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Abundance and diversity of plant parasitic nematodes associated with vegetable cultivation on various types of organic fertilizers

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Abstract. *Indarti S, Taryono, Purnomo CW, Wulandari AS, Maharani. 2023. Abundance and diversity of plant parasitic nematodes associated with vegetable cultivation on various types of organic fertilizers. Biodiversitas 24: 1010-1016.* Planting organic crops is inextricably linked to the presence of Plant-Parasitic Nematodes (PPNs) as a limiting factor. A survey was conducted to determine the abundance and prevalence of PPNs, their correlation with soil chemical properties, and their diversity index in October 2019 at the organic land of the Agrotechnology Innovation Centre, Universitas Gadjah Mada, Kalitirto, Sleman, Special District of Yogyakarta, Indonesia. The organic land was designed as three blocks of different organic fertilizers, including milk sludge fertilizer, cow manure fertilizer, and goat manure fertilizer. The samples were taken by simple randomized sampling method with replications and composited for each treatment in each vegetable plot. Three nematode genera of PPNs were associated with organic vegetable crops in Agrotechnology Innovation Centre, *Rotylenchulus* sp., *Meloidogyne* sp., and *Helicotylenchus* sp. The most common PPNs found was *Rotylenchulus* sp. with populations of 1388/5 gr roots and 1064/100 mL soil. The other genera, *Meloidogyne* sp. and *Helicotylenchus* sp. were found with less than 100 nematodes per 5 gr of roots and per 20 gr of soil. C-organic was positively correlated with the *Rotylenchulus* sp. population. The C/N ratio had a positive effect on the *Meloidogyne* sp. population, while N-total had a negative effect on the *Meloidogyne* sp. population. The PPN community diversity index value on the organic land was low (range 0.36-0.37). It indicated that the habitat conditions were relatively homogeneous. It is necessary to carry out integrated management of nematodes to suppress *Rotylenchulus* sp. population in addition of organic fertilizers on this land.

Keywords: Cow manure, goat manure, milk sludge, organic, plant parasitic nematodes

INTRODUCTION

Many countries have seen a tremendous rise in vegetable output and consumption in the last 20 years. Statistical data show that 71.5 million hectares, or 2.9 million farms, are now managed using organic methods (Willer et al. 2020). Australia has the largest area dedicated to organic farming (35.7 million ha), followed by Argentina (3.6 million ha) and China (3.1 million ha) (IFOAM-Organic International 2020). Indonesia also continues to see a sharp rise in its vegetable requirement and its organic farming practices. The expansion of organic farming methods in Indonesia should address local demand due to the increasing consumption of organic vegetables, fruits and rice in Indonesia (David and Ardiansyah 2017). According to 2016 statistics from the Indonesian Organic Agriculture Statistics, there was a 54% rise in consumer demand for organic goods over the previous year (BPS 2017). Organic farming in Indonesia has continued to develop since 1992, when organic products were first certified, and in 2015 the ASEAN Standards for Organic Agriculture (ASOA) harmonized organic farming standards among ASEAN countries (David and Ardiansyah 2017).

Organic farming offers the potential to regenerate farmland and counteract the loss of biodiversity by not using chemicals. The current agricultural practices in Indonesia indicated that around one-third of the farmers applied organic inputs such as processed manure or other types of organic fertilizer (Frits et al. 2021). The advantages of organic fertilizers are low cost, improving soil structure, texture and aeration that also stimulates healthy root development through the soil's water retention abilities. Organic fertilizer has many sources, such as minerals, animal sources, sewage sludge and plant. Vegetables, animals, and residue materials contribute to improve soil organic matter content in the soil. The use of organic fertilizers is expected to reduce the negative impact of inorganic fertilizers and to maintain soil health (Assefa and Tadesse 2019). The attention on environmental safety and human and animal health is also increasing due to the reduction and revision of pesticide use on agricultural crops based on European Legislations (Reg. CE 396/2005; 1095/2007; 33 and 299/2008 and 1107/ 2009). This also leads the researchers to investigate new alternatives for pest control strategies (Renco and Kovacik 2012). On the other hand, the application of animal and plant by-products to the soil is the best-recognized method of managing crops when synthetic inputs are prohibited, as such in organic agriculture. Numerous additions have been evaluated and suggested for the management of PPNs, even though the main goals of utilizing soil amendments are to improve nutrient supplementation, increase organic matter levels, and improve soil structure (Briar et al. 2016).

