Phytonematodes associated with the yellowing disease of Javanese long pepper plants in Sumenep, East Java

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Abstract. One of the major pathogen infecting the Javanese long pepper plantation is phytonematode. This study aims to identify the population of phytonematodes associated with the yellowing disease of Javanese long pepper. Root and soil samples were collected from Javanese long pepper with monoculture and polyculture cropping systems purposively in Sumenep Regency, East Java Province. Phytonematodes were extracted from root samples by mist chamber technique and soil samples by centrifugation flotation technique. Morphology character was observed from the permanent nematode slides. Based on morphological characters, six phytonematodes were found in the root and soil of Javanese long pepper, namely Paratylenchus nanus, Meloidogyne spp., Rotylenchulus reniformis, Rotylenchulus parvus, Coslenchus paramaritus, and Coslenchus cancellatus. The highest phytonematode population from root extraction is P. nanus with an average of 165 nematodes/10 g roots. R. reniformis was the highest phytonematode population from soil extraction with an average of 1314 nematodes/100 ml soil.

Keywords: Disease, Paratylenchus nanus, Rotylenchulus reniformis, morphology

1. Introduction
Javanese long pepper (Piper retrofractum Vahl.) is one of the most important spices crop in Indonesia. The production of Javanese long pepper is used domestically in traditional medicine industries, and herbal medicine [1]. However, in recent years Javanese long pepper is hampered by plant disease, resulting in yield reduction or complete crop loss. Disease symptom includes yellowing leaves, wilting, and root rot. These symptoms were also similar to those observed in the yellowing disease of black pepper. This is thought to be caused by the two plants are from the same genera. 

Yellowing disease or slow decline is one of the essential diseases in pepper plants. This disease causes yield loss significantly [2]. The pathogens associated with yellowing disease in pepper plants were M. incognita and Fusarium solani in West Kalimantan and Lampung, while in Bangka they were Meloidogyne sp., Fusarium sp., and Radopholus sp. [3, 4]. Several nematode genera associated with yellowing disease in pepper plants have been reported in various countries, such as Indonesia, China [5], Vietnam [6], India [7], and others. However, information regarding the yellowing disease of Javanese long pepper has not been widely studied in Indonesia. This study aims to identify the population of phytonematodes associated with Javanese long pepper in Sumenep Regency, East Java Province.
2. Material and methods

2.1. Sampling

Phytonematode sampling were taken from the Javanese long pepper plantation in Sumenep Regency, East Java Province. Root and soil samples were collected from Javanese long pepper with symptoms of yellowing disease. Samples were taken from two blocks and two sub-districts, namely the monoculture block in the Bluto sub-district (MB), the polyculture in the Bluto sub-district (PB), the monoculture in the Saronggi sub-district (MS), and the polyculture in the Saronggi sub-district (PS). Five symptomatic plants were sampled from each block.

2.2. Phytonematode extraction from soil

The centrifugation-flotation method was used to extract nematodes from soil samples. Soil samples were mixed evenly, taken 100 ml and put into a container with 800 ml of water, stirred and allowed to settle for 40 seconds. The soil suspension was then poured through a nematode sieve of 100 mesh and 400 mesh. Nematodes together with soil colloids caught on a 400 mesh sieve were then poured into a 15 ml centrifuge tube. The suspension was centrifuged for 5 minutes at 1500 rpm, then the water was eliminated and replaced with 40% sucrose solution, followed by a one minute centrifugation at 1700 rpm. The supernatant was rinsed with tap water and filtered through a 400 mesh sieve. Nematodes were poured into 35 ml collection bottles and stored at 10°C [8].

2.3. Phytonematode extraction from roots

The chamber method was used to extract nematodes from root samples. Root samples were cleaned with water and cut into 0.5 cm lengths. The root pieces (10 g) were placed in the extractor unit (nematode sieve + funnel + cup) and placed in a mist chamber. The fogging valve was opened and the sample was stored for 72 hours. The suspension was filtered through 100 and 400 mesh sieves and the nematode suspension was poured into 35 ml collection bottles and stored at 10°C [9].

2.4. Analysis of phytonematode population

Nematodes suspension were sampled into a counting dish and counted under a stereoscopic microscopic at 40 x magnification (protocol repeated three times). Further morphological identification was carried out with a light microscope with a magnification of 200 - 400 x. The Pictorial Key to Genera of Plant-parasitic Nematodes [10] and related journals were used to identify the nematode to the genus or species level. The formula of nematode population analysis is as follows

\[ \text{Phytonematode population} (p) = \frac{\sum_{i=1}^{n} p \times V}{n} \]

with (p) is the nematode population observed on counting dish, (V) is the total volume of nematode suspension, (v) is the volume of nematodes suspension on counting dish, (n) is a repeat observation (in this study 3 times).

