



## RESEARCH PAPER

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## Prevalence of plant parasitic nematode *Pratylenchus coffeae* in *Musa* in Tamil Nadu and assessment of resistant genotypes for its management

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### Abstract

Plant parasitic nematodes comprise one of the most economically important pests in banana production worldwide. Nematode secretes effector molecules to modify host cells through their stylet to ingest water and nutrients from plants, which are the primary source for plant growth and production. The resulting water and nutrient deficiency lead to poor plant growth, stunting and toppling of plants. However, plants also produce chemical constituents to combat nematode infestation, among which phenol compounds play a major role in the defence response against *Pratylenchus coffeae* nematodes. The incidence of plant parasitic nematodes associated with banana plantations has been encountered in different localities in Tiruchirappalli, Theni, Dindigul, Thanjavur, Namakkal, and Karur districts of Tamil Nadu, among which Tiruchirappalli and Theni districts had recorded 52% and 42.5% of population incidence of *P. coffeae*, respectively. Besides causing root lesions, the nematode *P. coffeae* affects the maximum population of the susceptible cultivar Nendran followed by Rasthali and Robusta. The resistant cultivars Pisang lilin and Yankambi Km5 were screened against *P. coffeae* and it was confirmed by population, reproductive factor and phenolic compounds ( $P < 0.05$ ). This implies that the infestation caused by nematodes varies among different genotypes, and the information obtained in this study would be further helpful in selecting resistant genotypes for the biological control of nematodes.

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## Introduction

Banana (*Musa paradisiaca* L.) is a large herbaceous perennial monocotyledonous plant belonging to the genus *Musa* of the family *Musaceae*. It is one of the fourth most important agricultural food crops produced globally after rice, wheat and maize (Aurore *et al.*, 2009; Alagesan *et al.*, 2019). Worldwide, India is the leading country in banana production with an estimated 40 million tonnes of fruits produced per annum (Sharangi and Acharya 2007; Justin *et al.*, 2008; Srinivasa Reddy *et al.*, 2015; FAO 2017). The banana clones used all over the world have been originally derived from the two wild species *M. acuminata* and *M. balbisiana*, which have contributed to A and B genomes, respectively. Banana provides a more balanced diet than several other fruits and has a high therapeutic value with low salt, cholesterol and fat content (Singh *et al.*, 2018). The major banana-growing states in India include Andhra Pradesh, Assam, Bihar, Gujarat, Karnataka, Kerala, Madhya Pradesh, Maharashtra, Orissa, Tamil Nadu and West Bengal. Tamil Nadu has the largest area under banana cultivation (0.114 million ha), followed by Maharashtra (0.08 million ha) and Andhra Pradesh (0.075 million ha). Tamil Nadu is the leading state for banana production, accounting for 24.06% of the total production in India, followed by Maharashtra (22.15%), Gujarat (13.39%), Andhra Pradesh (10.41%) and Karnataka (6.90%). Despite its importance, the production of banana is hampered by several parasitic factors such as plant-parasitic nematodes, insect pests and soil-borne fungi that seriously threaten the sustainability of these plantations by decreasing the yield, resulting in stunted growth and toppling of plants (Van Asten *et al.*, 2005; Queneherve 2009; Swennen *et al.*, 2013).

Plant-parasitic nematodes can devastate a wide range of crops, including bananas, causing losses of billions of dollars in agriculture each year (Barker and Koenning 1998; Ali *et al.*, 2013). In general, all plant parasitic nematodes are obligate parasites feeding exclusively on the cytoplasm of living plant cells. The most economically important groups of nematodes are the migratory endoparasites, which include the genus *Pratylenchus* (root-lesion nematodes), and the

sedentary endoparasites, which include the genus *Meloidogyne* (root-knot nematodes). Both root-lesion and root-knot nematodes have complex interactions with their host (Sarah 1993; Gowen *et al.*, 2005). Several studies have been conducted to evaluate the resistance of banana cultivars against *M. incognita*, whereas only a few reports are available related to *P. coffeae*. Root-lesion nematodes are widespread and comprise one of the most important groups of plant-parasitic nematodes (Jones *et al.*, 2013), which have been recognized as the major nematological constraints of primary economic importance due to their role in crop losses, a wide host range and their worldwide distribution (Hockland *et al.*, 2006; Castillo and Vovlas 2007). However, host-plant resistance is an important component in the development of an integrated pest-management strategy for the control of nematode pests due to anti-xenosis or anti-biosis.