Planting organic crops is inextricably linked to the presence of plant pest organisms, including Plant-Parasitic Nematodes (PPNs). PPNs are a very important limiting factor in agriculture production, especially in vegetable crops. Annual crop losses due to PPNs in the United States amounted to $8 billion in 2008 and $78 billion worldwide (Sikora and Fernande 2005). Chickpea (*Cicer arietinum*) yield losses because of nematodes in India reached 14 to 40% (Ali and Sharma 2003), while cucumber (*Cucumis sativus*) losses due to nematodes were 12% (Kumar et al. 2020), eggplant (*Solanum melongena*) losses reached 43% (Singh and Kumar 2015). Yield losses due to PPNs could reach up to 75%, especially in intensive cultivation, without proper implementation of crop protection management. The occurrence of PPNs in the agricultural field is generally undistinguished as crop pests because of their microscopic size and their existence in between soil particles and in the root system (Suyadi and Rofiansyah 2017). Several genera of PPNs have been reported to be associated with vegetable crops, such as *Meloidogyne*, *Rotylenchus*, *Nacobbus*, *Ditylenchus*, *Pratylenchus*, *Radopholus*, *Heterodera*, *Belonolaimus*, *Trichodorus*, and *Paratrichodorus* (Sikora and Fernandez 2005; Mutala'liah et al. 2019; Wulandari and Indarti 2020). *Meloidogyne* sp. causes significant losses in a variety of agricultural crops worldwide (Castillo et al. 2008), including chili (*Capsicum frutescens*), eggplant, okra (*Abelmoschus esculentus*), and long beans (*Vigna unguiculata* subsp. *sesquipedalis*) (Wallingford 2007). *Aphelenchoides*, *Aphelenchus*, *Helicotylenchus*, *Meloidogyne*, *Pratylenchus*, *Rotylenchulus* and *Tylenchus* were found in most of the organic vegetable crops. *Rotylenchulus* spp. and *Meloidogyne* sp. cause significant harm to ginger (*Zingiber officinale*), eggplant, celery (*Apium graveolens*), and black pepper (*Piper nigrum*) (Pascual et al. 2017). Given the lack of information regarding the abundance of PPNs in organic vegetable fields in Indonesia, a survey was conducted to determine the abundance and diversity of PPNs, their association with soil chemical properties, and their index of diversity.

MATERIALS AND METHODS

Sample collection and nematode analysis

Sampling was carried out on the organic vegetable cultivation land at the research field of the Agrotechnology Innovation Centre, Universitas Gadjah Mada, located in Kalitirto, Sleman, Special District of Yogyakarta, Indonesia. Two different vegetable plants (*Vigna unguiculata* ssp. *sesquipedalis*/ Yardlong bean and *Cucumis sativus*/ Cucumber) were planted with three treatments of organic fertilizers and three replications for each kind of plant in randomized complete block design. The three treatments of organic fertilizers were as follows: milk sludge fertilizer, cow manure fertilizer, and goat manure fertilizer with a dosage of 10 tons/ha. The total trial area was 105 m^2 and the space between plants was about 0.45 m. The cultivation was carried out organically with the addition of liquid organic fertilizers for each treatment and *Azadirachta indica* as organic control. The abundance and diversity of PPNs were observed in yardlong beans and cucumber plants during the dry season. Soil and root samples for each treatment were randomly taken and composited.

Nematodes were extracted using Whitehead Tray Technique with modification referred to El-Marzoky (2019). Each soil and root type underwent a similar procedure, which involved 100 mL of composited soil and 5 gr of the root samples. The bottom and sides of a large mesh plastic tray were covered with a single sheet of tissue paper before it was placed into another plastic tray. The obtained soil and root samples were placed on tissue paper, spread out into a thin layer, and then lightly misting them with tap water. After being collected in the plastic tray for 24 hours, the suspension was concentrated by being poured over a fine sieve (400 mesh screen), and nematodes were then ready for observation and identification.

