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PLANT-PARASITIC NEMATODES ASSOCIATED WITH COMMON HORTICULTURAL WEEDS

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ABSTRACT

Weed is plant species that interfere with cultivated crops, including horticultural crops. Weeds are competing with crops for nutrients, light, water, and other growth factors and ultimately reduce the crop yield. Weeds can be the alternative hosts for various plant pathogens (fungi, bacteria, viruses, and nematodes). This study aimed to observe the species of weeds and the genera of nematodes associated with them on horticultural crops (tomatoes, celeries, carrots, and carrot seedlings) found in the experimental garden of IPB University, Indonesia. Weeds collected from the horticultural land were identified based on their species. Nematodes associated with weeds were extracted and identified. Nematode extraction from the weed rhizosphere was performed following the floating-centrifugation method, whereas nematode extraction from weed roots was performed using the misting method. Weed species and nematode genera, as well as the number of nematodes, were obtained and processed into figures, tables, and graphs. Based on the study results, there were seven types of weeds on tomatoes, celeries, carrots, and carrot seedlings land. The seven weeds found were *Ageratum conyzoides*, *Ageratum houstonianum*, *Portulaca oleracea*, *Eleusine indica*, *Amaranthus spinosus*, *Borreria alata*, and *Borreria laevis*. In weed rhizospheres, it was also found seven genera of nematodes, namely *Meloidogyne*, *Helicotylenchus*, *Pratylenchus*, *Rotylenchulus*, *Hoplolaimus*, *Criconeoides*, and *Scutellonema*. In 100 ml of weed rhizosphere, the most number of nematodes were mainly found on weeds grown among celeries (105), followed by tomatoes (96), carrots (87), and carrot seedlings (58). Furthermore, based on the extraction result of 10 g weed roots to determine the nematode population, *A. conyzoides* weed root contained 165 nematodes/100 g roots, followed by *B. laevis* (146), *A. spinosus* (143), *P. oleracea* (122), *A. houstonianum* (121), *B. alata* (113), and *E. indica* (101). This study provides new information on the species of weeds growing on the horticultural land (tomatoes, celeries, carrots, and carrot seedlings) and plant-parasitic nematodes associated with them.

Keywords: abundance, carrot, celery, distribution, diversity.

INTRODUCTION

Weed is an undesirable plant that grows around the cultivated plants and potentially reduces crop yields. Yield loss caused by weeds is due to nutrients, water, light, CO₂, and space competition, besides the existence of allelopathy compounds produced by them that suppress the crop growth around it (Głąb *et al.*, 2017). There has been no special report nowadays on the

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amount of yield loss in Indonesia caused by the existence of weed. However, Milberg and Hallgren (2004) reported that the presence of weeds causes increased yield loss of cereal in Sweden from 1961 to 2014, reaching 31.3%.

In addition, the presence of weed can also cause indirect negative impacts. Weed can become the alternatives host to plant pests and pathogens (Wisler and Norris, 2005). An alternate host can be defined as plant material or other organisms utilized as a substitute by the plant-disturbing organism in obtaining nutrients and performing parasitism activities (Opoku *et al.*, 2002). One of the plant-parasitic microorganisms that utilize weed as an alternative host is a nematode (Ntidi *et al.*,

2016; Giraldeli *et al.*, 2017). Some types of nematodes are reportedly able to reproduce and use weed as a distributive agent in an agricultural ecosystem. Quénehervé *et al.* (2006) reported that there are 24 weed species as *Radopholus similis* host, 23 species as *Helicotylenchus* spp. host, 13 species as *Pratylenchus* spp. host, 13 species as *Hoplolaimus seinhorst* host, 29 species as *Meloidogyne* spp. host, and 24 species as *Rotylenchulus reniformis* host derived from the banana crop in Martinique fruit garden.

