

## Short Communication:

# Abundance and diversity of plant parasitic nematodes associated with BP 308 and BP 42 clones of robusta coffee in Java, Indonesia

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**Abstract.** Mutala'liah, Indarti S, Putra NS. 2018. Abundance and diversity of plant parasitic nematodes associated with BP 308 and BP 42 clones of robusta coffee in Java, Indonesia. *Biodiversitas* 19: xxxx. Plant parasitic nematodes are an important limiting factor in the productivity of coffee plantations. Clone resistance and soil texture influence the severity of plant infestation by parasitic nematodes. The aim of this research was to determine the diversity of plant parasitic nematodes in two clone of Robusta coffee (BP 308 and BP 42) on soils with different contents of sand. The research was carried out in Malangsari Field (East Java), Getas Field (Central Java) and Candirotto Field (Central Java). Nematodes were extracted from soil samples by the decanting method using a whitehead tray, while roots sample by the funnel spray method with a 'mistifier'. Differences in diversity of nematode genera between clone and location combinations were analyzed by ANOVA. The results showed that there were five genera associated with Robusta coffee, i.e. *Pratylenchus*, *Helicotylenchus*, *Radopholus*, *Rotylenchulus*, and *Meloidogyne*. With clone BP 308 at the Malangsari Field site where soil contained 31.3 % sand, *Pratylenchus* was the most abundant genus i.e. 6 nematodes/100 mL soil. On the same clone, BP 308, at the Getas Field site where soil contained 26.9 % sand, *Meloidogyne* was the most abundant i.e. 14.4 nematodes/10 g roots. With clone BP 42 at the Candirotto Field site with 25.5 % sand in the soil, *Pratylenchus* was highly abundant i.e. 60 nematodes/10 g roots.

**Keywords:** Abundance, diversity, plant parasitic nematodes, *Coffea canephora* clone.

## INTRODUCTION

Coffee (*Coffea* sp.) is one of the most important economic crops in Indonesia. In 2014, Indonesia exported about 382,750.3 ton of coffee (BPS 2017). The major type of coffee plantation in Indonesia is Robusta, because Indonesia has a tropical climate suitable for production of this coffee. The optimum environment for growing Robusta coffee is at an altitude of about 300-800 masl (Pohlan and Janssens 2016). South Sumatera is the major province producing Robusta coffee (29.3%), followed by East Java (5.5%) and Central Java (3.5%) (Nurbahar et al. 2014).

Plant parasitic nematodes (PPNs) are regarded as destructive pests that can damage coffee plantation (Campos and Vialin 2005). Fourteen genera of plant parasitic nematodes (PPNs) have been found in association with coffee: *Rotylenchulus*, *Pratylenchus*, *Meloidogyne*, *Radopholus*, *Helicotylenchus*, *Xiphinema*, *Macrophostonia*, *Discocriconemella*, *Hemicriconemoides*, *Diptherophora*, *Paratylenchus*, *Hoplolaimus*, *APratylenchus*, and *Longidorus* (Trinh et al. 2009), whereas the dominant genera that cause high yield losses are *Meloidogyne* spp. and *Pratylenchus* spp. (Campos and Vialin 2005). The nematode believed to be most limiting of coffee production in Indonesia is the species *Pratylenchus coffeae* (Wiryadiputra and Tran 2008).

Plant parasitic nematodes generally live in soil and roots. Soil abiotic factors significantly influence the development of PPNS. These include physical and chemical

soil characteristics such as soil texture, soil pH, soil RH, soil temperature, and organic matter. Each component can influence directly or indirectly PPN development (Norton 1989). The objective of this research was to determine the abundance and diversity of PPNS in two Robusta coffee clones.

## MATERIALS AND METHODS

A survey was conducted in three areas of Robusta coffee plantation in Java, i.e. East Java and Central Java. The survey was carried out between August and November in 2016. In East Java, Indonesia, the Malangsari Field owned by PTPN XII was chosen. In Central Java, Indonesia the Getas Field owned by PTPN IX and the Candirotto Field owned by an individual farmer were chosen. The planted clone in both government fields (PTPN XII and PTPN IX) was BP 308, whereas in the farmer's field it was BP 42.

