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The Effect of Abiotic Factors and Elevation on the Diversity of Plant Parasitic Nematodes in Garlic on Central Java, Indonesia

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Abstract | Plant-parasitic nematodes are one of the most prominent type of pests in garlic (*Allium sativum* L.). To determine the influence of elevation and soil abiotic factor to abundance and diversity of plant parasitic nematode in garlic crops, this research was conducted Sampling was carried out in four locations: Brebes, Magelang, Tegal and Temanggung, Central Java, Indonesia. Samples of soil, roots and tubers were collected. Soil abiotic factors are taken measurements such as pH, temperature, and organic matter. The altitudes are measured using the Geographical Positioning System (GPS). The results showed that there were five parasitic nematodes genera in garlic, that are *Ditylenchus* sp., *Helicotylenchus* sp., *Hoplolaimus* sp., *Rotylenchulus* sp., and *Pratylenchus* sp. Abiotic factors as temperature has a positive influence on the abundance of *Helicotylenchus* sp., pH has a negative influence on the abundance of *Hoplolaimus* sp., organic matter has a positive effect on the abundance of *Pratylenchus* sp.. The diversity index of parasitic nematodes in garlic at various elevations is relatively low, ranging from 0.90 to 1.36. The dominance of plant parasitic nematodes in garlic at various elevations varies at the elevations >1 600 m a.s.l, 1 201 m to 1 400 m a.s.l and < 1 000 m a.s.l. The dominating plant parasitic nematode was *Ditylenchus* sp., found at 1 401 m to 1 600 m a.s.l elevation, the predominant plant parasitic nematode was *Helicotylenchus* sp., found at 1 000 m to 1 200 m a.s.l elevation, and the dominating plant parasitic nematode was *Pratylenchus* sp. found at less 1 000 m a.s.l elevation.

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Keywords | *Allium sativum* L., Flagship horticulture, abiotic factor and elevation, Survey of plant parasitic nematodes

Introduction

Garlic (*Allium sativum* L.) is one of the most important flagship horticultures in Indonesia. Central Java is a suitable area for producing garlic in Indonesia. The area includes Magelang, Tawangmangu, Tegal and Temanggung. Garlic production is bound to be attacked by pests in the field. One kind of the pests causing a pretty high loss yet drawing

less concern is plant-parasitic nematodes. Plant-parasitic nematodes are a major type of pest that causes a huge loss on the agricultural production of plants in both tropic and sub-tropic region (Mahmoud and Wafaa, 2021). Garlic crop loss caused by plant-parasitic nematodes reaches 8.8 % to 14.6 % and results in economic loss amounting to USD 100 to 157 billion across the entire country (Nicol et al., 2011).

According to George and Muktan's (2010), garlic diseases caused by nematodes in New York, symptoms of the severe loss may lead to 80 % to 90 % of the crop loss. The development of nematodes is influenced by several abiotic factors. This becomes a major concern since nematodes life in soil allows direct contact with factors in the soil that may affect their persistence. Abiotic factors such as temperature, humidity and soil texture may affect the abundance of plant-parasitic nematodes. Plant-parasitic nematodes identification in garlic is extremely necessary since there is little information about plant-parasitic nematodes of garlic in Indonesia. Based on the lacking of information of the influence of elevation and soil abiotic factor to abundance and diversity of plant parasitic nematode in garlic crops, this investigation was done. This information may hopefully benefit people in controlling garlic-parasitic nematodes attack and preventing the quarantine pest's entry to the Republic of Indonesia.

Materials and Methods

Sample collection and soil abiotic factors measure

The survey was carried out at garlic plantations in Magelang, Temanggung, Brebes and Tegal areas in Central Java from November 2018 to May 2019. Soil abiotic factors observed were temperature, pH and organic matter. Temperature and pH are observed using a soil thermometer and pH meter (Mediatech Soil Survey Instrument Digital 4 in 1 Backlight) by sticking it in the soil with 10 cm in depth for 10. whereas organic matter was analysed in the laboratory. The location of the land was divided into five points based on their height above sea level (a.s.l) with the altitude of < 1 000 m a.s.l, 1 000 m to 1 200 m a.s.l, 1 201 m to 1 400 m a.s.l, 1 401 m to 1 600 m a.s.l and >1 600 m a.s.l the altitude are measured using the Geograpichal Positioning System (GPS). Samples were taken based on the morphology of the diseased plant (diagnostic method).

