

Short Communication: Nematodes associated with Robusta coffee plantations in Malang District, East Java, Indonesia

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Abstract. Tarno H, Marsudi EW, Widjayanti T, Setiawan Y. 2021. Short Communication: Nematodes associated with Robusta coffee plantations in Malang District, East Java, Indonesia. *Biodiversitas* 22: 3306-3312. Coffee (*Coffea* spp.) is an important commodity in Indonesia. Nematodes have different roles, such as plant parasites and non-parasites, and are commonly found in the soil of coffee plantations. This research determined the ecological role, diversity, and abundance of nematodes in three coffee plantations in Malang District, East Java. We used a Whitehead tray to extract and isolate nematodes from the soil and root samples. Nematodes were identified based on their morphological characteristics. Analysis of variance and diversity indices were used to identify the differences among three coffee plantations. We identified eight nematode genera in the Ngantang sub-district site. In the Jatikerto Agrotechnopark and University of Brawijaya Forest sites, six genera were identified. *Criconemoides*, *Pratylenchus*, *Xiphinema*, *Helicotylenchus* are plant-parasitic nematodes, and *Mononchus*, *Dorylaimus*, *Rhabditis*, and *Aphanolaimus* are non-plant parasitic nematodes. Non-plant parasitic nematodes were more abundant than plant-parasitic nematodes in all sites. Non-plant parasitic nematode, *Dorylaimus* was the dominant genus in this study (272 individuals), ca. 35% of the total nematodes collected. In this study, the differences between coffee plantations and soil pH influence nematode abundance. The nematode abundance increases when the soil pH is lower.

Keywords: *Coffea canephora*, coffee plantations, diversity, ecological role, nematode abundance

INTRODUCTION

Coffee crops that are widespread in tropical areas (Adhikari et al. 2020), provide income sources for farmers, materials for industrial production, a foreign exchange commodity, and employment, trading, and marketing activities (Sarirahayu and Aprianingsih 2018). Arabica (*Coffea arabica*) and Robusta (*C. canephora*) species have the highest economic value (Dias and Benassi 2015). *Coffea arabica* (ca. 75% of global coffee exports) is produced in more than 60 countries. Central and South America produce the most *C. arabica* (Krishnan 2017), more than 40% of all Arabica coffee is produced in Brazil (Zullo et al. 2011), and Robusta is produced mostly in West Africa and South Asia (Byrareddy et al. 2019). In Indonesia, Robusta, Arabica, and Liberica (*C. liberica*) are the commonly cultivated coffee plants (Byrareddy et al. 2019). The optimum environment for growing Robusta coffee is 300–800 meters above sea level (masl) (Adem et al. 2017). Most Robusta coffee is produced in the provinces of South Sumatra (29.3%), East Java (5.5%), and Central Java (3.5%) (Nurbahar 2015).

Soil provides a habitat for various macro and microorganisms, including nematodes. Some nematode species are free-living (non-plant parasitic) fungivores, bacterivores, predators, and omnivores (Hossain et al. 2016), while others parasitize plants, attacking their roots (Lazarova et al. 2021). Several parasitic nematode species

cause damage to their hosts, which can include trees, grasses, foliage plants, and agriculture crops (Matute et al. 2012). *Meloidogyne* (root-knot nematodes), *Radopholus* (root-cavity nematodes), *Pratylenchus* (root-lesion nematodes), *Rotylenchulus*, and *Helicotylenchus* are the main parasitic nematode genera threatening agricultural crops. Several genera of nematodes are associated with coffee plants, and in many countries (Brazil and Mexico in particular), *Meloidogyne* spp. nematodes are causing significant damage to coffee crops, resulting in huge economic losses (Jones and Fosu-Nyarko 2014). Parasitic nematodes have been cited as substantial limiting factors in coffee plantations and coffee-producing areas worldwide (Asyiah et al. 2020).

