	-47.1	0.48bc	-44.1
P11	50.9bc	-30.7	0.76c	-26.9	21.94de	-50.4	0.45bc	-47.7
P12	47.08bc	-45.6	0.61d	-41.3	20.04ef	-54.8	0.43bc	-50
P13	44.38bc	-48.7	0.60d	-42.3	19.98ef	-54.9	0.42bc	-51.2
P14	37.72c	-56.4	0.52e	-50	15.24f	-65.6	0.32c	-62.8
MSD	21.3		0.06		6.61		0.21	

Means within columns that share the same letter (s) are non-significant at $p = 0.05$.

WAI=weeks after inoculation, Cont.=control treatment, P8= 100 nematode inoculum (ni), P9=250 ni, P10= 500 ni, P11=1000 ni, P12=2000 ni, P13=3000 ni, P14= 5000 ni.
RTL= root length, RTWT= root weight, SHTLT= shoot length, SHTWT= shoot weight

Table 3: Effect of different mix nematodes inoculum densities (*Pratylenchus coffeae* and *Meloidogyne incognita*) on fresh root and shoot lengths (cm) and weights (kg) of *Musa paradisiaca* at 12 weeks after inoculation

Treatments	Fresh root and shoot lengths (cm) and weight (kg)							
	Root l	% reduction	Root wt	% reduction	Shoot l	% reduction	Shoot wt	% reduction
Contr	86.48a	0	1.04a	0	41.26a	0	0.87a	0
P15	75.54b	-12.7	0.10b	-90.4	29.88b	-27.6	0.65b	-25.3
P16	66.54c	-23.1	0.07b	-93.3	25.14bc	-39.1	0.46c	-47.1
P17	51.64d	-40.3	0.04c	-96.2	19.34b	-53.1	0.27d	-69
MSD	9.68		0.06		9.73		0.2	

Means within columns that share the same letter (s) are non-significant at $p = 0.05$.

WAI=weeks after inoculation, Cont.=control treatment, P8= 100 nematode inoculum (ni), P9=250 ni, P10= 500 ni, P11=1000 ni, P12=2000 ni, P13=3000 ni, P14= 5000 ni.