Nematode identification and observations

The number of PPNs obtained was calculated using a counting dish under a microscope. The population density of each genus of PPNs was calculated by multiplying the mean number of PPNs obtained in 5 mL (3 replication) of the total nematode suspensions (Rahman et al. 2014). The nematodes were identified based on their morphological characteristics. The nematodes to be identified were hooked up and transferred to a glass object. Specimens were observed with an Olympus CX-31 (Tokyo, Japan) light microscope at a magnification of 40-1000×, and images were captured using Optilab (Yogyakarta, Indonesia) for documentation. Morphological observations of each specimen were using the key morphological characteristics described in Mai (1988) and Mekete et al. (2012) to identify the samples to the genus level.

Soil chemical analysis

Soil analysis was carried out by sending samples to the Testing Laboratory of the Research Institute for Agricultural Technology, Yogyakarta, and the Department of Soil, Faculty of Agriculture, Universitas Gadjah Mada. C-organic, total-N, and the C/N ratio among the soil parameters were examined.

Statistical analyses

Relationships between the soil chemical parameters and plant-parasitic nematode abundance

The relationships between the soil chemical parameters and the abundance of the PPNs in the soil and roots were analyzed using linear regression analysis in Excel software (Microsoft Inc., Redmond, WA, USA).

Diversity of plant-parasitic nematodes

The diversity of the PPNs in the organic vegetable soil was analyzed using the Shannon-Weiner index (Ifo et al. 2016).

$$ID = H' = -\sum Pi \ln P_i \text{ where } Pi = \frac{ni}{N}$$

Where:
ni : individual number of each genus of nematodes
N : total number of all genera of nematodes
H′ : Shannon-Weiner diversity index
Pi : abundance index

RESULTS AND DISCUSSION

The abundance of the plant-parasitic nematodes

Three genera of PPNs were associated with the organic vegetable crops at the Agrotechnology Innovation Centre (PIAT), *Rotylenchulus* sp., *Meloidogyne* sp., and *Helicotylenchus* sp. (Table 1). This study showed different populations of PPNs in the two plant samples on organic farming practices. *Rotylenchulus* sp. was the most abundant in every organic fertilizer type and both plant species. This result was similar to Pascual et al. (2017)'s study that *Rotylenchulus* sp. had the highest population density in vegetable farms in the Philippines. Reniform nematodes (*Rotylenchulus* spp.) are semi-endoparasites nematodes and commonly found in tropical and subtropical regions. *Rotylenchulus* sp. causes serious losses to vegetable crops. Castillo et al. (2008) reported that *Rotylenchulus reniformis* has a wide host range, including legumes, cereals (maize, rice, sorghum, and wheat), vegetables (cucumber, eggplant, potato, and tomato), and fruits (bananas, oranges, and grapes). The greatest population of *Rotylenchulus* sp. in this study was found in milk sludge fertilizer on yardlong bean plants (1064 nematodes/20 g of soil and 1388 nematodes/5 g of root). Forge et al. (2005)'s study showed that sustained use of dairy slurry fertilizer increased the population density of PPNs (*Pratylenchus penetrans*).