The existence of nematodes can cause yield loss in the cultivation area. Nematode infection directly damages root tissue and inhibits water and nutrient absorption. A low supply of water and nutrients will cause a variety of symptoms such as withering, unoptimal growth, and suffering from severe infections, which leads to significant yield reduction (Jones *et al.*, 2013). Nematode infection also causes weak plant resistance, which is easily infected by other pathogens (fungi, bacteria, or viruses). Wounds caused by nematodes infection are reported as one of the plant pathogen entranceways (De Rooij, 1995). Root-knot nematode *Meloidogyne* spp. can synergize with bacteria *Ralstonia solanacearum* or fungi *Fusarium oxysporum*. The nematode synergy with bacteria or fungi will result in higher disease severity than a single infection. Complex plant diseases (caused by more than one type of pathogen) will be more challenging to control and lead to higher yield loss (Rocha *et al.*, 2018; Furusawa *et al.*, 2019).

Weed usually grew around the horticultural crops, some of which become the potential hosts for parasitic nematodes. Weeds that are widely found in horticultural lands in Indonesia include *Ageratum conyzoides*, *Ageratum houstonianum*, *Portulaca oleracea*, *Eleusine indica*, *Amaranthus spinosus*, *Borreria alata*, and *Borreria laevis* (Moenandir, 2010). Indonesian farmers generally clean weeds when the plant is still in the nursery phase. When the plant has passed the nursery phase, the weed typically grown around the plant is left to grow without being controlled. This phenomenon is undoubtedly less beneficial as the plant-parasitic nematodes can survive and reproduce in the weed roots that are grown.

Currently, there is still a lack of information about the plant-parasitic nematodes associated with weeds in Indonesia. This needs to be fixed to support the efforts of controlling plant-parasitic nematodes in the

horticultural crops holistically and continuously. This study aimed to identify the plant-parasitic nematodes associated with weeds on the tomatoes, celeries, carrots, and carrot seedlings land in the experimental garden of IPB University, Cianjur, West Java, Indonesia.

MATERIALS AND METHODS

Location: This study was conducted in the Plant Nematology Laboratory, Department of Plant Protection, Faculty of Agriculture, IPB University. Soil and weed root samples were obtained from four lands in the experimental garden, IPB University, Pasir Sarongge (106° 42' – 107° 25' EL dan 6° 21' – 7° 25' SL), Ciputri, Pacet, Cianjur, West Java, Indonesia. The sampling location was 1,000 m above sea level with 17-26°C temperature. The soil type in the sampling location was andosol.

Weed Roots and Soil Sampling: Weed roots were obtained by pulling out the weeds grown around the tomato, celery, carrot, and carrot seedling crops. The weeds collected were 40 samples of each type. Weeds were moved into the sampling plastic containing the same weed type. Samples were then moved into the icebox before being brought to the laboratory for species identification. Nematodes were extracted from the weed root after the weed species were determined. Besides weed roots, soil around the weed rooting area was also taken for laboratory analysis. Soil samples were obtained from ±10 cm depth using a small shovel. Soil samples were collected from 4 points with 50 ml volume on each sample. Soil samples were kept in the sampling plastic and icebox before being brought to the laboratory for nematode extraction (Been and Schomaker, 2006).

Soil Nematode Extraction: Nematodes were extracted using the floating centrifugation method described by Van Bezooijen (2006). A total of 100 ml of soil was taken and cleaned from leaves, stones, and other materials apart from the soil. The soil was moved into an "A" container with 800 ml water, then stirred until homogenous, and kept for 20 seconds. Water from the "A" container was poured into a "B" container by crossing a 20 mesh filter, then kept for 30 seconds. Water from the "B" container was moved into a "C" container by passing 50, 200, and 500 mesh filters. The 500 mesh filter was tilted at 30°.

Nematode suspension as the result of 500 mesh filters was centrifuged at 1,700 rpm for 5 minutes. Tube suspension was thrown away, while soil deposits and

nematodes were added with a 40% sugar solution, then shaken and recentrifuged for 1 minute. The suspension was filtered with a 500 mesh filter, then rinsed with water and retained into the collection bottles for the extracted nematode identification and calculation.