Parasitic nematode infections can be difficult to identify because they attack the below-ground parts of the plant. Early symptoms of nematode attacks can include wilting, slow declines of plant growth, and yellowing foliage (Khan 2020), followed by stunting, flower loss, and, eventually, death. In general, plants affected by nematodes wither easily during the day, especially during the dry season. Nematode attacks in conjunction with pathogenic fungi or bacterial infection can cause more severe crop damage (Mitiku 2018). Apart from parasitic nematodes, several genera of non-plant parasitic nematodes, such as the omnivorous genus *Dorylaimus*, are associated with coffee plants (Renco et al. 2019). In addition, predatory nematodes play an important role in nematode

communities, acting as agents of biological control and bio-indicators of ecological change (Cock et al. 2012). Ecological factors, such as altitude, plant diversity, precipitation, and temperature, affect nematode distribution, diversity, and abundance (Nisa et al. 2021). Soil abiotic factors, including temperature, pH, water-holding capacity, and mineral composition, also influence nematode abundance and communities (Sholeha et al. 2017; Van Den Hoogen et al. 2019). Low soil pH can affect soil communities and food webs (Matute et al. 2012; Kalinkina et al. 2019), and 5 to 7 of pH is ideal for the growth of both parasitic and free-living nematodes (Knox et al. 2020). The addition of inorganic and organic fertilizers can alter soil pH, as well as porosity and texture, increasing the distribution, diversity, and abundance of nematodes in soil habitats (Mahmood et al. 2017). The diversity of nematodes is not often studied in coffee plantations in Malang District. This research investigated the ecological role, diversity, and abundance of nematodes in coffee plantations in Malang District, East Java.

MATERIALS AND METHODS

Sampling area

Soil sampling was conducted at three coffee plantation areas located at different altitudes in Malang District, East Java, Indonesia: Jatikerto Agrotechnopark, Kromengan sub-district; Ngantang sub-district; and University of Brawijaya (UB) Forest, Karangploso sub-district (Figure 1 and Table 1). The sampling location had the different soil properties are shown in Table 1. At the Jatikerto Agrotechnopark, Kromengan sub-district site, the crops were composed by *Robusta* coffee, jumbie bean (*Leucanea* spp.), banana (*Musa* spp.), and ginger (*Zingiber officinale*). *Robusta* coffee, durian (*Durio zibethinus*), pineapple (*Ananas comosus*), banana, and bamboo (Bambusoideae) were identified at the Ngantang sub-district site. Finally, vegetation in the UB Forest consisted of *Robusta* coffee and pine (*Pinus merkusii*).

Sampling procedures

Soil and root samples were collected from ten randomly selected coffee trees at each location (Widowati et al. 2014). Soil and root samples were dug from the root area using a hoe at approximately 20-cm depth in April 2019.

Soil properties (soil temperature, organic matter, soil texture, soil moisture, and pH) and coffee cultivation systems were determined at each sampled location.

Nematode extraction and isolation

A Whitehead tray was used to extract and isolate nematodes from the soil and plant root samples. This method was modified from the Baermann funnel method (Cesarz et al. 2019). Soil (100 g) and root (10 g) samples were spread on tissue paper on top of a coarse filter and watered until fully soaked. The samples were incubated on the tray for 24 hours before the nematodes were extracted by sieving and decanting (Senthilkumar et al. 2021). Nematode extraction was repeated five times (ca. 24 hours for each). After decanting, the sediment was transferred onto a 365-mesh sieve placed on a petri dish (90-mm diameter). The suspension was homogenized using a syringe, and approximately 5 ml was poured into the counting dish. The number of nematodes was counted under a stereomicroscope. The nematodes were subsequently fixed in anhydrous glycerin and mounted on slides using a paraffin ring for identification (Ryss 2017). Nematode counts were calculated by multiplying the average number of individuals in each genus in 5 ml to reach the total volume of the sample (Rahman et al. 2014).

Nematode identification

Nematodes were identified using pictorial keys and identification books (Goodey and Goodey 1963; Tarjan et al. 1977; Mai 1988). The morphological characteristics used for identification were the lip shape, style shape, esophageal shape, reproductive organ type, knob shape, tail shape, and body length.

Data analysis

Nematodes population counts at each location were analyzed using Analysis of Variance (ANOVA) followed by Duncan's Multiple Range Test (DMRT). The Shannon–Wiener index (H'), Simpson's dominance index (1-D), and evenness index (E) (Krebs 1999; Tarno et al. 2016) were adopted to evaluate nematode diversity at each location. All data were analyzed using the vegan package in R version 3.3.3 (Oksanen 2015; R Core Development Team 2019). Results were considered statistically significant at $P < 0.05$.