The abundances of the other PPNs, Meloidogyne sp. and *Helicotylenchus* sp. were lower, <100 nematodes per 5 g roots and 100 g soil. According to Wulandari et al. (2021), some PPNs, such as root lesion nematodes, are more abundant when organic matter is present. While the lowest PPNs abundance was the nematode *Helicotylenchus* sp. with 2 nematodes/ 5 g on roots and 20 g of soil in yardlong beans and cucumbers among all of the types of organic fertilizer treatments. Wallingford (2007) reported that *Helicotylenchus* sp. is a minor nematode on eggplant and long bean plants. According to Sikora and Fernandez (2005), these nematodes do not cause serious damage to vegetables, even though *Helicotylenchus* sp. is often found in vegetable fields. *Helicotylenchus dihystera* is an active pathogen of cucumber, cauliflower, chinese cabbage, peppers, chickpeas, and onions, but there are no reports of pathogenicity in organic vegetables in the Philippines (Pedroche et al. 2013). In addition, the results of Damaryono et al. (2018)'s research showed a decrease in the abundance of PPNs in the field with organic management. Studies from Marull et al. (1997), Lamondia et al. (1999), Evert et al. (2006) showed that compost and raw sewage sludge had activity against some PPNs, but in other studies, compost was only affected slightly (Oka, 2010). The organic fertilizers' impact could be influenced by various factors, such as organic material type, composting of material, application rate, crop rotation, agronomic practices, soil type, climate, and other environmental factors (McSorley 2011).

Soil chemical characteristics of the organic fertilizers

The soil chemical characteristics were different for each kind of organic fertilizer. C-Organic content, N-Total and C/N Ratio of the organic fertilizers in the organic vegetable fields are shown in Table 2. It could be seen that the highest of C-Organic and N-Total content was found in milk sludge fertilizer. The high content of N-total was closely related to the plant's growth, where plants grew better when N availability was the highest (Tabarant et al. 2011). N availability in the soil determines the amount of nitrogen that can be absorbed by plants (Putra et al. 2018). It's indicated that milk sludge fertilizer had a nutrient content that was more easily absorbed by plants than goat manure. The addition of sewage sludge into the soil could provide high quantities of mineral N, beside this amendment was full of microorganisms (Tabarant et al. 2011).

Table 2. Soil chemical content of the organic fertilizers

Types of organic fertilizer	C-Organic (%)	N-Total (%)	C/N Ratio
Goat manure	30.06	1.49	20.31
Cow manure	17.69	2.02	8.76
Milk Sludge fertilizer	44.90	5.86	7.66

Table 1. The abundance of plant-parasitic nematodes in organic vegetable cultivation. Mean of 3 replications (range)

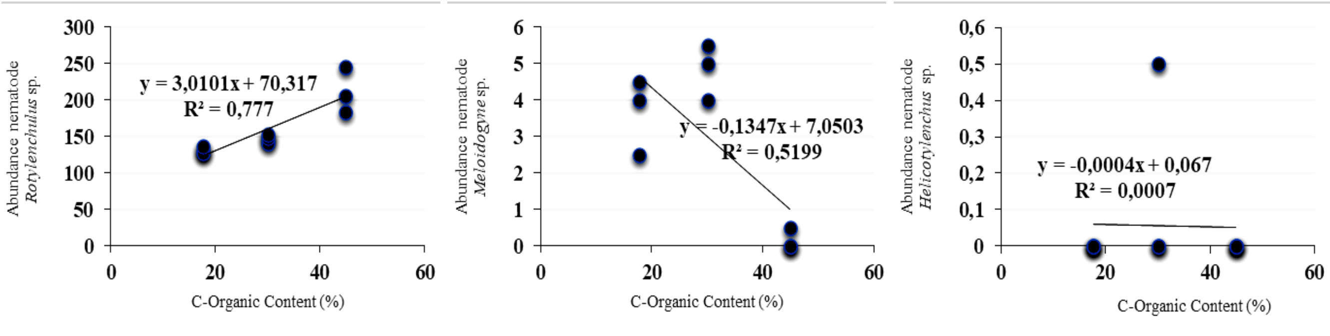
Plant type	Types of organic fertilizer	*Rotylenchulus* sp.		*Meloidogyne* sp.		*Helicotylenchus* sp.	
		Per 100 mL of soil	per 5 g of root	per 100 mL of soil	per 5 g of root	per 100 mL of soil	per 5 g of root
Yardlong beans	Goat manure	812 (744-900)	786 (702-852)	56 (42-66)	2 (0-6)	2 (0-6)	0 (0-0)
	Cow manure	916 (858-973)	588 (486-666)	24 (12-42)	20 (6-36)	0 (0-0)	0 (0-0)
	Milk sludge fertilizer	1064 (834-1452)	1388 (1320-1446)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
Cucumber	Goat manure	70 (36-96)	98 (84-108)	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)
	Cow manure	52 (36-66)	16 (6-24)	0 (0-0)	0 (0-0)	2 (0-6)	0 (0-0)
	Milk sludge fertilizer	44 (24-60)	44 (30-66)	0 (0-0)	2 (0-6)	2 (0-6)	0 (0-0)