Purposive sampling was used in this survey. Soil and root samples were collected around coffee plants, consisting of three soil cores. For determination of PPNS, soil samples were divided into two depths below ground level i.e. < 30 cm, and 50 cm. Five sub-samples were assessed within each of the two soil depths, while two sub-samples were assessed for each root sample. Nematode extraction from soil sub-samples was carried out using the decanting method with a white-head tray, whereas for root

sub-samples, nematode extraction was carried out using the funnel spray method with a 'mistifier' (Bezooijen 2006). The soil samples were incubated on the tray for one or two days, whereas root samples were incubated for four days.

Soil samples were thoroughly mixed. A 100 mL sub-sample of soil and 10 g of roots were taken for extraction, sieving and decanting (Cobb 1918) through a 40 µm sieve. Plant parasitic nematode genera were observed and counted in a counting dish under the stereo microscope. Population abundance was determined by multiplying the average number of each genus within 5 ml of a 50 ml nematode suspension (Rahman et al. 2014).

For soil texture analyzes, 20 mL samples of soil were sent to the Soil Laboratory in the Agriculture Faculty of Universitas Gadjah Mada, Yogyakarta, Indonesia.

Data were analyzed by ANOVA (R version 3.3.1).

## RESULTS AND DISCUSSION

The three observed fields were at medium altitude: 480, 659, 783 masl for Getas Field, Malangsari Field and Candiroto Field, respectively. The general soil texture was clay-loam in all fields, however there was a different content of sand fraction in each field. Soil of the Malangsari Field was highest in its content of the sand fraction, i.e. 31.3 %; the soil of the Getas Field contained 26.9 % sand and the Candiroto Field 25.5 %.

The data showed that there were five genera of Plant Parasitic Nematodes (PPNs) identified in the Robusta coffee plantations, i.e. *Pratylenchus*, *Helicotylenchus*, *Radopholus*, *Rotylenchulus*, and *Meloidogyne*. All fields were infected by *Pratylenchus*. *Helicotylenchus* was found in Malangsari and Candiroto Fields but not in the Getas Field, whereas *Meloidogyne* was found in the Getas and Candiroto Fields but not in the Malangsari Field. *Rotylenchulus* and *Radopholus* were found only in the Malangsari Field (Table 1).

Plant parasitic nematodes have been recognised as one of the limiting factors in coffee producing areas world-wide. Genera associated with coffee are both endoparasitic and ectoparasitic nematodes. In Nigeria, endoparasitic nematodes were found to be more dominant than ectoparasitic nematodes. The major endoparasite nematodes detected were *Meloidogyne* spp., *Pratylenchus coffeae*, and *Rotylenchulus reniformis* in about 82.51 and 5 % respectively of all soils sampled (Orisajo and Fademi 2012). Ectoparasitic nematodes such as *Helicotylenchus coffeae*, *Xiphinema* spp., *Radopholus* spp., *Criconeimoides xenoplax*, *Scutellonema brachyurus*, and *Trichodorus* spp. were also found.

In our survey, the data showed that endoparasitic nematodes were significant in the coffee plantations i.e. *Pratylenchus*, *Meloidogyne* and *Rotylenchulus*. Ectoparasitic nematodes found were *Helicotylenchus* and *Radopholus*. *Helicotylenchus* was found in both < 30 cm and 50 cm soil depth, whereas *Radopholus* was found only at 50 cm soil depth. Occurrence of *Pratylenchus* was dominant in all fields and the highest abundance was extracted from roots in the Candiroto Field.

**Table 1.** Occurrence of plant-parasitic nematodes in soil and roots of Robusta coffee

Nematode genera	Field location		
	Malangsari	Getas	Candiroto
<i>Pratylenchus</i>	+	+	+
<i>Helicotylenchus</i>	+	-	+
<i>Radopholus</i>	+	-	-
<i>Rotylenchulus</i>	+	-	-
<i>Meloidogyne</i>	-	+	+

Note: (+): exist, (-): absent

In the Malangsari Field, there were found 4 genera of PPN i.e. *Pratylenchus*, *Helicotylenchus*, *Radopholus*, and *Rotylenchulus* (Figure 1). The most abundant was *Pratylenchus* both in the < 30 cm depth of soil and in the roots i.e. 6 nematodes/100 mL soil and 5.57 nematodes/10 g roots, respectively. The abundance of two nematode genera (*Pratylenchus* and *Rotylenchulus*) in the roots differed significantly, i.e. 5.57 and 0.66 nematodes/10 g roots, respectively. There was not a significant difference between the abundances of the nematode genera at the 50 cm soil depth: *Pratylenchus* at about 1.34/100 mL soil; *Helicotylenchus* at about 0.66/100 mL soil; and *Radopholus* at about 2 /100 mL soil (Figure 1).