RTLT=root length, RTWT=root weight, SHTLT=shoot length, SHTWT=shoot weight

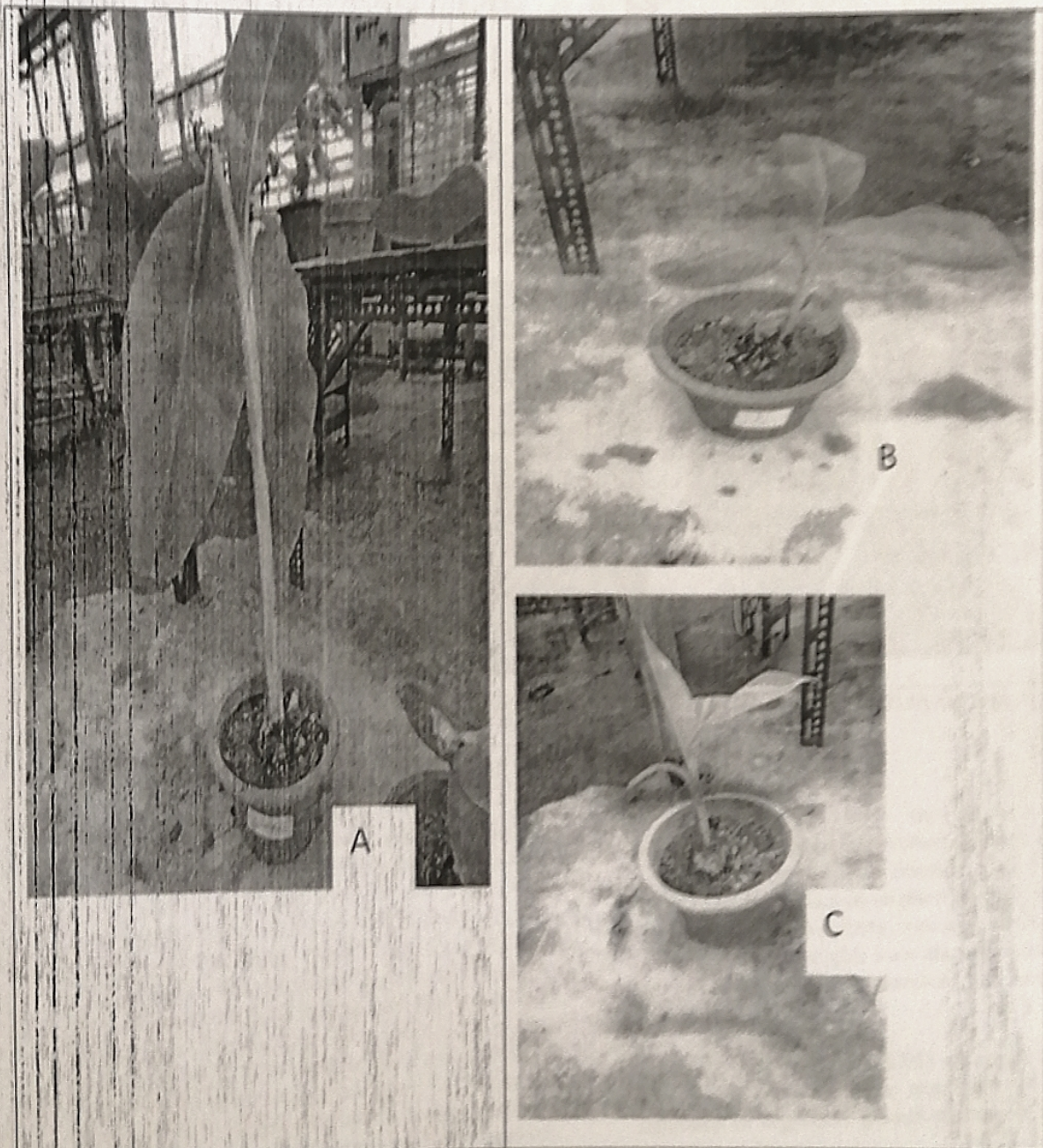


Figure 1: Above ground symptoms of *P. coffeae* (B), *M. incognita*(C) and control (A)



Figure 2: Below ground symptoms of (A) control, (B), *M. incognita*, (C) *P. coffeae* and (D) Mixed infection of A and B

For *Meloidogyne incognita*, the results presented in Table 2, show that the ranges of percentage reduction of plant parts were -25.8 to -56.3%, root length, -5.7 to -50% root weights, -32.9 to -65.7% shoot length, -32.9 to -65.7% shoot weight -38.2 to 62.7% compared to the control plants. Significant reduction ($P \leq 0.05$) was seen from various densities examined. Though, the significance was mostly in ranges between 100ni to 500ni on one hand and 1000ni to 3000ni on the other, and in few cases to 5000ni. In general, 5000 *P. coffeae* inoculum density recorded greater damages. All higher vegetative growth

parameter damage was recorded at 5000 *Meloidogyne incognita* inoculum density and the least on the lower inoculum densities. The *P. coffeae* recorded higher reduction in plant heights; leaf area and pseudo stems. Similarly, root length, weight and shoot length and weights both showed reduction at 5000 *P. coffeae* inoculum densities compared to the control plants.

Significant reduction ($P \leq 0.05$) was seen from the various inoculum densities examined. In general, reduction in vegetative growth parts were higher at

higher inoculum otherwise, inoculum densities were proportional to the reduction percentages across all densities. However, the significant differences among the inoculum densities were in the ranges of 100 to 500ni and 1000 to 3000ni and in some instances to 5000ni.

The result of pathogenicity trial in this study (Table 2) shows that *M. incognita* inoculum densities caused substantial damage to banana vegetative parts. There were significant differences among the treatments and the highest root length reduction of 37.7cm was recorded in P14, while the other treatments were not significantly different from each other as compared to the control P 14with 86.48 cm and the root weight followed the same trend. However, the shoot length and weight recorded significant reduction both in P7 and the rest were similar.

The present results are in agreement with the report of Pinochet *et al.*(1998) who reported banana damage by *Meloidogyne* spp in Canary Islands. Other workers also documented similar findings in North Africa, South Africa, West Africa, Martinique and in Brazil (Quénéhervé *et al.*, 2000 and Cofcewicz *et al.*, 2001).

For the mixture of the two strains, the treatments differed significantly with treatment P15 recording the lowest root reduction of 7 5.54 cm and P17 recording the highest root reduction compared to the control plants. The trend was the same for the other measured parameters, except for shoot weight where there were no significant differences among the treatments compared with the control. The percentage reduction of the measured parameters as affected by *P. coffeae* and *M. incognita* ranges from -27.5 to -53.1% on plant height, -18.2 to -50.8% in leaf area, -20.3 to -57.2% *pseudo stems*, -12.6 to -40.3% root length, -9.7 to -96.1% shoot length and -25.2 to -68.9% shoot weight, compared to the control plant. Significant reduction ($P \leq 0.05$) was recorded from the various densities examined. In general, 5000 *P. coffeae*/*M. incognita* inoculum density recorded higher effects on plant heights, leaf areas *pseudo stems*, root and shoot lengths and weights by reducing them drastically when compared to control plants.

The result shows that mixed population of *P. coffeae* and *M. incognita* inoculum densities caused substantial damage to banana vegetative parts in the same way they did individually. These results agree with the reports of De Waele and Davide (1998) that most local banana cultivars like Pisang Berangan (A.A. syn. Lakatan), Pisang Mas (AA, syn. Sucrier), Pisang Rastali (AAB, Silk subgroup), Pisang Nangka (AAB), Pisang Tanduk (AAB), and Pisang Embung (AAA, syn. Gros Michel) are good hosts to *M. incognita* either singly or in mix population with *P. coffeae* and others. Here they reduce *pseudo stems*, girth and plant height which are obviously seen in the present study.