3. Result and discussion

3.1. Disease symptom

Nematodes associated with Javanese long pepper cause yellowing disease or slow decline. The symptoms include wilting, slight to severe yellowing of leaves, browning and blackening of vines and stem, roots rot, premature falling of leaves and fruit. In serious cases, the main roots lose their feeder roots, resulting death of the vines (Figure 1).

Symptoms of Javanese long pepper infected with phytonematode have similarities to those observed in yellowing disease of black pepper plants. This is caused by the two plants are from the same genera. The symptoms of phytonematode infection on Javanese long pepper are foliar yellowing, premature leaves falling, wilting, and stunting. The root system shows necrosis, root galls, root rot, and reducing total root mass [2, 11]. In Sri Lanka, nematode infection inhibits plant and root growth. Others show
stunting and foliar yellowing [12]. *M. incognita* and *R. similis* associated with black pepper plants affect wilting, in severe cases can cause death [7].

Piper plants are hosts to parasitic nematodes. Several nematode genera have been reported infect black pepper (*Piper nigrum* L.) and betel (*Piper betle*) plants. According to Hieu et al. [5], there were fifteen parasitic nematode genera detected infecting pepper and spreading vertically. As many as 31 parasitic nematode species infected black pepper in Vietnam [6] and 14 genera of parasitic nematodes associated with black pepper in Kerala, India [13].

![Image](image1.png)

**Figure 1.** The overall phenotype of healthy and phytonematodes-infected Javanese long pepper plant (a) healthy Javanese long pepper plant, (b) early symptom of Javanese long pepper plant infected phytonematodes. The symptoms are leaves yellowing., (c) late symptom of Javanese long pepper plant infected phytonematodes. The symptoms are leaves yellowing, wilting, premature falling of leaves and fruit, root rot.

### 3.2. Morphological character of phytonematodes

*P. nanus, Meloidogyne* spp., *R. reniformis, R. parvus, C. cancellatus,* and *C. paramaritus* were phytonematodes obtained from root and soil extraction of Javanese long pepper (Figure 2). Morphological character of the female body of *P. nanus* is curve in the form of the letter C, the head region is round and not set off with somewhat truncate anterior end, stylet is strong, pharyngeal gland overlaps the intestine. Vulva located more than a third of the body’s posterior. The tail terminus was subacute and blunt but not digitate [14, 15].

*Meloidogyne* spp. juvenil 2 morphological character is vermiform, with a well-developed and prominent stylet and an oval median bulb. The esophagus is difficult to see. The tail tip has a jagged pattern [8, 16].

Morphological characteristics of an immature female *R. reniformis* have a ventrally curved body with a spiral shape, a conoid lip, a moderately developed stylet, rounded basal knobs, a two-genital branch reproductive system, immature ovaries, and a tail that tapers to a narrow and rounded terminus [17]. The male body is similar to that of an immature female, except for the genital system and a more curved posterior part of the body. Mature males have a weaker stylet, labial framework, and pharynx. Male tail is broad and rounded in shape, with a rounded tip, bursa reduced, spicules and gubernaculum well developed, ventrally arcuate, subterminal [8, 16].

The immature female of *R. parvus* has a vermiform shape, with a body that curves ventrally in the shape of the letter C. The lip area is conoid, flattened, somewhat rounded, and not set off. The mouth
part has a sclerotized stylet with rounded knob slanting posteriorly. The pharyngeal gland overlaps the intestine ventrally. Reproductive system is didelphic. Vulva is located in around 60 to 66% of the body length from the anterior end, post medially. Tail is tapers to a rounded, with coarsely annulated tip. Males have a somewhat longer and slender body than immature females. In comparison to immature females, the anterior portion is less developed, with a shorter and weaker stylet and knob. Tail is slightly longer than that of immature females. Spicules are arcuate ventrally [18].

Figure 2. Phytonematode morphology of Javanese long pepper plants. (a) immature female *Rotylenchulus reniformis*, (b) male *R. reniformis*, (c) female *Paratylenchus nanus*, (d) male *P. nanus*, (e) juvenil 2 *Meloidogyne* spp., (f) immature female *R. parvus*, (g) male *R. parvus*, (h) female *Coslenchus paramaritus*, (i) male *C. paramaritus*, (j) female *C. cancellatus*. Scale bar= a-j= 50 µm

The female of *C. paramaritus* has a straight to slightly ventrally curved body shape. With a short and delicate stylet, the head is slightly offset. The median bulb of the esophagus is oval. Females have a
monodelphic reproductive system. The tail is elongated, with a filiform terminal that is strongly pointed. The male body is straight until it curves ventrally. Spicules have a sharp tip and are slightly curved ventrally. Tail elongated to a pointed filiform terminus [19].

The female body of *C. cancellatus* has a straight to slightly ventrally curved morphology. The head is slightly set off. Stylet is delicate and short. The median bulb of the esophagus is oval. The esophageal and intestinal valves are distinct. Females have a monodelphic reproductive system. Tail is conical in shape, with a delicately rounded to pointed tip. The male was not found [19].