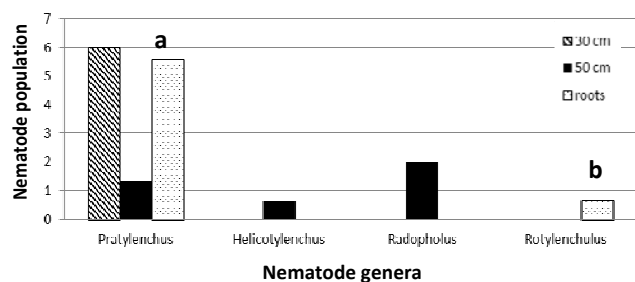
Figure 2 shows that two genera of PPN were found in the Getas Field i.e. *Pratylenchus* and *Meloidogyne*. On the roots, *Meloidogyne* was dominant i.e. 14.43 nematodes/10 g roots, but its abundance was not significantly different from the abundance of *Pratylenchus* at about 6.7 nematodes/10 g of roots. In the soil, *Pratylenchus* was found at about 2 nematodes/100 mL soil in < 30 cm depth. No nematode genera were detected at the 50 cm soil depth.

In Candiroto Field with BP 42 clone, there were found three genera of PPN i.e. *Pratylenchus*, *Helicotylenchus*, and *Meloidogyne*. Abundance of *Pratylenchus* in the roots was dominant as about 60 nematodes/10 g roots and it was significantly different from the abundance of *Meloidogyne* i.e. 1.65 nematodes/10 g roots. In the soil at < 30 cm depth, abundance of *Helicotylenchus* (32 nematodes/100 mL soil) was higher, but not significantly, than abundance of *Pratylenchus* (26.68 nematodes/100 g of soil). There were no nematode genera found at the 50 cm soil depth (Figure 3).

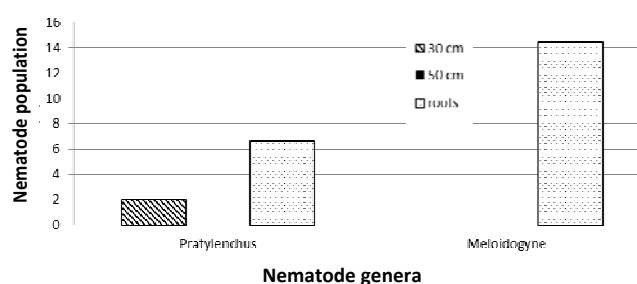
In Getas Field, the abundance of *Meloidogyne* from extracted roots sampled was higher than for *Pratylenchus*, whereas in the Candiroto Field, *Pratylenchus* abundance was higher than *Meloidogyne* abundance. It is likely that this difference is a result of differences in factors such as the kind of coffee clone grown and the textures of the soil in the fields.

BP 308 is a clone that was developed in order to be highly resistant to infection by *Pratylenchus coffeae* and *Radopholus similis*, whereas BP 42 was assessed to be a clone susceptible to attack by PPNs (Mawardi et al. 2004). Clone BP 42 was the clone planted in the farmer's field at Candiroto whereas BP 308 was the clone planted in the two government fields. Data for the BP 308 clone showed that the Malangsari Field was not infected by *Meloidogyne* but the Getas Field was infected by *Meloidogyne*. This suggests that the BP 308 clone is not resistant to

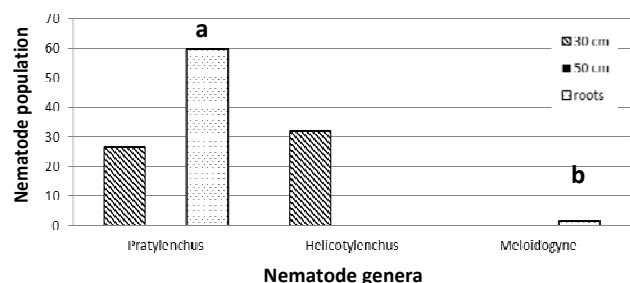
*Meloidogyne*; its abundance in the Getas Field was higher than the abundance for *Pratylenchus*. Despite the reputed resistance of BP 308 clone to *Pratylenchus*, we detected some presence of *Pratylenchus* in this clone, particularly in the Malangsari Field. This could be a result of the optimal environmental conditions for the development of *Pratylenchus*.



