Phytonematodes associated with arabica coffee in Bondowoso, East Java

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Abstract. Phytonematode is one of the important pathogen infecting Arabica coffee plantation. This study is aimed to identify phytonematodes associated with the declining Arabica coffee plantation in Bondowoso, East Java. Root and soil samples were taken from the symptomatic coffee plants at Belawan and Kalisat Jampit plantations. Phytonematode were extracted from root samples by mist chamber and soil samples by centrifugation flotation methods. Five phytonematodes, that are Radopholus similis, Meloidogyne sp., Paratylenchus sp., Criconemoides sp., and Helicotylenchus sp., were identified based on their morphological characters. The highest phytonematode population is R. similis with average of 260 nematodes /10 g roots.

Keywords: morphology, populations, Radopholus similis

1. Introduction

Coffee is one of the main plantation commodities in Indonesia. Arabica coffee has good taste and high prices. Plant disease infections can cause low productivity of Arabica coffee. Leaf rust disease (Hemileia vastatrix) has been known as the main plant disease that cause the most severe damages on Arabica coffee plants [1]. Information related to other diseases caused for instance phytonematodes has not been widely studied in Indonesia. Preliminary study on phytonematodes associated with coffee was reported in 1991 in Indonesia [2]. After the report, there have not been many reports regarding phytonematodes associated with Arabica coffee in Indonesia. Nematodes associated with arabica coffee were previously reported in several countries such as Hawaii [3], Ethiopia [4], Vietnam [5], Nigeria [6], Brazil [7], and Kenya [8].

Phytonematodes associated with Arabica coffee plants have not been widely known in coffee plantations in Bondowoso, East Java. Lack of information about the phytonematodes abundance in coffee plants is a major challenge in the management of phytonematodes [9]. This study aims to determine the population of phytonematodes associated with Arabica coffee in Bondowoso, East Java.
2. Material and Methods

2.1. Sampling
Phytomonadodes sampling were carried out in Arabica coffee plants. Samples were taken from roots and soil. There are 4 observed plantation blocks. Each block was sampled 4 symptomatic plants. The characteristics of symptomatic plants are the less number of main branches and leaves, yellowing leaves starting from the leaves near the main stem, and when the main stem is shaken the plants easily sway [10]. The four plantations blocks are Belawan BP1 (S 8 ° 0.869’E 114 ° 9.654’ 1094 m asl), Belawan BP2 (S 8 ° 0.869’E 114 ° 9,654’ 1088 m asl), Kalisat Jampit KAJA1 (S 8 ° 0.202’E 114 ° 8.456’ 1110 m asl) and Kalisat Jampit KAJA2 (8 ° 0.507’114 ° 8,478’ 1125 m asl) all of which located in Bondowoso, East Java.

2.2. Phytonematode extraction from soil
Extraction of nematodes from the soil used the centrifugation flotation method. Soil samples are mixed and refined until homogeneous. A 100 ml soil sample is mixed with 900 ml of water in a container. The soil suspension is stirred until homogeneous and deposited for ± 40 seconds. The soil suspension is poured into the stack of nematode sieves with pore size of 50, 100 and 400 mesh, respectively. The suspension was poured into a 15 ml capacity centrifugation tube. The suspension was centrifuged for 5 minutes at a speed of 1500 rpm, then the water was removed, and the precipitate was added with a 40% sugar solution then homogenized. The suspension was centrifuged for 1 minute at 1700 rpm. The supernatant was poured through a 400 mesh nematode sieve, rinse with tap water and kept into a 50 ml bottle containing 35 ml distilled water. The nematode suspension is stored in a collection bottle containing 25 ml of a nematode suspension to be immediately observed or stored in a refrigerator at a temperature of 10ºC [11].

2.3. Phytonematode extraction from roots
Extraction of root phytonematodes used a modified mist chamber method [12]. Root samples were washed from soil, then cut with ± 0.5 cm length then weighed as much as 10 g. The root sample is placed on a funnel that has been coated with a 0.2 cm diameter filter. Funnels that contain a root sample are placed inside the mist chamber. Collecting of phytonematodes is carried out after four days of misting with water. The phytonematodes suspension is filtered in the stack with a 50 mesh and 400 mesh nematode sieves. The phytonematodes suspension is stored in a collection bottle containing 25 ml of water. Phytonematodes suspension is ready to be identified or stored in the refrigerator at 10ºC.

2.4. Phytonematode population analysis
The phytonematodes suspension inserted into the counting disk was then observed using a stereoscopic microscope to a magnification of 40 times. Each sample was observed three times (n). Phytonematode was identified morphologically by using the Pictorial Key identification To Genera of Plant-parasitic Nematodes book from Mai and Lyon [13] and related journal for identification to genus or species. The phytonematodes obtained were photographed and the morphological characters described. The calculation formula for phytonematode population is as follows [14]:

\[
\text{phytonematode populations} (p) = \frac{\left(\sum_{i=1}^{n} \frac{p_x V}{v}\right)}{n}
\]

with (p) is the population of phytonematodes species observed on counting dish, (V) is the volume of the suspension of phytonematodes extracted, (v) is the volume of phytonematodes suspension when counting dish, (n) is a repeat observation (in this study 3 times).
3. Result

3.1. Disease symptoms
Soil samples and roots of Arabica coffee plants were taken from symptomatic plants infected with phytonematodes. Symptomatic plants are characterized by the less numbers of primary branches and leaves, and yellowing leaves starting from the leaves close to the main stem (Figure 1).

![Figure 1](image_url). Arabica coffee plants infected phytonematode. The symptoms are characterized by the fewer numbers of primary branches and leaves and yellowing leaves starting from the leaves close to the main stem.