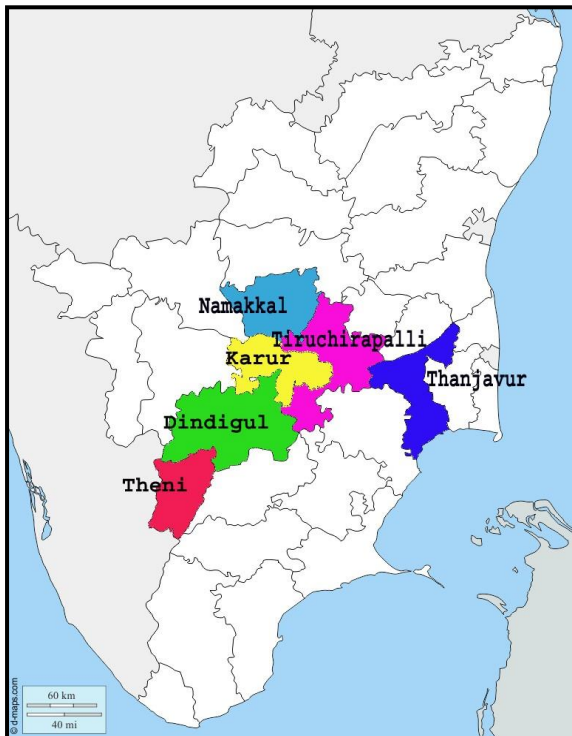
The control of plant-parasitic nematodes relies heavily on the use of synthetic insecticides such as carbofuran, which has led to several adverse effects on the environment and induced nematode resistance and toxicity to non-target species. However, biological control is a feasible, lifelong pest control strategy for the management of pests (Sharmila Bharathi and Mohan 2015). Breeding for the development of banana genotypes that are resistant to plant-parasitic nematodes encompasses numerous challenges that originate from the broad genetic diversity of *Musa* genotypes. Hence, screening to identify a resistant cultivar may provide a source for defence-resistant gene for controlling plant parasitic nematodes. Therefore, in the present study, different genotypes were screened using different parameters based on nematode population, lesion index, root weight and total phenol content in roots. It is expected that the results of this investigation would be helpful in determining the nematode-resistant cultivar to identify the defence gene to control the plant-parasitic nematodes in *Musa*.

## Materials and methods

### *Study area and sample collection*

The distribution of the nematode species in Tamil Nadu, India, was assessed from data collected from

sampling activities conducted in household gardens and individual and commercial farms from July 2016 to January 2017. Nematode isolates were obtained from untreated soil of banana plantations/farms in six hot spot locations in the districts of Dindigul (10°1'N and 77°7'E), Karur (10°9'N and 78°4'E), Namakkal (11°2'N and 78°3'E), Thanjavur (10°8'N and 79°1'E), Theni (10°0'N and 74°94'E) and Tiruchirappalli (10°8'N and 78°9'E) in Tamil Nadu (Fig. 1).



**Fig. 1.** Districts of Tamil Nadu assessed for distribution of root-lesion nematodes *P. coffeae*, in *Musa* commercial genotypes.

Based on the nematode infestation data in the field and literature survey, nematode-free suckers and root samples of different genotypes such as Nendran (ABB), Rasthali (ABB), Robusta (AAB), Yankambi Km5 (AAA) and Pisang liliin (AA) were collected from the major banana-growing regions in Tamil Nadu. The collected suckers were planted into pot cultures for further *in vitro* screening of nematode-resistant cultivars.

#### Morphological identification

Based on morphological identification of structures on the root-lesion nematodes, *P. coffeae* collected from banana-growing regions at different hot spot locations in Tamil Nadu were distinguished from other nematode

species by microscopic observation according to the method described by Castillo *et al.* (2012).

#### Nematode culture

The plant parasitic nematode *P. coffeae* was isolated from the infected root samples and cultured with the susceptible host for mass production. It was used for screening and characterization of the resistant cultivar by introducing the nematodes into different genomic cultivars under atmospheric conditions. Nematodes were extracted through sieve methods from roots and used for screening studies. The Second stage juveniles (J2s) were obtained from handpicked in sterile distilled water at  $27 \pm 2$  °C.