Table 1. Sampling location soil properties, site coordinates, and elevations

Locations	pH	Soil moisture (%)	Soil temp. (°C)	Soil texture	Organic matter (%)	Latitude	Longitude	Altitude (masl)*
Jatikerto Agrotechnopark	5.4	52.50	28.00	Silty clay	3.05	8°07'31.0"S	112°31'47.1"E	340
Ngantang	5.8	98.70	22.14	Clay loam	4.60	7°50'08.7"S	112°22'34.8"E	761
UB Forest	6.0	73.50	25.60	Silty clay loam	3.80	7°49'28.2"S	112°34'44.7"E	1,237

Note: *m asl is meters above sea level

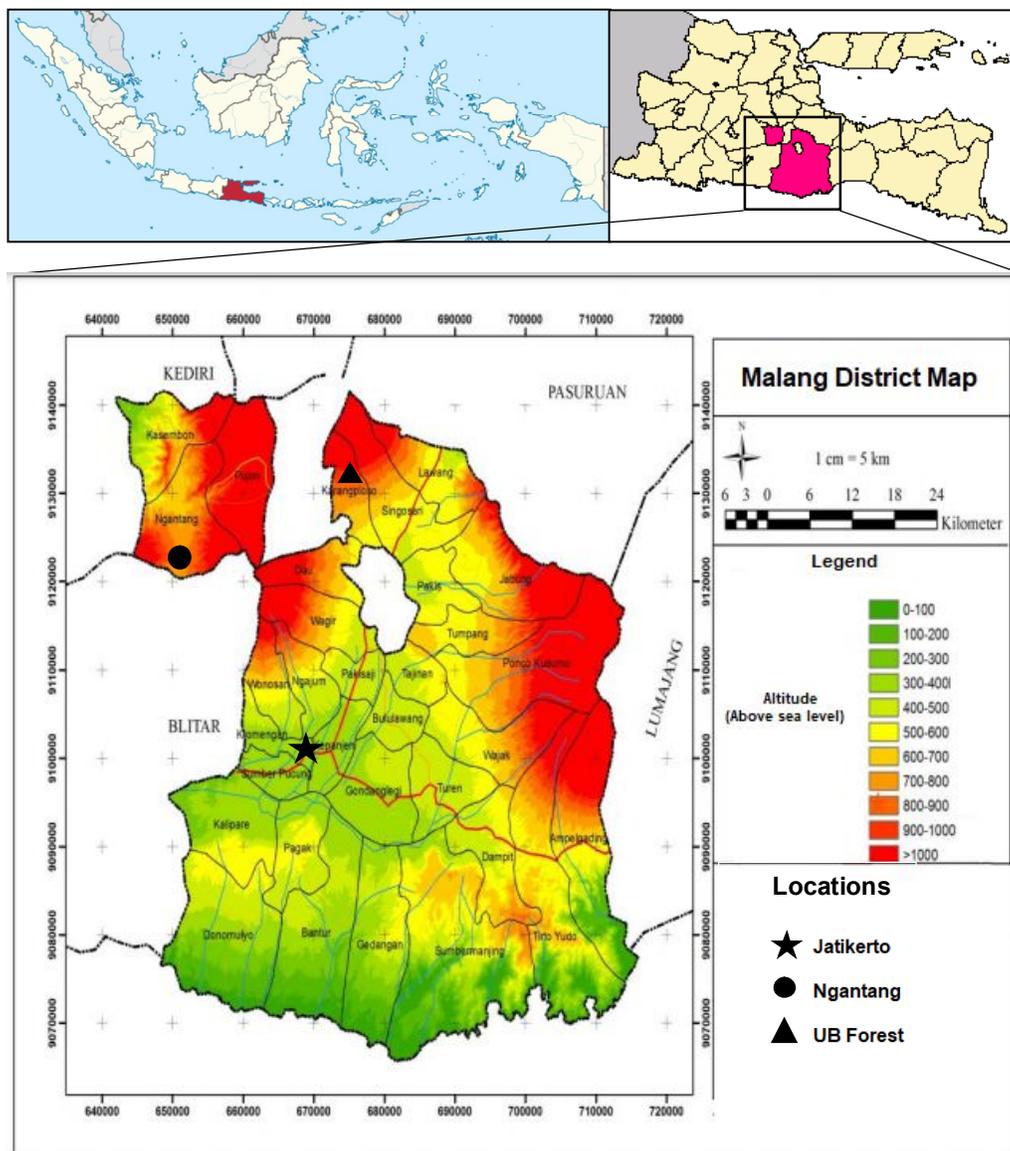


Figure 1. Study sites where nematodes in soil and root samples were collected from coffee plantations in Malang District, Indonesia

RESULTS AND DISCUSSION

The ecological roles of nematodes in coffee plantations in Malang District

In Jatikerto Agrotechnopark, six genera of nematodes were identified: *Criconemoides*, *Pratylenchus*, *Mononchus*, *Dorylaimus*, *Aphanolaimus*, and *Rhabditis*. In Ngantang sub-district, eight genera were identified: *Xiphinema*, *Criconemoides*, *Helicotylenchus*, *Pratylenchus*, *Dorylaimus*, *Mononchus*, *Rhabditis*, and *Aphanolaimus*. Six genera, *Criconemoides*, *Pratylenchus*, *Mononchus*, *Helicotylenchus*, *Rhabditis*, and *Dorylaimus*, were collected in UB Forest (Table 2). All identified genera were classified into three orders, Tylenchida, Dorylaimida, and Rhabditida. The nematodes collected in this study play various ecological roles. *Criconemoides*, *Pratylenchus*,

Xiphinema, and *Helicotylenchus* genera are plant-parasitic nematodes (Swibawa 2010). *Pratylenchus* spp. were the dominant plant-parasitic nematodes (95 individuals), accounting for 12.22% of all nematodes collected. *Criconemoides*, *Xiphinema*, and *Helicotylenchus* are ectoparasitic nematodes. *Dorylaimus* spp., which are non-plant parasitic nematodes, were the dominant species collected, accounting for 35% (272 individuals) of the total nematodes in the soil and roots. *Mononchus*, *Dorylaimus*, *Rhabditis*, and *Aphanolaimus* genera are all classified as free-living nematodes (Mirsam et al. 2020). The ecological roles of *Mononchus* and *Dorylaimus* are predatory and omnivorous, respectively; *Rhabditis* and *Aphanolaimus* are bacterivorous (Mirsam et al. 2020).