Analyses of nematode population and identification

The Isolation-Extraction of nematodes from soil samples was carried out using a modification of the Whitehead Tray method (Bezooijen, 2006), while the Isolation-Extraction of nematodes from plant tissues (roots and tubers) was carried out by incubation method (Bezooijen, 2006). Sample with approximately 200 g of soil and 5 g of root in each location was taken. Population abundance parasitic nematode counted by multiplying the average number of parasitic nematodes from 5 mL in 50 nematodes pop-

ulation (Rahman *et al.*, 2014). Nematode identification till genus level was done based on morphological characteristics referred to Key to Genera of Plant Parasitic Nematodes (Mai and Peter, 1996).

Relation between abiotic factors and parasitic nematodes abundance

Relation between abiotic factors such as temperature, pH, organic matter with parasitic nematodes abundance in soils, tubers and roots was analysed using correlation analysis and linear regression through Ms. Excel program.

Diversity of parasitic nematodes analysis based on elevation

Diversity of parasitic nematodes in garlic on various elevation analysed using Shannon diversity index (Ifo *et al.*, 2016), explain in Equation (1).

$$H' = - \sum [P_i \ln P_i] \dots\dots(1)$$

Where;

H' = Shanon diversity index; P_i = Abundance index; Noted: P_i=n_i/N, n_i = individual number of each genus of nematodes; N = total number of all genera of nematodes.

Results and Discussion

Soil abiotic factor characteristics

The soil pH of the collected samples varied between 5.4 to 5.9. The concentration of H ions in soil may vary and fluctuate due to the weather and the vertical distribution of colloidal material on the soil profile (Mulyadi, 2009). The temperature of each altitude were also varied between 22.5 °C to 25.5 °C. In wet soil, an increase in temperature is slower than that in dry soil although the input of heat on the surface is the same. The heat from the sun will penetrate deeper into wet soil rather than dry soil (Mulyadi, 2009). Organic matter contents in each soil sample were various with range 5.33 % to 11.55 % and the value were categorized in high level when reach more than 5 % (Eviati and Sulaeman, 2009)(Table 1).

Table 1: Soil abiotic factors characteristics at garlic plantations in Central Java.

Location	Elevation (m a.s.l)	Temperature (°C)	pH	Organic matter (%)
Magelang	>1 600	22.5	5.9	5.33
Brebes	1 401 to 1 600	23	5.8	6.41
Temanggung	1 201 to 1 400	23.5	5.4	5.45
Temanggung	1 000 to 1 200	24	5.8	11.05
Tegal	< 1 000	25.5	5.4	11.55