3.2. Extraction results and morphological characters of phytonematodes
Phytonematodes obtained from soil and root extraction of Arabica coffee plants are *Radopholus similis*, *Meloidogyne* sp., *Helicotylenchus* sp., *Paratylenchus* sp., and *Criconemoides* sp. (Figure 2). The morphology key of female *R. similis* is described by the position of the vulva in the middle of the body with the didelphic reproductive system. The male posterior part has dimorphism to its reduced size. The male lip area is shaped rounded, tall, hemispherical, and setoff. The male stylet is unclear and without a basal knob. The long bursa reaches more than two thirds the length of the tail [15]. Key description of Juvenile 2 *Meloidogyne* sp. stylet and knob are well developed and clearly visible, the median bulb is oval, the esophagus is not clearly visible. The tail tip is textured like a jagged pattern and hyaline is clearly visible [16]. The key description of *Helicotylenchus* sp. is a spiral body, hemispherical lip region, well-developed stylet, tail dorsally convex-conoid [13]. Key decryption of *Criconemoides* sp. body stout and slightly curved ventrally, annuli head not setoff, stylet strong with anchor-shaped base, tail conoid and last annuli lobed [13]. Description of the key *Paratylenchus* sp. female head rounded with somewhat truncate anterior end, stylet strong, vulva located in posterior more than a third of the body, tail is conoid, strongly curved ventrally with rounded terminus [17].

3.3. Population density of phytonematode
The population of phytonematodes resulting from the extraction of Arabica coffee roots is presented in Figure 3. *R. similis*, *Meloidogyne* sp. and *Paratylenchus* sp. were extracted which. *R. similis* is found in all Arabica coffee plantation blocks. The highest *R. similis* population was in the BP2 block with a total of 260 nematodes/10 g of roots. The population of phytonematodes extracted in Arabica coffee soil is presented in Figure 4. *Meloidogyne* sp., *Helicotylenchus* sp., *R. similis* and *Criconemoides* sp. which is
Meloidogyne sp. was found in all four blocks observed. The highest population of Meloidogyne sp. was in block BP1 with a population of 23 nematodes/100 ml of soil.

**Figure 2.** Morphology of phytonematode found in Arabica coffee plants. (a) female *Radopholus similis*, (b) male *R. similis*, (c) anterior female *R. similis*, (d) anterior male *R. similis*, (e) vulva *R. similis*, (f) bursa and spicula *R. similis*, (g) Juvenile 2 *Meloidogyne* sp., (h) anterior *Meloidogyne* sp., (i) *Meloidogyne* sp. tail, (j) *Criconemoides* sp., (k) *Paratylenchus* sp., and (l) *Helicotylenchus* sp. Scale bar= a, b, g, j, k, l = 50 µm and c-f, h, i = 20 µm.
Phytonematodes found in each sample were *R. similis* and *Meloidogyne* sp. The results of root extraction in blocks BP1, BP2, and KAJA2 were dominated by *R. similis* while in KAJA1 block was dominated by *Meloidogyne* sp. Phytonematodes with high population at root extraction are in BP2 and KAJA1 blocks. The highest result of soil extraction of phytonematodes population is BP1 block. BP1 block was dominated by *Helicotylenchus* sp., *Meloidogyne* sp., and *Criconemoides* sp.

**Figure 3.** Phytonematode population in Arabica coffee roots (mean ± SE). BP1 = Belawan plantation 1, BP2= Belawan plantation 2, KAJA1 = Kalisat Jampit plantation 1, KAJA2 = Kalisat Jampit plantation 2.

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**Figure 4.** Phytonematode population in Arabica coffee soil (mean ± SE). BP1 = Belawan 1, BP2= Belawan 2, KAJA1 = Kalisat Jampit 1, KAJA2 = Kalisat Jampit 2.

4. Discussion

Arabica coffee observed is grown at an altitude of 600-1500 m asl, which is in accordance with the requirements for Arabica coffee growth [18]. The sample conditions of Arabica coffee planted have a
fewer number of branches and leaves, some yellowing leaves starting from the leaves near the main stem. Symptoms of coffee plants infected with phytonematode have similarities to those described by Hulupi [10]. These symptoms are certainly not only caused by a single factor of phytonematode infection. Symptoms of phytonematode infection are similar to nutrient deficiencies. The general difference between nutrient deficiency and phytonematode infection is if a nutrient deficiency occurs in a large area, whereas nematode infection is usually clustered in small plant groups [19].

Phytonematodes associated with Arabica coffee are categorized based on feeding behavior, *R. similis* as migratory endoparasites, *Meloidogyne* sp. as sedentary endoparasites, *Helicotylenchus* sp., *Paratylenchus* sp. and *Criconemoides* sp. as ectoparasites [20]. Migratory and sedentary endoparasites are the most plant-damaging phytonematode in general [21]. Phytonematode associated with Arabica coffee in Bondowoso are dominated by two endoparasites nematodes, namely *R. similis* and *Meloidogyne* sp. many found in roots. This phytonematodes are considered to play a major role in infection in the roots of Arabica coffee.

*Radopholus similis* and *Meloidogyne* sp. are predominately infecting nematodes in Arabica coffee. Previously, *Meloidogyne* spp. has also been reported to predominantly infect Arabica coffee [5-8], and *Radopholus* spp. also reportedly predominantly infect Arabica coffee [5, 6, 22]. Endoparasites phytonematodes dominate the phytonematodes found in coffee roots. *Helicotylenchus* sp. found the highest population of the ectoparasite phytonematodes group. Previous reports of *Helicotylenchus* were found in Arabica coffee roots in Ethiopia and Hawaii [3, 4]. Although *Helicotylenchus* sp. as an ectoparasite phytonematode was found to be associated with coffee, the phytonematode is minor in coffee [23].

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