The phytonematodes species on sugarcane plants (*Saccharum officinarum* L.) at Subang Sugar Factory PT PG Rajawali II, West Java

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**Abstract.** Phytonematodes are an important plant pest in sugarcane. The presence of phytonematodes can cause root damage and eventually reduce plant productivity. This study aimed to identify and calculated the abundance of sugarcane phytonematodes species in the Subang Sugar Factory of PT PG Rajawali II, West Java. The symptoms of sugarcane plants infected by phytonematodes include stunting plants and slender stems, and necrotic roots with blackish purple spots that spread unevenly on young roots. The diagnostic samples, consisted of roots and soil, were taken from the second ratoon cane. The stages of the research included nematode extraction from soil sample by centrifugation flotation method and extraction nematode from the root using the mist chamber method, nematode staining inside root tissue, and nematode identification. The nematode species were identified based on the morphological characters. Eight phytonematodes species, namely *Pratylenchus zeae*, *Xiphinema setariae*, *Hoplolaimus indicus*, *Rotylenchulus reniformis*, *Criconemoides morgenensis*, *Helicotylenchus* sp., *Coslenchus paramaritus* and *Tylenchus* sp. were identified. Based on the highest absolute population in sugarcane roots was *Pratylenchus zeae*.

**Keywords:** necrotic root, *Pratylenchus zeae*, stunted plants

1. **Introduction**

Sugarcane (*Saccharum officinarum* L.) is an important plantation commodity and cultivated in many tropical and subtropical countries [1]. Sugarcane is generally known as the raw materials of sugar industry. Sugar is one of the strategic commodities in Indonesian economy and currently, domestic sugar production has not met the national sugar needs [2]. During the period 2016-2019 the Indonesian sugar production tends to fluctuate due to a decrease in sugarcane harvested area and disturbances of pests and diseases, one of which is phytonematodes [3]. Disturbance by phytonematodes in sugarcane caused a decrease in production [4]. Phytonematodes as the cause of plant diseases are widely reported in almost all sugarcane growing countries [5]. Phytonematodes in sugarcane have been widely reported in several other countries, including China [6], South Africa [7], India [8], Nigeria [4], Japan [9], and Brazil [10]. In Indonesia, the initial study of phytonematodes in sugarcane was first reported in 1976-1978 in seven sugar mill areas in East Java [11].

Many phytonematodes species have been reported to be important parasites of sugarcane, including *Pratylenchus zeae*, *Pratylenchus brachyurus*, *Hoplolaimus indicus*, *Xiphinema elongatum*, Helloctylenchus sp., *Coslenchus paramaritus* and *Tylenchus* sp.
Meloidogyne javanica, Helicotylenchus dihystera, Mesocriconema, Hemicicliophora, Tylenchus sp., and Aphelenchus sp. [12, 13]. These phytonematodes infected sugarcane roots. Root disease symptoms include damage to the vascular tissue that causes necrotic spots to appear purple and then turn black. These symptoms usually occurred on young roots [13]. In Indonesia, the identification of sugarcane phytonematodes to the species level has not been widely carried out. This study aimed to identify and calculate the abundance of phytonematodes species in sugarcane at the Subang Sugar Factory of PT PG Rajawali II, West Java.

2. Material and methods
2.1. Sampling
Sampling was carried out at the Subang Sugar Factory plantation of PT PG Rajawali II from November to December 2020. Root and soil samples were taken from second ratoon sugarcane crops located in plot 38 (6°25'30.8"S 107°42'36.9"E) and plot 40 (6°25'37.9"S 107°42'17.1"E). Five points of plant groups/clusters with phytonematode symptoms were taken in the sample plot, including stunted plants and yellowing leaves. Root and soil samples were taken with a diagnostic sampling pattern at the center of the circle, the edges, and outside of the circle of diseased plant clusters. Root samples were taken from the main stem, about 30 cm with a depth of ± 20 cm from the soil surface. Soil samples were taken as much as 200 mL and roots as much as 10 g.

2.2. Phytonematode extraction from soil
Nematodes were extracted from soil samples using centrifugation flotation method. Soil samples were mixed until homogeneous, then 100 mL of soil was taken and added with 900 mL of distilled water, then stirred and allowed to stand for ± 20 seconds. The suspension was poured into graded sieves at sizes of 50, 100, and 400 mesh. The suspension accommodated in a 400 mesh sieve was then poured into a 15 mL Eppendorf tube. The nematode suspension was centrifuged at 1500 rpm for 5 minutes, then the water was removed and the residue was added with 40% sugar solution, then centrifuged again at 1700 rpm for 1 minute. The supernatant was poured on a 400 mesh sieve, rinsed with distilled water until the sugar water solution disappeared and the suspension was put in a 40 mL collection bottle and stored in the refrigerator at 10 °C [14].