**Figure 1.** Total population of nematode genera (mean of 5 replications) on each parameter observed (< 30, 50 cm of soil depth, and on roots) in BP 308 clone Malangsari Field. The different letters above the columns indicate significant differences between them, according to Duncan's multiple range test ( $P < 0.05$ ). Columns without letters in each parameter indicate no significant differences.



**Figure 2.** Total population of nematode genera (mean of 5 replications) on each parameter observed (< 30, 50 cm of soil depth, and on roots) in BP 308 clone Getas Field.



**Figure 3.** Total population of nematode genera (mean of 5 replications) on each parameter observed (< 30, 50 cm of soil depth, and on roots) in BP 42 clone Candirotto Field. The different letters above the columns indicate significant differences between them, according to Duncan's multiple range test ( $P < 0.05$ ). Columns without letters in each parameter indicate no significant differences.

The existence of *Meloidogyne* and *Pratylenchus* in coffee plantations has been studied in Nicaragua under two kinds of management system, i.e. conventional and organic systems (Herrera et al. 2011). Abundance of *Meloidogyne* was greater than that of *Pratylenchus* in the five management systems investigated but the relative abundance of the two nematode genera was affected by the particular ecological conditions prevailing under the management systems. The results of the study by Herrera et al 2011 align with the findings in our study that in the Getas Field *Meloidogyne* abundance was higher than *Pratylenchus*. However, they are contrary to what we found in the Candirotto Field, where the abundance of *Pratylenchus* was significantly higher than the *Meloidogyne* abundance. It is likely that differences exist between *Pratylenchus* and *Meloidogyne* as to their preferred ecological niches. *Pratylenchus* is a migratory endoparasite that usually lives in the cortex of roots and is able to penetrate primary, secondary as well as tertiary roots. However, *Meloidogyne* is a sedentary endoparasite that lives in the stele of roots and is only able to penetrate the tip of secondary and tertiary roots system (Duyck et al. 2009). When *Pratylenchus* has previously infected roots, *Meloidogyne* are not able to infect the same roots. It could be that the *Pratylenchus* infection of the roots forms lesions that inhibit subsequent penetration by *Meloidogyne*. However, it is found that if roots have previously been infected by *Meloidogyne* then *Pratylenchus* does have some chance to subsequently infect the roots.

Soil texture is an abiotic factor that is likely to affect the potential damage to coffee plants caused by PPNs. Most PPNs have optimum development on sandy soils. On cereal crops in Uganda, *Meloidogyne* spp. and *Pratylenchus zae* are dominant in sandy soils and their abundance decreases in clay soils (Talwana et al. 2008). *P. zae* was found to be highly abundant on loamy soil of sugarcane crops in South Africa (Dana et al. 2002). Reproduction of *M. incognita* was greater in soils with 80% sand than in soils with a 70% sand fraction. In a study of cotton crops in Arkansas, it was found that the higher the sand content of the field the more severe were the infestations by *M. incognita* (Jaraba et al. 2009). In our study, the data showed sand content of the Getas Field soil was higher than the Candirotto Field soil and the *Meloidogyne* abundance was also higher in Getas than in the Candirotto Field.

*Pratylenchus brachyurus* and *M. incognita* have been found to dominate at 15-30 cm soil depths in soybean crops in Florida (McSorley and Dickson, 1990). Hulupi and Mulyadi (2007) demonstrated that *P. coffeae* was dominant around the root systems in soils of < 30 cm depth. Our data showed that *Pratylenchus* was found in the Malangsari Field at 50 cm soil depth. This perhaps is due to a greater content of the sand fraction at this depth in the Malangsari Field. The higher sand content of the Malangsari Field perhaps promotes greater *Pratylenchus* infection of the roots, because BP 308 clone has a long root system, about 76 cm in length (Mawardi et al. 2004).

This is the first report about genera diversity of PPNs associated with Robusta coffee clones grown on soils of different soil textures. *Pratylenchus* was the nematode

genus with highest abundance in the Malang Sari Field of the BP 308 coffee clone and the Candiroto Field of the BP 42 clone, whereas in the Getas Field of the BP 308 clone the highest abundance was in the genus *Meloidogyne*.

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