Root Nematode Extraction: Nematodes in the weed roots were extracted following the misting method using the mist chamber. A total of 10 g of weed roots were cleaned from soil particles and cut into 1 cm lengths. Roots were kept on the rough filter with 100 µm diameter, then placed on a funnel, in which plastic glass was put under it to retain the nematode suspension. Retained nematodes in the plastic glass were kept in the misting place for 48 hours. Nematodes were harvested using a 500 mesh filter. Filtered nematodes were moved and kept in the collection bottles for the next observation (Van Bezooijen, 2006).

Nematodes Calculation: Nematode suspension was placed on a Syracuse dish and calculated under the stereo microscope with 40x magnification. Only the plant-parasitic nematodes were calculated. Data obtained were the number of nematodes per 10 g weed roots and 100 ml soil.

Semipermanent Nematode Sample Construction: Nematode suspension from soil and roots were moved into the Syracuse dish and given FAA with FAA volume and water ratio of 1:1 (v/v). The paraffin ring was formed on the object-glass, then 0.03% lactophenol solution was dropped in the middle of the paraffin ring formed. Nematodes from soil and root extraction were placed on the object-glass, then closed with the cover glass. Nematode samples were heated on Bunsen for 5 seconds to melt the paraffin. The cover glass was layered with thin liquid paraffin after the paraffin was dried. Constructed samples were used for nematode identification (Orajay, 2003).

Nematode Morphological Identification: The nematode was identified based on the morphological characteristics under the light microscope. Nematode identification was performed based on Plant Parasitic Nematodes: a Pictorial Key to Genera (Mai, 1966) and Horticultural Nematology (Ravichandra, 2014).

DATA ANALYSIS

Data were analyzed and presented in the form of descriptions, tables, and figures.

RESULTS AND DISCUSSION

Nematode Infection Symptoms on Weeds: Nematodes infection in weeds induce similar

symptoms as their infection in the cultivated plant. Their infection in weeds comprises primary and secondary symptoms. The primary symptoms that appear on weed roots are knot and necrosis. The observation results on the soil showed the dominant symptom is root-knot caused by *Meloidogyne* spp. (Figure 1). The symptom dominance of root-knot on weed had a positive correlation with the population of root-knot nematode among the entire nematode genera found. The type of root-knot appears to be round and small in size. Secondary symptoms were found above the soil surface, showing yellow and wilt leaves.

The weeds infected by the root-knot nematodes (RKN) were found on four cultivated crops. Weeds found associated with (RKN) are *A. conyzoides*, *A. houstonianum*, *P. oleracea*, *E. indica*, *A. spinosus*, *B. alata*, and *B. laevis* (Figure 2). Root-knots are formed due to *Meloidogyne* spp. infection on seven weed species obtained in small size. Root-knot is influenced by the weed age and the number of infective nematodes.

Weed Distribution: Based on the known observation results, there were seven types of weed grown around the tomato, celery, carrot, and carrot seedling. Seven weeds are *A. conyzoides*, *A. houstonianum*, *P. oleracea*, *E. indica*, *A. spinosus*, *B. alata*, and *B. laevis* (Table 1). The most diverse types of weeds are found on celery (5 species), tomato and carrot seedlings (4 species), then carrot (3 species). The observation showed that *A. conyzoides* were found throughout the observed land. Weeds grew irregularly, then seemed to be no special control efforts by farmers (Figure 3).

Plant Parasitic Nematode in Soil Sample: *Meloidogyne* spp. was the most encountered parasitic nematode on the soil around the weed roots. Weed rhizosphere grown around tomato and carrot seedlings contained seven genera of plant-parasitic nematodes, such as *Meloidogyne*, *Helicotylenchus*, *Pratylenchus*, *Rotylenchulus*, *Hoplolaimus*, *Criconemoides*, and *Scutellonema*. However, the weed rhizosphere around celery and carrot showed the inexistence of *Scutellonema*. Based on the total nematode found per 100 ml of soil around the weed roots, the most nematode number was found on the rhizosphere of weed around celery (105), followed by weeds around tomato (96), carrot (87), and carrot Seedlings (58) (Table 2).



Figure 1. Symptoms found on weeds; (a) root-knot on *A. conyzoides*, (b) yellow and wilt leaves, (c) root-knot on *A. houstonianum*, (d) root-knot on *P. oleracea*, (e) root-knot on *E. indica*, (f) root-knot on *A. spinosus*, (g) root-knot on *B. alata*, and (h) root-knot on *B. laevis*.