2.3. Phytonematode extraction from roots
Nematodes were extracted from sugarcane root samples using a modified mist chamber method. The modifications were on the weight of the root sample and the length of fogging. The sugarcane roots samples were washed and cut into ±1 cm in size. The roots were weighed as much as 5 g and placed on a funnel that had been lined with a 0.2 cm diameter sieve. The funnel containing the roots was placed in the mist chamber and harvested every 24 hours for 7 days of fogging. The suspension was filtered gradually using graded 100 and 400 mesh sieves. The suspension was stored at 10 °C in a 40 mL collection bottle [15].

2.4. Nematode staining in root tissue
The sugarcane roots were cleaned and cut to 1-2 cm, then soaked in 10 mL of 5.25% chlorox for 4 minutes. The roots were rinsed with running water until the chlorox aroma disappeared and soaked in distilled water for 15 minutes. The roots were put in a heat-resistant bottle containing fuchsin acid solution (3.5 g fuchsin acid, 250 mL acetic acid, and 750 mL distilled water). The bottle is then placed in a vessel filled with boiling water for 30 seconds and allowed to cool at room temperature. The fuchsin acid solution was discarded, the roots were rinsed with running water, added with glycerin until the roots were submerged and added two drops of HCl solution. Subsequently, it was heated again until the red color on the roots fades. Root pieces were arranged on a glass slide, and the red nematodes could be observed with a binocular light microscope [16].
2.5. Phytonematode population analysis
Nematodes were identified using the Commonwealth Institute of Helminthology (C.I.H.) identification key book. The number of phytonematodes was calculated from a sample of 2 mL of nematode suspension, inserted into the counting disk and observed using a stereoscopic microscope with four times magnification. This procedure was repeated three times, and the absolute population (P.A.) [17] was calculated using the following formula:

\[
\text{Phytonematode populations (PA) } p = \frac{\sum_{i=1}^{n} p_i \times V_i}{n}
\]

\(p\) = the population of phytonematodes species observed on counting dish, \(V\) = the volume of the suspension of phytonematodes extracted, \(v\) = the volume of phytonematodes suspension when counting dish, \(n\) = a repeat observation.

2.6. Morphological character
The identification of phytonematodes was carried out on permanent preparations by observing the key morphological characters. Nematodes were observed using an Olympus B.X. 51 binocular microscope with a 2 MP CCD camera, and image visualization was observed using ToupView© software. Nematodes were identified using the Commonwealth Institute of Helminthology (C.I.H.) identification key book.

3. Result and discussion
3.1. Symptoms in sugarcane
Diseased sugarcane plants have shorter stems and yellowing leaves. On the roots, necrotic symptoms appear in purple-black spots on the roots that spread unevenly. Spots, especially on young roots, developed into rot with darker in color. In addition, in sugarcane plants that did not show symptoms in the plant crown, it turned out that there were a few necrotic spots at the tips of the young roots (Figure 1).

\[\text{Figure 1. Sugarcane plants infected by phytonematode. (A) infected plants, (B) phytonematodes infected roots, (C) necrotic symptoms on young roots, (D) symptoms of purple spots on young root area.}\]

The symptoms of phytonematode-infected sugarcane roots are similar to those reported by Cadet & Spaull and Wang et al. [6, 13] which explained that the symptoms of phytonematode disease in sugarcane were necrotic in the form of patches that were unevenly spread in the area of young roots that were purple-black and resulted in damage to root tissue.
3.2. Staining in root tissue
In the fuchsin acid staining technique, red-stained phytonematodes were detected in cortical tissue, especially young roots. This result confirms the observation that phytonematodes are the primary cause of necrotic symptoms in roots, as shown in (Figure 2).

![Figure 2](image)

Figure 2. Acid fuchsin stained young roots of *Saccharum officinarum* L. (A) Symptom lesions caused by phytonematodes on the root (B) phytonematode infection in young sugarcane roots, (C) phytonematodes migration within root tissue, (D) phytonematodes entered in epidermis tissue, (E) phytonematodes were stained outside root tissue. Scale bar= B = 40x, C-E = 20 µm.