The highest C/N ratio was found in goat manure at 20.31, while the lowest C/N ratio was detected in the milk sludge fertilizer at 7.66. A lower C/N ratio is easier to decompose than a higher C/N ratio so the nutritional content is more easily available to the plants (Putra et al. 2018).

Effect of soil chemical properties on the abundance of parasitic nematodes

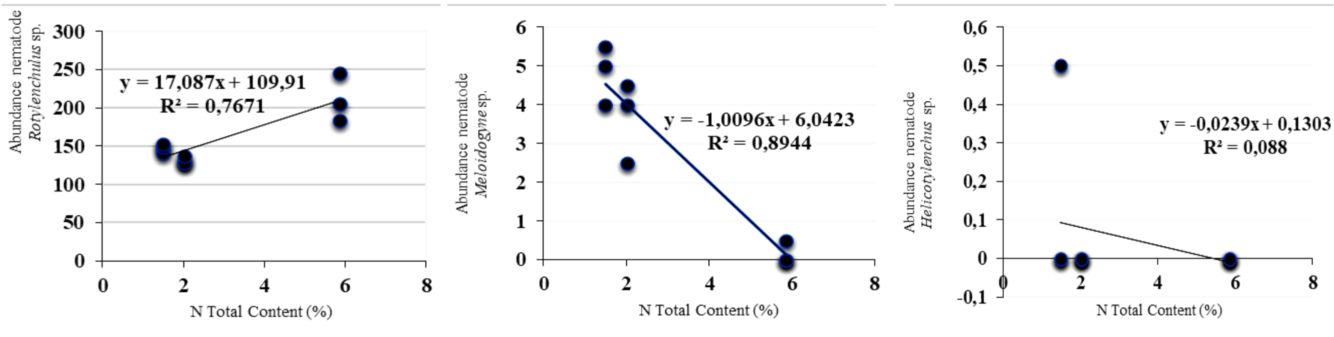
C-organic affected the three parasitic nematodes found (*Helicotylenchus* sp., *Rotylenchulus* sp., and *Meloidogyne* sp.) in varying degrees (Figure 1). C-organic had the greatest effect on *Rotylenchulus* sp. with a correlation coefficient of 0.77 compared to the effect of C-organic on the other PPNs found. The regression equation shows that C-organic had a direct positive effect on the abundance of *Rotylenchulus* sp. nematodes in the soil. The positive effects between PPNs and soil mineral nutrients (K, Ca, Na, and C) and organic matter content were reported by Zoubi et al. (2022). The abundance of *Meloidogyne* sp. and *Helicotylenchus* sp. was negatively correlated with C-organic (Figures 1B-1C). Thus, increasing C-organic content will reduce the *Meloidogyne* sp. and *Helicotylenchus* sp. abundance. This negative correlation was reported by Hu and Qi (2013) and Benjlil et al. (2020), who stated that the accumulation of soil organic matter significantly reduces the abundance of PPNs via decreasing their vital proprieties (Zoubi et al. 2022).