Figure 2. Weed species associated with plant nematode parasites on this study; (a) *A. conyzoides*, (b) *A. houstonianum*, (c) *P. oleracea*, (d) *E. indica*, (e) *A. spinosus*, (f) *B. alata*, and (g) *B. laevis*.

Table 1. Parasitic nematode infected weed distribution on tomato, celery, carrot, and carrot seedling land

Land	Parasitic nematode infected weed
Tomato	<i>A. conyzoides</i> , <i>A. houstonianum</i> , <i>P. oleracea</i> , <i>A. spinosus</i>
Celery	<i>A. conyzoides</i> , <i>P. oleracea</i> , <i>A. spinosus</i> , <i>E. indica</i> , <i>B. alata</i>
Carrot	<i>A. conyzoides</i> , <i>B. alata</i> , <i>B. laevis</i>
Carrot seedling	<i>A. conyzoides</i> , <i>A. houstonianum</i> , <i>P. oleracea</i> , <i>B. laevis</i>



Figure 3. Land condition and weed distribution on the observational land; (a) tomato, (b) celery, (c) carrot, and (d) carrot seedlings

Table 2. The total number of nematode per 100 ml soil around weed rooting area on cultivated tomato, celery, carrot, and carrot seedling

Nematode	Land			
	T	S	W	PW
<i>Meloidogyne</i>	52.20 ± 4.32	65.00 ± 4.74	43.40 ± 1.30	22.00 ± 1.87
<i>Helicotylenchus</i>	16.20 ± 1.48	8.20 ± 1.48	12.80 ± 1.64	6.00 ± 1.22
<i>Pratylenchus</i>	4.20 ± 1.30	2.60 ± 1.14	1.80 ± 1.30	1.60 ± 1.34
<i>Rotylenchulus</i>	10.60 ± 1.14	13.00 ± 2.54	16.40 ± 2.30	11.40 ± 2.30
<i>Hoplolaimus</i>	4.40 ± 1.14	14.60 ± 1.67	8.20 ± 1.30	12.80 ± 1.48
<i>Criconemoides</i>	7.20 ± 1.64	0.60 ± 0.54	4.80 ± 1.30	2.80 ± 0.83
<i>Scutellonema</i>	2.20 ± 1.30	0.00 ± 0.00	0.00 ± 0.00	0.60 ± 0.54

Note: T = tomato, S = celery, W = carrot, PW = carrot seedling

Plant-Parasitic Nematode on Root Samples: The most nematode populations were found at *A. conyzoides* root with 165 nematodes per 100 g root, followed by *B. laevis* (146), *A. spinosus* (143), *P. oleracea* (122), *A. houstonianum* (121), *B. alata* (113), and *E. indica* (101). On each of the observed weeds,

Meloidogyne always dominated the population with 118 on *A. conyzoides*, 77 on *B. alata*, 75 on *P. oleracea*, 58 on *E. indica*, 106 on *A. spinosus*, 82 on *A. houstonianum*, and 86 on *B. laevis*. Other nematodes found were *Pratylenchus*, as 9 on *A. conyzoides*, 3 on *B. alata*, 8 on *P. oleracea*, 4 on *E. indica*, 5 on *A. spinosus*, 3 on *A. houstonianum*, and 15 on *B. laevis*. Furthermore, the population of *Rotylenchulus* was 14 on *A.*

conyzoides, 6 on *B. alata*, 18 on *P. oleracea*, 15 on *E. indica*, 7 on *A. spinosus*, 3 on *A. houstonianum*, and 17 on *B. laevis*. *Hoplolaimus* nematode was also found with a total of 7 on *A. conyzoides*, 8 on *B. alata*, 3 on *P. oleracea*, 6 on *E. indica*, 5 on *A. spinosus*, 15 on *A. houstonianum*, and 16 on *B. laevis*. *Helicotylenchus*

nematodes were also found on the weed roots with 12 on *A. conyzoides*, 16 on *B. alata*, 15 on *P. oleracea*, 15 on *E. indica*, 18 on *A. spinosus*, 14 on *A. houstonianum*, and 10 on *B. laevis*. Other nematodes from *Scutellonema* and *Tylenchulus* genera were found in very low populations around the weed roots (Figure 4).