Disease severity

Reproductive factor (RF)

Results presented in Tables 4-6 show significant differences among the different inoculum densities with RF in proportion with the different densities. In both *P. coffeae* and *M. incognita*, lower RFs were recorded in 100ni and the highest in 5000ni respectively and same was observed in the mix populations. Similarly, root necrotic and galling indices followed the same trend with the RF increasing with increased inoculum densities.

From this study, increase in initial inoculum density resulted in increase in RF in all the treatments. Castillo *et al.* (2001) and DiVito *et al.* (2004) reported similar findings as obtained from this study. Reports that increasing initial nematode inoculum densities resulted in increased nematode reproductive levels have been documented on *Meloidogyne* spp infections on several crops. Kheir *et al.* (2004) reported proportional increase in final nematode population density of *M. incognita* with increase in initial inoculum densities on banana cultivars but observed that all densities suppressed banana growth. Contrary to result obtained from this study, Olabiyi *et al.* (2009) reported negative correlation of RF with *Meloidogyne* spp. to the initial inoculum density. The final *M. incognita* population increased proportionally with increase in initial population densities and all densities showed high damaging status.

Table 4: Reproduction and percentage root necrosis caused by different inoculum densities of *Pratylenchus coffeae* on *Musaparadisiaca*

<i>Pratylenchus coffeae</i> population	Average number of nematodes RF		Root necrotic lesion index (%)
	Absolute mean population (200 cc soil+10 g root)		
Control	00	00	00
100nempop	23d	1.7d	5.10
250nempop	102.2d	2cd	5.70
500nempop	228d	2.2c	13.10

1000nempop	484d	2.4c	14.30
2000nempop	1359.6c	3.24b	21.00
3000nempop	2076.8b	3.4b	22.30
5000nempop	4052a	4.1a	45.80
MSD	693.53	0.48	

RF = Reproductive Factor.

Table 5. Reproduction and percentage root galling caused by different inoculation levels of *Meloidogyne incognita* on *Musa paradisiaca* at 12 WAI

Pratylenchus population	<i>coffae</i>	Average	Number	of	RF=PI/PI	Root galling index
		Nematodes	Absolute mean population (200 cc soil+10g root)			(%)
Control		00			00	00
100nempop		23d			1.7d	5.10
250nempop		102.2d			2cd	5.70
500nempop		228d			2.2c	13.10
1000nempop		484d			2.4c	14.30
2000nempop		1359.6c			3.24b	21.00
3000nempop		2076.8b			3.4b	22.30
5000nempop		4052a			4.1a	45.80
MSD		693.53			0.48	

RF = Reproductive Factor.

Table 6: Reproduction and percentage root necrosis caused by different inoculum densities of *Pratylenchus coffeae* and *Meloidogyne incognita* combined on *Musa paradisiaca*

<i>P. coffeae</i> / populations	<i>M. incognita</i>	Average Number of Nematodes	RF
		Absolute mean population (200ccsoil+10g root)	
Control		00	00
3000 Mix nem pop (<i>P. coffeae</i>)		4432c	2.1d
" (<i>M. incognita</i>)		347.4c	1.8c
3000 Mix nem pop <i>P. coffeae</i>		940.8b	3ab
<i>M. incognita</i>		853.6b	2.8b
5000 Mix nem pop <i>P. coffeae</i>		1721.4a	3.4a
<i>M. incognita</i>		1518.8a	3ab
MSD		238.75	0.43

RF = Reproductive Factor

In the present report, root necrotic percentages due to *Pratylenchus coffeae* were greater than 5% in all the inoculum densities evaluated. This implies that the damage was high across the treatments. This agrees with the work of Speijer *et al.* (1994) who reported necrosis of root cortex above 5% as high. Coyne *et al.* (2007) and Peregrine and Bridge, (1992) described the extent of root cortical necrosis as a determinant of yield loss. Similarly, toppling is the outcome of the destruction of the root system of a plant by plant parasitic nematode (Barkeye *et al.*, 2000). The highest percentage root necrosis score of 45% recorded from the 5000-inoculum density in this study indicates what banana growers will go through, when nematode population reaches this density on their farms.

On the other hand, galling severity in all the inoculum concentrations increased with increase in initial nematode population in the present study. This is in agreement with earlier report by

Mekete *et al.* (2003) who showed that galling severity of tomato root and pepper increased with increase in initial inoculum density of *M. javanica*. Udo and Ugwuoke, (2010) reported that *M. incognita* recorded more pathogenic effect on turmeric plants with increased nematode density as more galls were recorded at higher inoculum densities compared to lower ones

CONCLUSION AND RECOMMENDATION

Tissue cultured *M. paradisiaca* plants were highly sensitive to *P. coffeae* and *M. incognita* diseases infecting their roots thus leading to significant suppression of the vegetative growth in the present study. Root lesion and galling indices showed higher disease severity at all inoculum densities evaluated. At both minimum 100ni and maximum 5000ni densities, damage to the test banana by the nematodes was severe. Future studies should involve screening of banana land races against these

nematodes with the view to developing resistant varieties to them for use by farmers.

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