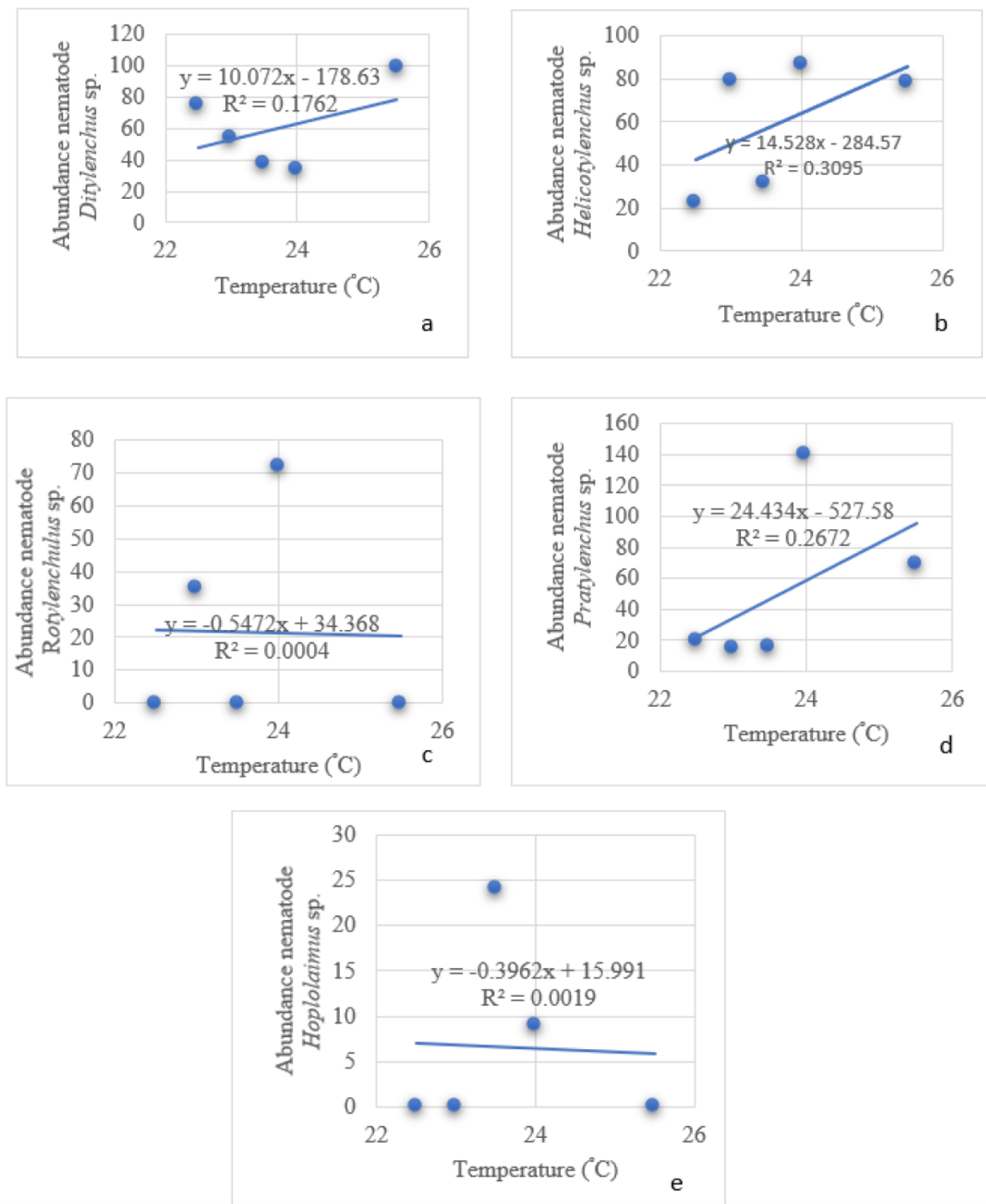


Figure 1: Temperature influences on parasitic Nematodes abundance (a): *Ditylenchus* sp.; (b) *Helicotylenchus* sp.; (c) *Rotylenchulus* sp.; (d) *Pratylenchus* sp.; (e) *Hoplolaimus* sp.

Parasitic nematodes abundance on garlic

This research found five genera of parasitic nematodes on garlic at various elevations in Central Java that is *Ditylenchus* sp., *Helicotylenchus* sp. *Hoplolaimus* sp., *Rotylenchulus* sp., and *Pratylenchus* sp. The result

showed that parasitic nematodes on genus *Ditylenchus* sp. were mostly found at < 1 000 m a.s.l elevation from the root or soil, with 66.67 individuals 5 g^{-1} tuber and 33 individuals 100 g^{-1} soil (Table 2). Genus *Helicotylenchus* sp. was mostly found at < 1 000 m a.s.l

Table 2: Parasitic nematodes abundance on garlic at various elevation.

Location	Elevation (m a.s.l)	Population /5 g tuber				Population/ 100 g soil				
		Dit	Hel	Roty	Praty	Dit	Hel	Roty	Praty	Hoplo
Magelang	> 1 600	64.42	22.5	0	0	10.83	0	0	19	0
Brebes	1 401 to 1 600	46.07	52.5	35	0	8.4	27	0	18	0
Temanggung	1 201 to 1 400	25.55	20	0	0	12	0	0	16	24
Temanggung	1 000 to 1 200	24.17	30	50	140	9	57	22	0	9
Tegal	< 1 000	66.67	53.75	0	45	33	24	0	23.5	0

Abbreviations: *Ditylenchus* sp. (Dit), *Helicotylenchus* sp.(Hel), *Rotylenchulus* sp. (Roty), *Pratylenchus* sp. (Praty), *Hoplolaimus* sp. (Hoplo).