#### *In vitro* screening of resistant *Musa* genotypes for plant parasitic nematodes

This experiment was conducted in a net house condition to investigate the physiological and biochemical changes induced by *P. coffeae* in the above-mentioned five genotypes of *Musa*. Banana suckers aged 3 months were collected (in triplicates) from the mother plant in hot spot locations in Tamil Nadu, India. The suckers were made free from nematode infestation by peeling off the roots and the outer corm layers followed by immersion in a water at 53°C-55°C for 20 minutes and then planted into 20-cm-diameter earthen pots containing 1 kg sterilized soil and farmyard manure in a ratio of 3:1. Then, the mass culture of nematodes was directly added to each pot onto the mixture of soil by inoculating 2mL of aqueous suspension at a depth of 3cm containing 50 freshly hatched juveniles. Observations were made after 90 days post-inoculation (dpi) along with control.

#### Root lesion index

Roots collected from each genotype were assessed root damage at 90 days of post-inoculation. Resistance rating for nematode screening under pot culture was performed according to the method described by Lopez Gomez *et al.* (2016) and Sundararaju (1996).

#### Estimation of phenolic substances

The total phenolic content of root samples was determined using the modified method described by

Sulaiman *et al.* (2011). Approximately 1 g of root sample was ground and extracted by shaking continuously in a 2-mL aqueous methanol (50%) solution for 1 hour at room temperature. The extract was filtered through a 0.45- $\mu$ M PTFE syringe filter and stored at  $-20^{\circ}\text{C}$ . Then, 200 $\mu$ L of the root extract was mixed with 5mL water in a glass test tube. To each of the samples, 500 $\mu$ L of Folin-Ciocalteu reagent was added. Samples were thoroughly mixed and after 3 min, 1mL saturated sodium carbonate (35% in water, w/v) was added. Samples were further diluted to 10mL with water and left in the dark at room temperature for 1 hour. Absorbance was measured at 727 nm against water using a spectrophotometer. A standard calibration curve of gallic acid was determined, and the results were expressed as gallic acid equivalents per gram fresh roots.

#### Statistical analysis

Data were presented as mean values after analysis using the SPSS software (Version 16.0). The differences between genotypes were evaluated by analysis of variance (ANOVA) and the least significant difference (LSD) test at  $P < 0.05$ .

#### Results

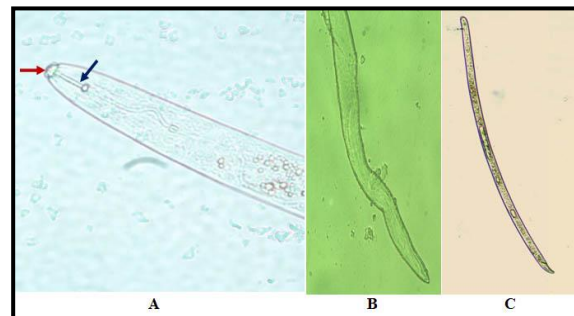
The nematode population was assessed in the commercial genotypes in the fields of major banana-growing regions in Tamil Nadu. Among these genotypes, the population incidence of *P. coffeae* was higher in the cultivar Poovan and Nendran root samples collected from Tiruchirappalli followed by Theni district, which showed incidences of 52% and 42.5%, respectively (Table 1).

**Table 1.** Incidence of *P. coffeae* in root samples collected from hot-spot locations of banana-growing regions in the state of Tamil Nadu, India.

Districts	Percent of incidence ( <i>P. coffeae</i> ) (Mean $\pm$ S.E)
Dindigul	30.0 <sup>bc</sup> $\pm$ 5.47
Karur	10.0 <sup>d</sup> $\pm$ 2.16
Namakkal	21.0 <sup>cd</sup> $\pm$ 1.91
Tanjavur	22.0 <sup>cd</sup> $\pm$ 4.96
Theni	42.5 <sup>ab</sup> $\pm$ 5.73
Tiruchirappalli	52.0 <sup>a</sup> $\pm$ 4.69

\*Mean values followed by the same letters are not significantly different ( $P < 0.05$ ).

The morphological structure of *P. coffeae* is similar to that of other *Pratylenchus spp.* and their length varies between 0.33 and 0.47 mm. The body wall consists of a cuticle with annulations, hypodermis or epidermis and somatic muscles. They possess a short, stout, hollow mouth stylet or spear with well-developed stylet knobs (Fig. 2).



**Fig. 2.** Morphological features of female head and tail of *Pratylenchus coffeae* nematodes. A) Head section; lip region (red arrow) and stylet (blue arrow). B) Tail section (Female). C) *Pratylenchus coffeae* (Male).