The abundances of nematode genera in each ecological role varied among the three sampling locations. The

number of *Rhabditis* individuals was higher in Jatikerto Agrotechnopark compared to the other locations. The 65 known species of *Rhabditis* are bacterivores and fungivores that decompose soil (Rønn et al. 2012). At the Ngantang sub-district location, *Mononchus* dominated the community and was found in greater numbers than at the other locations. The abundance of the *Mononchus* spp. may be influenced by the presence of prey. Widowati et al. (2014) stated that as predators of other nematodes, the *Mononchus* genus is typically found in areas where other nematodes are present, especially plant-parasitic nematodes. Predatory nematodes contribute to nitrogen mineralization in integrated agricultural systems and conventional agriculture (Yadav et al. 2018). At the UB Forest site, *Dorylaimus* species were more abundant than in other locations. Species belonging to the *Dorylaimus* genus are omnivorous nematodes (Eskandari et al. 2016). McSorley (2012) reported that several genera of omnivorous nematodes, such as *Aporcelaimellus*, *Eudorylaimus*, and *Mesodorylaimus*, are found in nearly all successions, often reaching the greatest numbers at the succession stage.

Individuals of the *Pratylenchus* genus were present in all three locations in this study as plant-parasitic nematodes. Huyen et al. (2020) reported that the most common nematode species found in coffee plants is *Pratylenchus coffeae*. *Pratylenchus* species are the most commonly studied nematodes on coffee plants, as they are destructive pests (Thiep et al. 2019). *Pratylenchus coffeae*, initially described as attacking coffee plantations in Java, is among the most widely distributed species of root-lesion nematodes in coffee plantations worldwide (Budiman et al. 2019). Fanelli et al. (2018) reported that *Pratylenchus* nematodes are migratory endoparasites, commonly called root-wound or root-lesion nematodes.