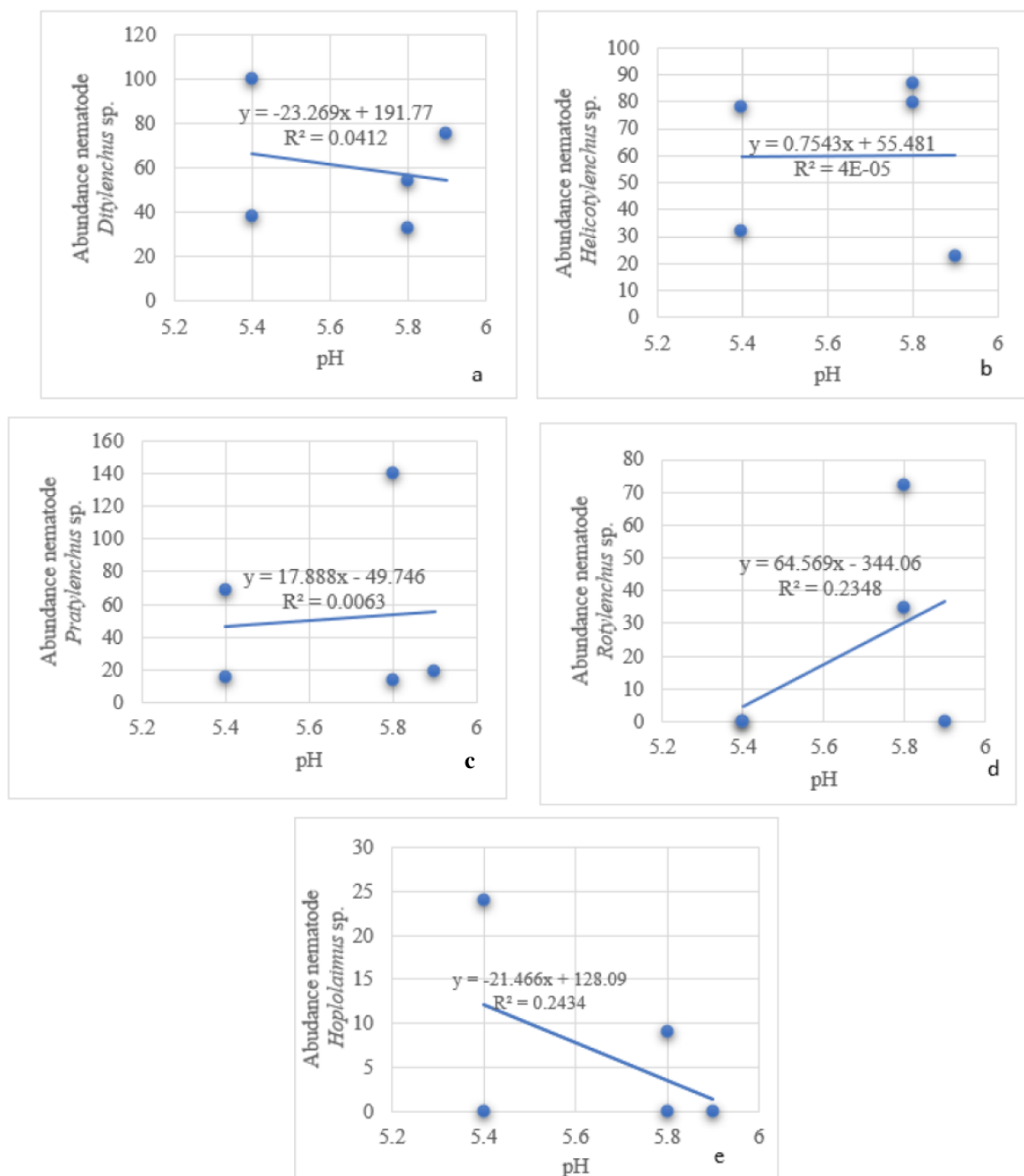


Figure 2: pH Influences on Parasitic Nematodes Abundance (a): *Ditylenchus* sp.; (b) *Helicotylenchus* sp.; (c) *Rotylenchulus* sp.; (d) *Pratylenchus* sp.; (e) *Hoplolaimus* sp.

from the root with 53.75 individuals 5 g^{-1} tuber. As from soil, 57 individuals 100 g^{-1} soil were found at 1 000 m to 1 200 m a.s.l. Genus *Rotylenchulus* sp. was mostly found at 1 000 m to 1 200 m a.s.l from soil and root with 50 individuals 5 g^{-1} tuber and 22 individuals 100 g^{-1} soil. Genus *Pratylenchus* sp. was mostly found at 1 000 m to 1 200 m a.s.l from root with 140 individuals 5 g^{-1} tuber, as from soil, 23.5 individuals 100 g^{-1} soil were found at < 1 000 m a.s.l. Genus *Hoplolaimus* sp. was mostly found at 1 201 m to 1 400 m a.s.l from soil with 24 individuals 100 g^{-1} of soil.