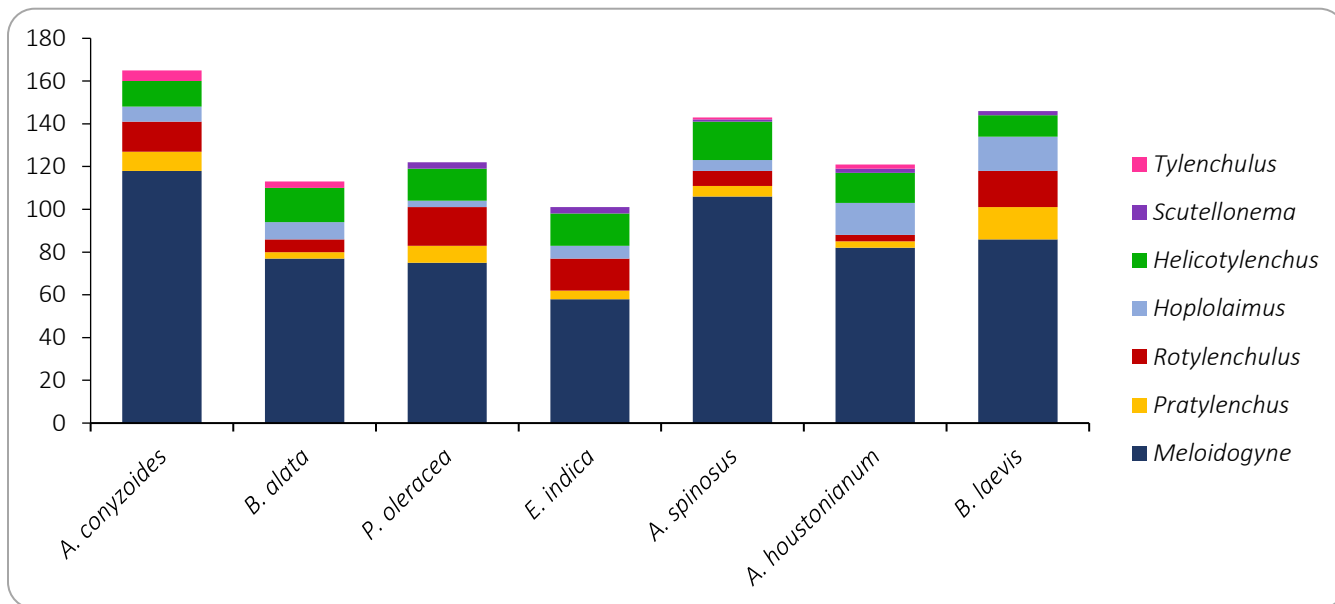


Figure 4. The total population number of nematode per 10 g roots on some weed species around the tomato, celery, carrot, and carrot seedling

DISCUSSION

Weed is plant species grown around the cultivated crops, causing decreased crop production during the cultivation period. Weed is generally an herbaceous herb, comprising from bush and trees. The presence of weed can reduce the agricultural yield, as requiring the same growth factor with the main crops, namely: water, light, CO₂, O₂, and nutrients (Benvenuti *et al.*, 2017; Zimdahl, 2018). To obtain the growth factor, weed competes with cultivated plants, therefore leading to inoptimal growth of cultivated plants. The interaction between weeds and plants is often referred to as competition or negative interaction (Dille *et al.*, 2016; Gharde *et al.*, 2018). Furthermore, weeds also have the effect of allelopathic compounds, which negatively impact the cultivated plants (Zohaib *et al.*, 2016).