However, *Musa* germplasm varieties are infected with a different range of nematode infestations, among which most of the genotypes are susceptible and only a few are resistant depending on the infestation in roots due to their host acceptability. In addition, the nematode-resistant genotype was identified using various parameters such as the population of nematodes, root weight, root damage and root necrotic lesions from the host, and it was also characterized through *in vitro* screening. Accordingly, in each pot culture containing three month old plant (suckers) of Nendran, Rasthali, Robusta, Yankambi Km5, and Pisang lilin was inoculated with 50 numbers of *P. coffeae* nematodes and its reproductive factor was calculated based on their population development.

The mean population of *P. coffeae* nematodes was estimated in a range of 35.6-209.3 individuals/10g of infected roots associated with five different genotypes in the culture pots at 90 days post-inoculation (dpi). The nematode population from the cultivars Nendran and Rasthali showed 209.3 and 139.3 nematodes, respectively. Similarly, the cultivars Pisang lilin, Yankambi Km5 and Robusta exhibited 35.6, 46.0, and 78.3 nematodes,

respectively. This indicated a significantly reduced number compared with susceptible cultivars ( $P < 0.05$ ). In addition, the fresh root mass of 119.37g was showed in resistant cultivar Pisang

lilin and the susceptible cultivar Nendran which showed 56.83g in 90 dpi. This mass was directly proportional to the nematode population in the different genotypes (Table 2).

**Table 2.** Host status of *Musa* to *P. coffeae* under controlled conditions 90 days post-inoculation of nematodes.

SN	Cultivars	Initial Nematode inoculum (1 kg of soil)	Nematode Population from infected roots (10 g)	RF= Pf/Pi	Root weight	Root lesion index	Host status (R)
1.	Nendran	50	209.3±21.52	4.18	56.83 <sup>c</sup> ±5.21	4.3	GH
2.	Rasthali	50	139.3±14.49	2.78	82.43 <sup>b</sup> ±2.42	4.1	GH
3.	Robusta	50	78.3±15.24	1.56	80.64 <sup>b</sup> ±4.56	3.6	MH
4.	Yankambi Km5	50	46.0±7.23	0.92	107.84 <sup>a</sup> ±3.15	2.0	PH
5.	Pisang lilin	50	35.6±4.33	0.71	119.37 <sup>a</sup> ±5.56	1.0	PH

\*Mean values followed by the same letters are not significantly different ( $P < 0.05$ ).

\*RF= Reproductive Factor; Pf = Final population; Pi = Initial population

R = Host status: RF<0.5 = Non - host; RF<1 = Poor host; RF<2 = Moderate host;

RF ≥ 2 = Good host

The reproductive factor (RF) values of the cultivars Nendran and Rasthali were 4.18 and 2.78, respectively, whereas those of Pisang lilin, Yankambi Km5 and Robusta were 0.71, 0.92 and 1.56, respectively. Based on the reproductive factor, cultivar Nendran and Rasthali was considered as good host (GH), Pisang lilin and Yankambi Km5 were determined as poor host (PH) and Robusta with an RF value of 1.56 was considered as a moderate host (MH) to *P. coffeae*. Moreover, the root lesion index (Rli) was evaluated from the nematode infestation in *Musa* genome. Cultivars with the highest infestation score index (5) were represented as susceptible, whereas those with the lowest score index (1) were considered as resistant. Among the five cultivars, Nendran was found to be highly susceptible (4.3 Rli) and Rasthali was found to be moderately susceptible (4.1 Rli). Similarly, the cultivars Pisang lilin (1.0) and Yankambi Km5 (2.0) were found to be highly resistant, whereas Robusta (3.6 Rli) was moderately resistant.

The level of phenol content was significantly higher in the uninfected resistant cultivar (0.76mg/g of fresh root) than in the susceptible cultivar, with a phenol content of 0.65mg/g of fresh root tissue (Fig. 3). The total phenol content in resistant cultivars after inoculation with *P. coffeae* indicated that the level of phenols progressively increased at 90 dpi ( $P < 0.05$ ). In contrast, the susceptible genotypes Nendran and

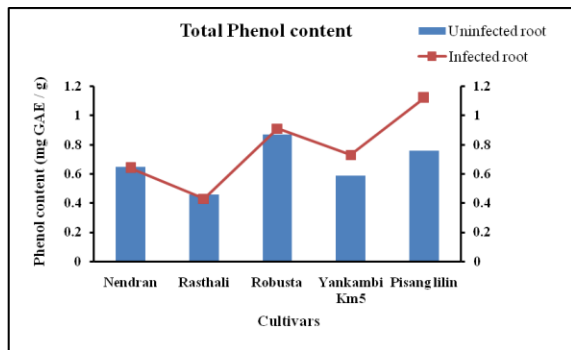
Rasthali showed lower phenolic contents in the infected root tissue than the uninfected root tissues. The resistant factor of phenolic content was higher in the infected cultivars such as Pisang lilin and Yankambi Km5, which were 1.12 and 0.73mg/g, respectively (Table 3). The phenolic content in the infected resistant cultivars was higher compared to the uninfected resistant cultivars.