Nematode diversity and abundance in coffee plantations in Malang District

The Shannon–Wiener diversity index in the Jatikerto Agrotechnopark, Ngantang sub-district, and UB Forest were 1.39, 1.28, and 1.31, respectively (Figure 2). The index values, which range from 1 and 3, were categorized

as moderate diversity and moderate species distribution (Tarno et al. 2016) at all three sites. The evenness values in the Jatikerto Agrotechnopark, Ngantang sub-district, and UB Forest were 0.75, 0.61, and 0.73, respectively, indicating moderate evenness (Tarno et al. 2016). The Simpson's dominant index values in the Jatikerto Agrotechnopark, Ngantang sub-district, and UB Forest were 0.30, 0.33, and 0.34, respectively. According to Krebs (1999), values less than 0.5 indicate no dominance in genera and species.

The total number of individual nematodes collected in the UB Forest sample (112 individuals) was significantly lower ($F = 4.36$, $P = 0.03$) than in Jatikerto Agrotechnopark (350 individuals) and Ngantang sub-district (315 individuals) samples (Figure 3). The vegetation at each location also had different plant species. The UB Forest site only had coffee plants and pine trees, representing lower vegetative diversity than the Jatikerto Agrotechnopark and Ngantang sub-district sites. This finding suggests that the lower plant diversity may have affected the nematode populations at the UB Forest site. Nematode abundance is strongly influenced by plant communities and diversity (Sohlenius et al. 2011; Viketoft and Sohlenius 2011; Wang et al. 2019). Yan et al. (2020) reported that declining plant diversity decreases the abundance of nematodes in the soil. Other factors that influence nematode abundance include soil temperature, moisture, organic matter, and pH. Our analysis showed that soil moisture ($r = -0.08$, $P = 0.829$), temperature ($r = 0.03$, $P = 0.993$), and organic matter ($r = -0.12$, $P = 0.762$) were not correlated with nematode abundance. However, soil pH was negatively correlated with nematode abundance ($r = -0.84$, $P < 0.001$). Siepel et al. (2019) reported that increased pH (soil acidity) can induce faster reproduction in some nematode genera and species. Matute et al. (2012) also reported that the abundance of nematodes associated with *Brassica rapa* (fungivores, bacterivorous, predatory, and plant-parasitic) was negatively correlated to soil pH. Soils with acidic pH increase nematode reproduction and enhance soil microbes (Nisa et al. 2021).

Table 2. Numbers of individuals in each nematode genera collected from coffee plantations in Malang District, East Java, Indonesia

Genera	Number of nematodes (Individuals/100 ml suspensions)			Total no. of individuals (N)	(% of N total)	Ecological role
	Jatikerto	Ngantang	UB Forest			
<i>Dorylaimus</i>	118	100	54	272	35.00	Omnivorous
<i>Rhabditis</i>	138	33	21	192	24.71	Bacterivorous
<i>Mononchus</i>	50	132	8	190	24.45	Predatory
<i>Pratylenchus</i>	24	46	25	95	12.22	Plant Parasite
<i>Criconemoides</i>	14	1	1	16	2.05	Plant Parasite
<i>Aphanolaimus</i>	6	1	0	7	0.90	Bacterivorous
<i>Helicotylenchus</i>	0	1	3	4	0.51	Plant Parasite
<i>Xiphinema</i>	0	1	0	1	0.12	Plant Parasite
Total no. of individuals	350	315	112	777	100	
Total no. of genera	6	8	6			