Total-N affected the three parasitic nematodes (*Helicotylenchus* sp., *Rotylenchulus* sp., and *Meloidogyne* sp.) to varying degrees (Figure 2). Total-N had the greatest effect on *Meloidogyne* sp. nematodes (89%) compared to the effect of total-N on the other parasitic nematodes. Based on the regression equation, total-N negatively affected the abundance of the *Meloidogyne* sp. nematodes. This is in contrast to Asif et al. (2015), Noronha et al. (2020), Ngeno et al. (2019), who showed that N content is positively correlated with the *Meloidogyne* spp. population. Nevertheless, the efficiency of nitrogen fertilizer has been reported before (Karajeh and Al-Nasir 2012; Wei et al. 2012; Patil et al. 2013). N content is known to affect the pH of plants, which affects the plant's susceptibility to nematode infection (Patil et al. 2013). Second stage juvenile (L2) of nematode is affected by its ability to locate plant roots (Noronha et al. 2020). N supply may have increased plant sensitivity to pathogens. Although the N availability was the highest, it did not necessarily reduce the PPNs impacts on the roots. Damage on roots depended on the abundance of PPNs (Tabarant et al. 2011). The effect of total-N on the abundance of *Helicotylenchus* sp. was weak at 8%. This result shows that the effect of N on nematodes will depend on where it was available in the soil (Sánchez-Moreno and Ferris 2018).



A B C

Figure 1. Correlation of C-organic content and the abundance of plant-parasitic nematodes: A. *Rotylenchulus* sp.; B. *Meloidogyne* sp.; C. *Helicotylenchus* sp.



A B C

Figure 2. Correlation of total-N and the abundance of the plant-parasitic nematodes: A. *Rotylenchulus* sp.; B. *Meloidogyne* sp.; C. *Helicotylenchus* sp.

The C/N ratio elicited different responses by the three parasitic nematodes (*Helicotylenchus* sp., *Rotylenchulus* sp., and *Meloidogyne* sp.) (Figure 3). The C/N ratio had only a minimal effect on the abundance of *Rotylenchulus* sp. at 13% (Figure 3A). Based on the regression equation, the C/N ratio had a direct negative effect on the abundance of the *Rotylenchulus* sp. nematodes. Soil physiochemical properties such as organic matter, the C/N ratio, pH, and texture greatly affect the community structure and abundance of parasitic nematodes in the agrosystem (Godefroid et al. 2013). Adding organic matter increases the C/N ratio and increases plant vigor, which indirectly affects nematode activity (Wildmer et al. 2002). The C/N ratio had the greatest effect on the *Meloidogyne* sp. nematodes at 50% (Figure 3B). C/N ratio had a direct positive effect on the abundance of the *Meloidogyne* sp. nematodes. C/N ratio was one of the other factors which correlated strongly with the abundance and distribution of nematodes (Ilieva-Makulec et al. 2014).

Prevalence, relative abundance, and mean intensity of the plant-parasitic nematodes

The results of the population analysis on the prevalence, relative abundance, and mean intensity of the PPNs on the organic vegetable crops are shown in Table 3. Our results showed similar results to Adam et al. (2013)'s study in Egypt that *Helicotylenchus* sp., *Meloidogyne* sp., and *Rotylenchulus* sp. was also found in different kind of organic crops with 100% occurrence. The genera of PPNs detected on organic and conventional farms tend to be similar under similar environmental conditions (Adam et al. 2013; Briar et al. 2016). While in our result, the highest prevalence only occurred in *Rotylenchulus* sp. Different results were also observed in Krif et al. (2020)'s study that *Meloidogyne* spp. was the most frequent nematode found in organic vegetables and medicinal and aromatic plants in Morocco. The abundance and distribution of nematodes were strongly correlated to the C/N ratio, soil acidity, plant cover and biomass, soil temperature and soil depth (Ilieva-Makulec et al. 2014).

Plant parasitic nematodes diversity index

Hariyadi et al. (2019) proposed several criteria for diversity index values between 0-7: 0-2 (low), 2-3 (medium), and >3 (high). Stable, broad, and homogeneous communities have a lower diversity index than mosaic forest ecosystems or areas with periodic disasters (Hariyadi et al. 2019). Our study showed a relatively similar diversity index of PPNs for the different fertilizers, and it was low (Table 4), indicating that the habitat conditions and the nematodes for organic vegetable crops were relatively homogeneous at different fertilizers. This showed that the characteristic of the land with different kind of organic fertilizers was the same. Besides that, the nematodes found were quite the same or homogeneous, with *Rotylenchulus* sp. as the most abundant nematode. It is necessary to carry out integrated management of nematodes to suppress *Rotylenchulus* sp. population in addition of organic fertilizers on this land. Further studies regarding the integrated nematode management based on organic and about the *Rotylenchulus* sp. impact to the plants need to be carried out to suppress the PPNs populations, although other PPNs found were low by applying the organic fertilizers.