**Table 3.** Total phenol content in (mg gallic acid equivalents (g fresh root weight)<sup>-1</sup>) infected and uninfected roots of susceptible and resistant *Musa* genotypes against *P. coffeae*.

SN	Cultivars	Genotypes	Total phenol content (mg GAE/ g fresh roots)		Percent differentiation of Total phenol content
			Uninfected	Infected	
1	Nendran	ABB	0.65 <sup>ab</sup> ± 0.08	0.64 <sup>bc</sup> ± 0.07	-1%
2	Rasthali	ABB	0.46 <sup>b</sup> ± 0.05	0.43 <sup>c</sup> ± 0.05	-3%
3	Robusta	AAB	0.87 <sup>a</sup> ± 0.16	0.91 <sup>ab</sup> ± 0.12	4%
4	Yankambi Km5	AAA	0.59 <sup>ab</sup> ± 0.09	0.73 <sup>b</sup> ± 0.08	14%
5	Pisang lilin	AA	0.76 <sup>ab</sup> ± 0.05	1.12 <sup>a</sup> ± 0.08	36%

\*Mean values followed by the same letters are not significantly different ( $P < 0.05$ ) between the *P. coffeae*-infected and uninfected roots of the same genotype.





**Fig. 3.** Evaluation of total phenol content in *Musa* germplasm cultivar roots during pre-infection and post-infection of *Pratylenchus coffeae* nematodes.

### Discussion

Plant-parasitic nematodes constitute an important component of the micro-fauna associated with the rhizosphere of banana plants. The most common species of plant parasitic nematodes associated with banana are *Meloidogyne incognita*, *Pratylenchus coffeae* and *Radopholus similis* in India. These nematode species with different feeding habits generally exist as mixed nematode populations in the banana fields. Of these, *P. coffeae* is an economically important root lesion nematode that infests more than 40 plants, including banana cultivars, and causes more yield losses (Ploetz *et al.*, 2003; Queneherve *et al.*, 2009; Araya *et al.*, 2016).

In the present investigation, the field survey and screening studies suggested that the incidence of nematode damage was higher among the commercial genotypes Nendran and Poovan, which are susceptible under suitable conditions ( $23 \pm 2^\circ\text{C}$ ). Nematode invasion into the root paves the way for other nematodes and secondary pathogens from the soil that can further infect the roots through the wound made by the nematodes. Nematode infestation causes reddish brown lesions on the roots at the place of entry, migration and feeding, which leads to reduction of root growth, stunting and root distortion (Kyndt *et al.*, 2013; Fosu-Nyarko and Jones 2015).

In the present study, nematodes were identified based on morphology and were characterized according to the method of Luambano *et al.* (2019) and Castillo *et al.* (2012). However, data on nematode incidence

revealed that a large number of plant-parasitic nematodes are present in the banana roots in the fields of major banana belts in Tamil Nadu. Of these, *P. coffeae* and *M. incognita* were found in the fields, and in particular *P. coffeae* was one of the most abundant and prominent nematodes affecting banana cultivars. It poses a serious threat to banana production in Tamil Nadu and, most probably, throughout India.

In the present investigation, the *Musa* germplasm cultivars of Nendran, Rasthali (ABB) and Robusta (AAB) were found to be largely affected by the root-lesion nematodes due to their susceptibility, whereas a range of other *Musa* cultivars such as Pisang lilin (AA) and Yankambi Km5 (AAA) are resistant due to some defense responses. However, host resistance is an important factor in nematode management and is an alternative to chemical control. Resistant genotypes are defined as those supporting lower levels of nematode population compared with susceptible genotypes (Roberts 2002; Cook and Starr 2006). Previous studies have also reviewed the level of nematode-resistant crop varieties by Williamson and Roberts (2009) and Starr *et al.* (2010).