Based on the leaf types, weeds can be divided into three groups, namely grass, broadleaf, and sedge weeds. Moreover, based on the life cycle, weeds can be divided into annual, biennial, and perennial weeds (Zimdahl, 2018). Weeds generally grow around horticultural crops are grass, broadleaf, and sedge weeds, while weed based on the life cycle that is often encountered around

horticultural crops is the annual weeds (Hamma and Ibrahim, 2013). This statement reinforces this study's results. Furthermore, the separate report mentioned that common weeds growing up around the horticultural crops in Indonesia are *E. indica*, *B. laevis*, *B. alata*, *Centella asiatica*, *A. conyzoides*, *A. houstonianum*, *A. spinosus*, and *P. oleracea* (Rosmanah *et al.*, 2017). This study result strengthens the previous report, which obtained seven weed genera as reported in the study.

The weed that grows on the horticultural land can lead to declined crop yields. Nevertheless, there is still no specific report mentioning the amount of dropped results caused by weed competition with the cultivated crops. The presence of weed in the horticultural land can indirectly be the alternative host for some pathogens, namely bacteria, fungi, viruses, and nematode (Sales Júnior *et al.*, 2019). *A. conyzoides* can be an alternative host for *Begomovirus* (Leke *et al.*, 2012). Besides *A. conyzoides*, other weeds such as *Sida* spp., *Macroptilium lathyroides*, and *Wisadula amplissima* are reported to become the alternative host for *Geminivirus* in Jamaica (Roye *et al.*, 1997). As the alternative host of pathogenic *Boletus*, *Euphorbia* weeds are reported to become the

host of *Fusarium* spp (Caesar *et al.*, 1998).

In previous studies, weed has been reported to be an alternative host of the nematode. Djiwanti and Supriadi (2011) reported that *A. conyzoides*, *Acalypha lanceolata*, *Oxalis sepium*, *Borreria* sp., *Lindernia* sp., and *Pleocnemia* sp. were the alternative host of *Aphelenchoides fragariae* nematode causing burnt leaf. Weeds infected by *A. fragariae* showed the symptoms of a burning leaf. The same study also reported in other weeds with approximately 2,000 to 12,274 *A. fragariae* nematode. In separate reports, *Centrosema pubescens*, *Chromolaena odorata*, *Cyperus iria*, *C. kyllingia*, *E. indica*, *Imperata cylindrica*, *Mimosa pudica*, *Tagetes erecta*, *Tridax procumbens*, and *Vernonia cinerea* are reported to become the alternative host for root-knot nematode *Meloidogyne* spp. (Widiyanto and Indarti, 2016; Mehmood and Khanum, 2021; Ullah and Khanum, 2022). Furthermore, Quénéhervé *et al.* (2006) reported that 24 types of weeds could potentially be the alternative host of *Radopholus similis*, 23 as the alternative host of *Helicotylenchus* spp., 13 as the alternative host of *Pratylenchus* spp., 13 as the alternative host of *Hoplolaimus sinhorsti*, 29 as the alternative host of *Meloidogyne* spp., and 24 as the alternative host of *Rotylenchulus reniformis*.

Symptoms that appeared on the weed infected with nematode are vary depending on the species of nematode. Weeds infected with root-knot nematodes will show the root knotted. The amount of knot formed after being infected with *Meloidogyne* spp. depends on the reproduction factor of the nematode (Rich *et al.*, 2009). Weed roots infected with *Radopholus similis* and *Pratylenchus* spp. suggests typical symptoms, i.e., necrosis and wound presence at roots (Edwards and Wehunt, 1971). These reports support this study's results, showing several symptoms on the infected weed root by nematodes.

Nematodes found on the weed rhizosphere and living inside of weed roots were quite diverse. The root-knot nematode *Meloidogyne* spp. was the most found nematode both on root rhizosphere and tissues. *Meloidogyne* spp. is reported to be the polyphage, infecting 2,000 to 3,000 plant species. The infective phase of the root-knot nematode is at Juvenile-2 (J2). *Meloidogyne* spp. J2 penetrates the growing edge of root tissue. The infection activity reaches the cell in the cortex area to find the feeding site. Larvae use stylet to damage the surrounding cell walls and secrete mucus

from the esophagus gland that causes giant cell formation as a food source (Abad *et al.*, 2003).