Many spots as a result of nematode infection. The nematodes enter through the root tissue using their stylet which then migrates to the tissue. According to Wang et al. [6] who stated that every spot lesion usually contains more than one nematodes. This is similar to the result obtained, if more than one nematode was detected along the young root tissue in sugarcane.

3.3. Morphological characters of phytonematodes in root and soil sugarcane
Eight phytonematodes species were identified from soil and root of sugarcane, that are *Pratylenchus zeae*, *Xiphinema setariae*, *Hoplolaimus indicus*, *Rotylenchulus reniformis*, *Criconemodes morgensis*, *Coslenchus paramiratus*, *Helicotylenchus sp.*, and *Tylenchus sp.* (Figure 3). The morphology of *Pratylenchus zeae* is characterized by flat lips, lip region is not set off from the body, slightly squared, short stylet, flat stylet knob, oval median bulb, monodelphic vulva position, vulva splitting, and splitting protruding from the cuticle of the body, vulva position 60-77%, spermatheca round and small, post-vulval uterus sac is short, tail slightly tapered at the end, and this nematode is parthenogenic [10]. The female morphology of *Xiphinema setariae* is characterized by having an odontostile type, a body that is longer than other nematodes, in the anterior stylet, there is a needle-shaped odontostile that extends towards the anterior lip, the lip region has an indentation that separated it from the rest of the body, and tail shaped like a conoid with digitate terminus [18].

The key to identification of *Hoplolaimus indicus* is a hemispherical lip area, strong stylet, round stylet knob, a round median bulb that does not meet the ventral wall, large and long body, vulva position in the middle of the body with a didelphic reproductive system, round tail shape with slight annulation. Rough and jagged on the cuticle [19]. The key to morphological identification of immature
female *Rotylenchulus reniformis* is the body shape is curved to form the letter G in the resting position, the stylet is weak, and the basal knob is well developed, the lips are conoid and not set off, the vulva is slightly split into the body, the reproductive system is monodelphic, the tail is tapering with rounded terminus. The male *Rotylenchulus reniformis* has a body shape like the letter C and G in the resting position, the shape and size of the male body is relatively similar to that of immature female nematodes. Male stylet are shorter and thinner than immature female nematodes. Male lips are slightly curved at the ends. Besides that, there is a pointed bulge at the end of the lips. The tail is tapering with rounded terminus and contains spicules. Male has hyaline at the end of the tail. [20].

Morphology *Criconemoides morgensis* female has a cylindrical body and slightly curved ventral, strong stylet and rounded basal knob, oesophageal gland ventrally overlapping with intestine, vulva reproductive system with monodelphic type, and tail dorsally convex-conoid with the tail end slightly protrusion [22]. Morphology *Coslenchus paramaritus* female has a straight body shape slightly curved in the resting position. The stylet is thin, short, with a basal knob visible and belongs to the stomatostylet. The head is fused to the stylet and body contour, and there is a slight indentation. The median bulb is slightly oval. The shape of the lips is a bit conoid that blends with his body. The oesophageal glands are seen to overlap with the intestines. The median bulb is slightly oval. The reproductive system of the vulva is monodelphic, and the female vulva is positioned posteriorly close to the anus. The lips of the vulva open and part inward. The post-vulval uterine sac is absent in female nematodes. The tail is elongated with a sharp tapered tip. Morphology *C. paramiratus* male has a body straight to ventrally curved. Stylet knobs well developed and thin. Oesophageal median bulb oval, and have a spicule. Spicules slightly curved ventrally, with pointed tip and the tail elongated ending [23]. *Tylenchus* sp. female morphology has a straight body shape in the resting position. thin stylet, weak, and short in size. Lips blend directly with body contours, not set off, reproductive system has a monodelphic vulva, and the tail is slightly curved. The tail tip like rounded to the acute terminus. Male *Tylenchus* sp. has a body almost the same as the female that differs only in the reproductive system. Males have spicules and gubernaculum. Tail end dorsally bent like scythe shaped [24].