The cultivars Nendran and Rasthali supported a high nematode population density of 209.3 and 139.3, respectively, as observed in 10g of root tissues. A very low population density was found in Pisang lilin (35.6) and Yankambi Km5 (46), which could presumably be due to a suppressive effect or a resistance response on *P. coffeae*. This result further indicated that these cultivars could be resistant to *P. coffeae* and could be used in nematode control management. A similar concept was reported by Sundararaju *et al.* (2003). Moens *et al.* (2005) also observed that the population of *P. coffeae* was reduced in Yankambi Km5 compared to that in the susceptible cultivar Grande naine. Similarly, the cultivar Nendran was a more suitable host than Poovan for nematode multiplication (Sundararaju *et al.*, 2002). Khan *et al.* (2010) also observed that the rhizospheres of Matti, Krishna Kanthali and Kanchkela have a high population density of *P. coffeae*. A very low population density of *P. coffeae* was found in the wild-type

banana. This indicated that the wild-type-seeded banana was used for the development of nematode-resistant plants through breeding.

Our pathogenicity trial indicated that *P. coffeae* inoculation levels caused substantial damage to the susceptible cultivars Nendran and Rasthali, and there was low infestation in the resistant genotypes Pisang lilin and Yankambi Km5. Price (1994) reported that some triploid cultivars such as AAA, AAB and ABB are partially resistant to the root-lesion nematode *P. goodeyi*. However, the cultivars Paka and Kunnan were also found to be resistant to *P. coffeae* (Collingborn and Gowen 1997; Gowen *et al.*, 2005; Nguyen *et al.*, 2015). Queneherve *et al.* (2009) also reported that 12 of 30 diploid cultivars, including Pisang lilin and Calcutta 4, exhibited partial resistance to the root-lesion nematode.

Devarajan and Rajendran (2002) reported that the response of banana cultivars to nematodes is most probably due to the presence of chemical constituents. Phenols are secondary metabolites extensively found in plants and have been identified as important compounds in the plant defence response to the root-lesion nematode *P. coffeae* (Vaganan *et al.*, 2014). Resistant accessions had more phenolic compound contents and higher lignification. Post-infectious resistance response such as the accumulation of phenolic compounds was induced in the cultivars Gros Michel and Yankambi Km5 (Vallette *et al.*, 1997; Collingborn *et al.*, 2000). Fogain (1996) and Valette *et al.* (1997) found higher amounts of phenol compounds in the resistant cultivar Yankambi Km5. Nicholson and Hammerschmidt (1992) reported that in the defence mechanism of plants against pathogen invasion, phenolic compounds play a key role in limiting nematode population in host tissues. Phenols are primarily produced through the shikimic acid-phenyl propanoid pathway and range from simple phenols, such as cinnamic acid, to complex phenol polymers such as lignin (Kubalt 2016). Phenol is a bio-molecule that is rapidly synthesized after microbial infection and can be polymerized by oxidative enzymes into cell walls as lignin (Matern and Kneusel 1988).

Phenols also help in neutralizing reactive oxygen species that are produced in plants as an immediate defence mechanism against pathogenic attack (Sharma *et al.*, 2012). Nithya Devi *et al.* (2009) reported that phenolic and lignified compounds were observed in the damaged cortex and cavities in the cortical parenchyma of susceptible genotypes after the invasion of nematodes. Phenols are also known to be involved in plant pigmentation, growth, signalling molecules and reproduction (Mandal *et al.*, 2010).

### Conclusion

Based on our study results, we suggest that banana roots are susceptible to attack from the severe nematode species of *P. coffeae*, which occupy large densities and form root lesions to reduce the yield of banana. We conducted screening studies to identify resistant genotypes to develop nematode management strategies. Nematode infection appears to activate secondary metabolites in roots that in turn trigger the defence response against the pathogen. In this metabolism, the phenyl propanoid pathway is of critical importance for phenolic compounds to protect the plants against nematodes. Our results have clearly indicated that phenolic compounds also have strong potential against nematodes and are hence involved in the defence mechanism of the banana plant. This information can be further useful for breeding programmes to develop cultivars that are resistant against *P. coffeae* to improve banana production.

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### Competing Interests

The authors declare no competing interests.

### Authors' contributions

This study is the part of the Ph D thesis of the first author GT who carried out the research under the

guidance of Assistant Professor SM. GT, PB and AA helped during sample collection. The article was drafted by GT, SM and SJ. The revision was made by SM, PB, SJ and GT. All authors have read and approved the final version of the manuscript.

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