Table 3. Prevalence, relative abundance, and mean intensity of the plant parasitic nematodes in the organic vegetable fields

Nematode Species	Prevalence (%)	Relative abundance (%)	Mean intensity
Rotylenchulus sp.	100	88.22	86.56
Meloidogyne sp.	50	3.06	5.75
Helicotylenchus sp.	16.67	0.17	13.4

Table 4. Diversity index values of the parasitic nematodes on the different organic fertilizers

Types of organic fertilizer	Parasitic nematodes diversity index
Goat manure	0.36
Cow manure	0.36
Milk sludge fertilizer	0.37

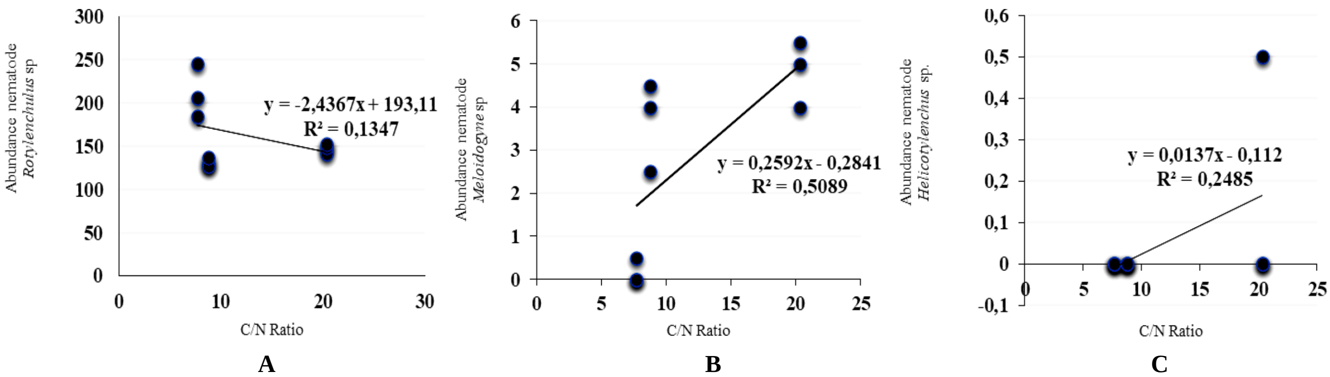


Figure 3. Correlation of the C/N ratio and the abundance of the plant-parasitic nematodes: A. *Rotylenchulus* sp.; B. *Meloidogyne* sp.; C. *Helicotylenchus* sp.

Organic material type, composting of material, application rate, crop rotation, agronomic practices, soil type, climate, and other environmental factors could influence the impact of organic fertilizers (McSorley 2011). Organic field treatment tends to decrease the PPNs besides increasing the abundance of earthworms and non-parasitic nematodes, increasing soil respiration and increasing aggregate stability (Damaryono et al. 2018). The composition of the nematode functional group may reflect the diversity and heterogeneity of resources and environments (Ferris and Tuomisto 2015) that reflect to the different ecosystem or land use characteristics (Li et al. 2019). The changes in the soil's microfauna, microflora, population, and activities of nematodes antagonistic microorganisms may suppress the soil nematodes. The suppression of the soil nematodes can be affected by manure type and dose, as well as its endogenous microflora (Oka 2010).

In conclusion, plant-parasitic nematodes (PPNs) on vegetable fields with organic fertilizer management showed a low population and low diversity, indicating that the habitat conditions were relatively homogeneous. Three nematode genera of PPNs were associated with organic vegetable crops in Agrotechnology Innovation Centre, *Rotylenchulus* sp., *Meloidogyne* sp., and *Helicotylenchus* sp. The most abundant PPNs was *Rotylenchulus* sp. in goat manure, cow manure, and milk sludge fertilizer.

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