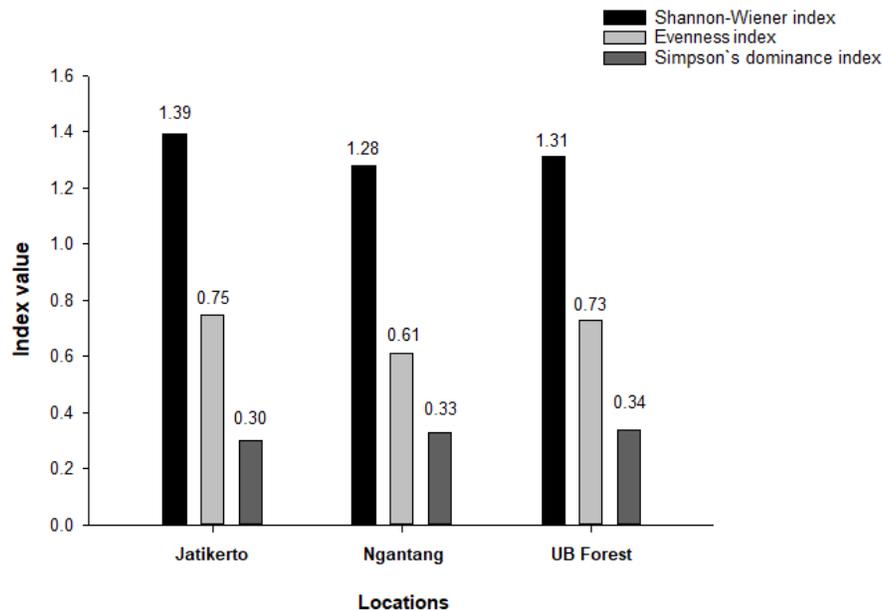


Figure 2. Shannon–Wiener, Evenness, and Simpson’s indices for total nematodes collected in three coffee plantation locations in Malang District, East Java. (Shannon–Wiener Diversity index: < 1 , low diversity, 1–3, moderate diversity, > 3 , high diversity. Species evenness index: $0.00 < E < 0.50$, low evenness; $0.50 < E < 0.75$, medium evenness; $0.75 < E < 1.00$, high evenness. Simpson’s dominance index: $0 < D \leq 0.5$, no dominance; $0.5 > D \geq 1$, dominance.)

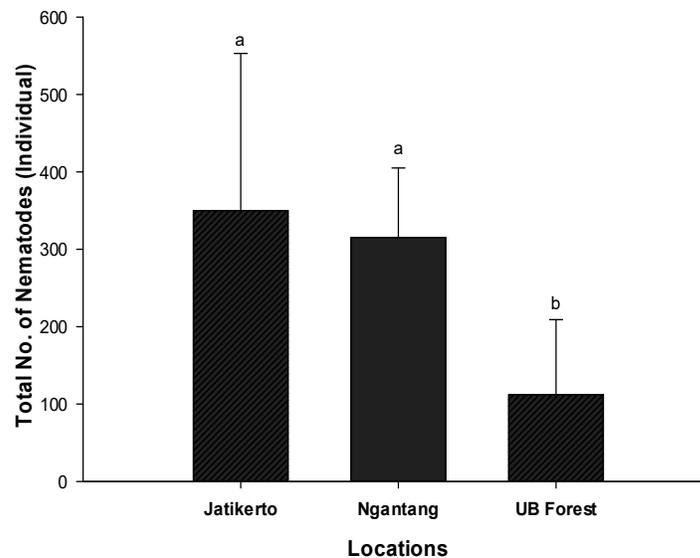


Figure 3. The total number of nematodes collected in root and soil samples from coffee plantations in Jatikerto Agrotechnopark, Ngantang sub-district, and UB Forest. Different lowercase letters denote significant differences at $P < 0.05$ (ANOVA; DMRT)

In conclusion, nematodes belonging to eight genera were identified in the Ngantang sub-district and six genera in the Jatikerto Agrotechnopark and UB Forest. *Criconemoides*, *Pratylenchus*, *Xiphinema*, and *Helicotylenchus* are plant-parasitic nematodes, and *Mononchus*, *Dorylaimus*, *Rhabditis*, *Aphanolaimus* are non-plant parasitic nematodes. Non-plant parasitic nematodes were more abundant than plant-parasitic

nematodes. The omnivorous *Dorylaimus* genus was most dominant (ca. 35% of all nematodes collected). Plant diversity and soil pH are the influence factors on the abundance of nematodes in this study. Nematode abundance increased with lower pH. The plant-parasitic nematodes in each location can potentially damage coffee plants.

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