3.4. *Phytonematode of community analysis*

The population of phytonematodes found in sugarcane plantations at Subang Sugar Factory was eight species. Based on the extraction results of root samples, *Pratylenchus zeae*, *Hoplolaimus indicus*, *Xiphinema setariae*, *Coslenchus paramaritus*, and *Tylenchus* sp. were found. The phytonematode population extracted from sugarcane root samples is presented in Figure 4-5. *Pratylenchus zeae* was the most common phytonematode found in all sugarcane plant samples. *P. zeae* had the highest absolute population values in plot 38, namely SO1 and SO3 points with 426 and 342 nematodes/5 g roots, while the highest absolute population in plot 40 was SO1 points with 613 nematodes/5 g roots. Extraction of phytonematodes from sugarcane soil samples found *Pratylenchus zeae*, *Rotylenchulus reniformis*, *Helicotylenchus* sp., *Criconemoides morgensis*, *Hoplolaimus indicus*, *Xiphinema setariae*, *Coslenchus paramaritus* and *Tylenchus* sp. Phytonematode populations extracted from sugarcane soil samples are presented in Figures 6-7. The abundance of phytonematodes based on absolute population values in the soil sample in plot 38 was SO5 point as much as 159 nematodes/ 100 ml of soil. In plot 40, the highest population was at SO4 point of 156 nematodes/ 100 ml of soil.
Figure 3. Morphology character of phytonematodes found in sugarcane plants. (a) female *Pratylenchus zeae*, (b) anterior female *Pratylenchus zeae*, (c) vulva *Pratylenchus zeae*, (d) posterior female *Pratylenchus zeae*, (e) male *Rotylenchulus reniformis*, (f) anterior male *Rotylenchulus reniformis*, (g) spicule *Rotylenchulus reniformis*, (h) immature female *Rotylenchulus reniformis*, (j) vulva immature female *Rotylenchulus reniformis*, (k) female *Hoplolaimus indicus*, (l) anterior female *Hoplolaimus indicus*, (m) posterior female *Hoplolaimus indicus*, (n) female *Coslenchus paramaritus*, (o) female *Tylenchus sp.*, (p) male *Tylenchus sp.*, (q) anterior betina *Xiphinema setariae*, (r) anterior J4 *Xiphinema setariae*, (s) female *Criconemoides morgensis*, (t) anterior female *Helicotylenchus sp.*, (u) female *Helicotylenchus sp.* Scale bar= a, e, h, k, n, o, p, q, s, u = 50 µm and b-d, f-g, l-j, l-m, r, t = 10 µm.
P. zeae was a phytonematode found in all sample plots. Based on the results of root extraction, P. zeae was the dominant species in plots 38 and 40. The highest phytonematode populations in root extraction were at points SO1 and SO3. The results of nematode extraction from the soil in plots of 38, the highest phytonematode population was SO5 point. In plot 40 the highest phytonematode population was at SO4, dominated by Pratylenchus zeae, Criconemoides morgensis, and Helicotylenchus sp.
Figure 7. Phytonematode population in plot 38 sugarcane soil (mean ± SE). SO1 = first point, SO2 = second point, SO3 = third point, SO4 = fourth point, SO5 = fifth point.

Phytonematodes associated with sugarcane were categorized based on feeding behavior, each species having different feeding behavior. *Pratylenchus zeae* is categories as migratory endoparasite nematodes [25], *Hoplolaimus indicus* as ectoparasite nematode [26], *Rotylenchulus reniformis* as semi-endoparasite nematode [20], *Helicotylenchus* sp. and *Criconemoides morgensis* as ectoparasite nematode [27], and *Xiphinema setariae* as ectoparasite nematode [28].

*P. zeae* became the dominant phytonematode with the abundant population in sugarcane root and soil samples, this has also been reported in sugarcane in Tanzania and Brazil [10, 12]. In addition, the abundance of *Helicotylenchus* sp. and *Criconemoides morgensis* populations became the dominant phytonematodes in the soil samples. Previous reports of *Helicotylenchus* sp. found in sugarcane are less pathogenic than other ectoparasitic nematodes. The presence of a high population of *Helicotylenchus* sp. in the soil did not cause severe damage [29].

The highest abundance of phytonematode populations in root samples in both plots was indicated by *P. zeae*, a migratory endoparasitic nematode that lives in the roots and migrates through the soil to healthy plants to transmit infection [30]. Phytonematodes can entered and penetrated the roots using a stylet. Penetration carried out by endoparasitic nematodes moved by penetrating the tips of young roots, then made a longitudinal tunnel to the parenchyma. The cells around the penetration site will turn dark in color due to infection that necrotic symptoms are seen in the roots [31].

4. Conclusion

The population of phytonematodes found in sugarcane plantation at Subang Sugar Factory was eight species and *Pratylenchus zeae* was the most common phytonematode found in all sugarcane plant samples.

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