### 3.3. Population density of phytonematodes

The population of root phytonematodes of Javanese long pepper is presented in Figure 3. Phytonematodes obtained from root extraction of Javanese long pepper were *Paratylenchus nanus*, *Meloidogyne* spp., *Rotylenchulus reniformis*, and *Costenochus cancellatus*. *P. nanus* was the highest nematode population in the monoculture in Saronggi (MS) block with a total of 165 nematodes per 10 g of roots. The next highest nematode population was *Meloidogyne* spp. in the polyculture in Bluto (PB) block with a total of 105 nematode per 10 g of roots.

The population density of parasitic nematodes in the four blocks did not differ much. This is presumably because the two types of land are in the same environmental conditions, the same soil conditions, close distances, the same irrigation water, and the same land management. This has similarities to those described in Norton [20] that passive dispersal such as water, wind, animals, and agricultural tools can impact nematode migration.

![Phytomonematode population in Javanese long pepper roots (mean ± SD).](image)

**Figure 3.** Phytomonematode population in Javanese long pepper roots (mean ± SD).

MB= Monoculture in Bluto sub-district, PB= Polyculture in Bluto sub-district, MS= Monoculture in Saronggi sub-district, PS= Polyculture in Saronggi sub-district.

*R. reniformis*, *R. parvus*, *C. cancellatus*, and *C. paramaritus* are phytonematodes obtained from soil extraction of Javanese long pepper, as shown in Figure 4. *R. reniformis* was discovered in all block observed, which the highest population was in MS and PS blocks, with a total of 1140 and 1314 nematodes/ 100 ml of soil, respectively.

*Meloidogyne incognita*, *Radopholus similis*, *Trophoylenchus piperis*, *Rotylenchulus reniformis*, and *Helicotylenchus* sp. are the most common parasitic nematodes associated with black pepper in India [13]. Meanwhile, the most dominant nematodes of black pepper in China are *Meloidogyne, Ditylenchus,*
Rotylenchulus, Filenchus, Discocriconemella, Hemicriconemoide, and Helicotylenchulus [5]. In Sri Lanka, Black pepper was associated with M. arenaria, M. incognita, M. javanica, Hoplolaimus seinhorsti, and Xiphinema ifacolum [12]. The nematodes that infect pepper plants in Indonesia are M. incognita and R. similis. In this study, the most dominant nematodes based on population density were P.nanus and Meloidogyne spp. However, the species Radopholus similis was not found.

Parasitic nematodes associated with Javanese long pepper are categorized based on feeding behavior. P.nanus, C. cancellatus, and C. paramaritus as ectoparasites, Meloidogyne spp. as sedentary endoparasites, R. reniformis and R. parvus as sedentary semi-endoparasites. In general, the most plant-damaging phytonematode are sedentary endoparasites. In this study, there was one sedentary endoparasite nematode and two sedentary semi-endoparasite nematodes associated with Javanese long pepper. Parasitic nematode infection affects various disease symptoms, Meloidogyne spp. produces galled roots, R. reniformis causes leaf yellowing and root rot [21], P. nanus causes yellowing, late flowering, and root necrosis [22].

Phytonematodes associated with Javanese long pepper have a wide host range, such as annual plants, horticultural crops, ornamental plants, and grasses. Zinnia elegans [23], Poa annua L., Dactylis glomerata L., Lolium multiflorum L., L. perenne L., and Festuca arundinacea Shreb. [24], apples [25], cherries, grapes, potatoes, carrots, celery, corn, and alfalfa are hosts of P. nanus. Meloidogyne spp. attack different kinds of crops, like vegetables, forage crops, ornamentals, fruit trees [26], and weeds [27]. Ipomoea reptana, chicory, pakcoy, spinach, cowpea, string bean, eggplant, celery, local Cianjur cucumber, local siam squash, red beet, local radish, Japanese radish, cosmos, lampenasa, Ageratum conyzoides, Commelina diffusa, Emilia sonchifolia, Gnaphalium, Polygonum nepalense, Galinsoga parviflora, Centula asiatica, Erichites palerionofolia, Crotalaria juncea, and T. patula are hosts of R. reniformis [28].
4. Conclusion
Phytonematodes obtained from root extraction of Javanese long pepper were *Paratylenchus nanus*, *Meloidogyne spp.*, *Rotylenchulus reniformis*, and *Coslenchus cancellatus*. The highest nematode population in the monoculture in Saronggi (MS) block was *P. nanus* with a total of 165 nematodes per 10 g of roots. Phytonematodes obtained from soil extraction of Javanese long pepper were *R. reniformis*, *R. parvus*, *C. cancellatus*, and *C. paramaritus*. The highest nematode population in MS and PS (polyculture in Saronggi) blocks was *R. reniformis* with a total of 1140 and 1314 nematodes/100 ml of soil, respectively.

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