In addition to *Meloidogyne*, other nematodes found on the weed rhizosphere and roots were *Pratylenchus*, *Rotylenchulus*, and *Hoplolaimus*. *Pratylenchus* is called root-lesion nematodes. This nematode attacks the plant host and raises yellow wounds that will turn brown and lateral root decay. According to Thompson *et al.* (2008), *Pratylenchus* is an endoparasitic nematode that generally develops in the cortex tissue of the plant host. *Pratylenchus* has a wide host range. In addition to the cultivated crops, Been and Kutuyayo (2006) reported the *Pratylenchus* association with seven weed species on the mung bean cultivation area.

Rotylenchulus is a semi-endoparasite nematode, which is also called reniform nematodes due to the female shape being kidney-like. *Rotylenchulus* associated with weed root found was the second stadia larvae, showing curved shapes towards the ventral as letter C. The cone-shaped head shape with rounded edges and no border curves between the head and the posterior body (not set off). *Rotylenchulus* has a short-type stomato stylet with a rounded basal knob (Van Den Berg *et al.*, 2016). According to Lawrence *et al.* (2008), *Rotylenchulus* has a wide host range spreading onto tropical countries. The microscopic symptoms show to be the sinessy development in the root tissue as the nematode feeding site, while the infected roots show necrosis condition.

Hoplolaimus is often referred to as javelin nematodes as having stilet and a strong head frame. *Hoplolaimus* has a cylindrical, obese, and long shape. The anterior part is characterized by a thick head frame with a curved sucker, followed by a strong and long stilet with a large knob and oesophageal gland overlapping the intestines in dorsal and lateral parts. Annulation of the cuticle is apparent with four or several lateral lines. The posterior part has a rounded tail with annulation extending to the end (Firoza *et al.*, 1990). According to Quénéhervé *et al.* (2006), most members of *Hoplolaimus* genera are ectoparasitic nematodes. *Hoplolaimus* has a wide host range, including weeds.

Helicotylenchus is also called spiral nematodes as having spiral shape after being given heat treatment (dead condition), but sometimes also shaped like the letter C. *Helicotylenchus* demonstrates the characteristic of spiral morphology at resting phase, with blunt cone-shaped head, as well as long and strong stilet with a rounded knob like a bowl, and short tail with dorsal-like part of

the cone, tail end there is a bulge, then vulva located at 70% of the total body length (Marais *et al.*, 2005). According to Quénéhervé *et al.* (2006), *Helicotylenchus* is ectoparasitic, semi-endoparasitic, and endoparasitic nematode. All phases of these nematodes can be found in the cortex root tissue. The symptoms cause minor injuries and evolve into necrosis after the secondary invasion.

Scutellonema nematode is generally shaped like letter C or opened spiral at dead or resting phase. The anterior part indicates the hallmark of the semi-spherical notched head. Stilet is strong and long with a round-shaped knob. The cuticle annulation is visible with lateral lines. The esophageal gland slightly overlaps with the dorsal part of the intestines. The posterior section shows the wide-rounded tail characteristic of annulation surrounding the entire tail. The vulva is located behind the mid-length body. *Scutellonema* is an ectoparasitic nematode, which becomes an important pest of tropical crops. These nematodes generally attack the tuber crops (Humphreys-Pereira *et al.*, 2014).

This study conclude that weeds found on tomato, celery, carrot, and carrot seedlings were *Ageratum conyzoides*, *Ageratum houstonianum*, *Portulaca oleracea*, *Eleusine indica*, *Amaranthus spinosus*, *Borreria alata*, and *Borreria laevis*. Seven weed species found can be utilized by the parasitic nematodes as the alternative host and potentially increase crop yield loss. Plant-parasitic nematodes found associated with seven weed species were *Meloidogyne*, *Pratylenchus*, *Helicotylenchus*, *Hoplolaimus*, *Rotylenchulus*, *Scutellonema*, and *Tylenchulus*.

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Abdul Munif	:	Conceived ideas and designed experiments
Erikson Butarbutar	:	Reviewed manuscript and helped in conducting research
Ankardiansyah P. Pradana	:	Data analysis and results interpretation
Ahmed I. A. Yousif	:	Translate the manuscript and helped in data analysis