Influence of soil abiotic factor on parasitic nematodes

Temperature influences on parasitic nematodes abundance:

Temperature is one of the soil factors that influences microbe's activities in the soil, not to mention the nematodes. The average temperature for nematodes' activity falls between 5°C to 40°C . Nematodes activity will increase at 25°C and decrease at 30°C . It will also inactivate at 40°C (Mulyadi, 2009). Based on the research, temperature influenced the five found parasitic nematodes (*Ditylenchus* sp., *Helicotylenchus* sp., *Pratylenchus* sp., *Hoplolaimus* sp. and *Rotylenchulus* sp.) at different temperature. The average temperature in this research fell between 22.5°C to 25.5°C . This corresponds Mulyadi's opinion (2009) stating that the optimal temperature for nematodes activities is between 15°C to 30°C . Temperature influences *Helicotylenchus* sp. greatly by 30 %. According to the linear regression, the temperature positively influenced *Helicotylenchus* sp. abundance. This corresponds to a research by Pinochet and Cisneros (1986) stating that *Helicotylenchus* sp. population reaches the highest number in summer with 320 nematodes in 500 cm^3 soil. As well as in linear regression of *Pratylenchus* sp. which is known to positively influence the abundance of *Pratylenchus* sp. nematode. This corresponds to Inomoto and Olivera's research (2008) showing positive influence of the temperature between 23.8°C to 26.7°C on *Pratylenchus brachyuru* development and reproduction.

pH influences on parasitic nematodes abundance:

pH is one of the crucial factors in soil that influences its physical, chemical and biological characteristics and may affect plant growth and organism life in soil such as nematodes (Calanska et al., 2006). Soil pH left diverse influence on some nematodes genera. Every nematode genus possesses its optimal pH to grow. The result showed that pH influenced those five

nematodes (*Ditylenchus* sp., *Pratylenchus* sp. *Hoplolaimus* sp. *Rotylenchulus* sp., and *Helicotylenchus* sp.) at various elevations. pH influenced *Hoplolaimus* sp. more greatly by 24 % than other nematodes. Based on the linear regression above, pH directly influenced *Hoplolaimus* sp. abundance negatively. This conforms Matute (2013) showing that pH negatively influences several nematodes such as *Hoplolaimus galeatus*, and survived best at pH 6.0. pH influence on *Helicotylenchus* sp. abundance is known to be very weak which is only by 0.004 %. Simon et al. (2018) state in their research that *Helicotylenchus* sp. is never found in soil pH under 4.3, neither in organic or mineral soil. It is mostly found in soil with pH above 6.0. This proves that *Helicotylenchus* sp. is mostly found in pH higher than 6, while the average pH in this research was only 5.6. This caused low encounter of *Helicotylenchus* sp. If the environmental condition such as pH is not in its optimal condition for nematodes to reproduce, many nematodes may fail to reproduce and reach their adult phase and the plant-parasitic nematodes population may as well decrease (Northon, 1989).

Organic matter influence on parasitic nematodes abundance:

Organic matter directly gives negative influence on plant-parasitic nematodes growth and development. Organic matter decomposition results as a toxic chemical compound to some genera of parasitic nematodes (Mulyadi, 2009). Based on the result of the research, organic matter influenced those five parasitic nematodes (*Ditylenchus* sp., *Helicotylenchus* sp., *Pratylenchus* sp., *Hoplolaimus* sp., and *Rotylenchulus* sp.). It left diverse influence on each parasitic nematode. In this research, organic matter influenced *Pratylenchus* sp. greatly by 72 %. Based on the above linear regression, organic matter is known to directly and positively influence *Pratylenchus* sp. nematode abundance. This conforms Mutala'iah et al. (2017) saying that organic matter positively correlates with *Pratylenchus* sp. nematode population. Cadet and Thioulouse (1998) state in their research that *Pratylenchus coffeae* prefers all types of soil with high organic matter content. When the organic matter is high, *Pratylenchus* sp. nematode abundance will also be high. As in potato plants, there is no real difference between giving organic matter and not giving organic matter on *P. penetrans* and *M. hapla* population as it does not decrease their population (Kimpinski et al., 2003). In bean plants, however, additional organic matter may decrease *M. incognita* population by 90 % (Akhtar, 2000).

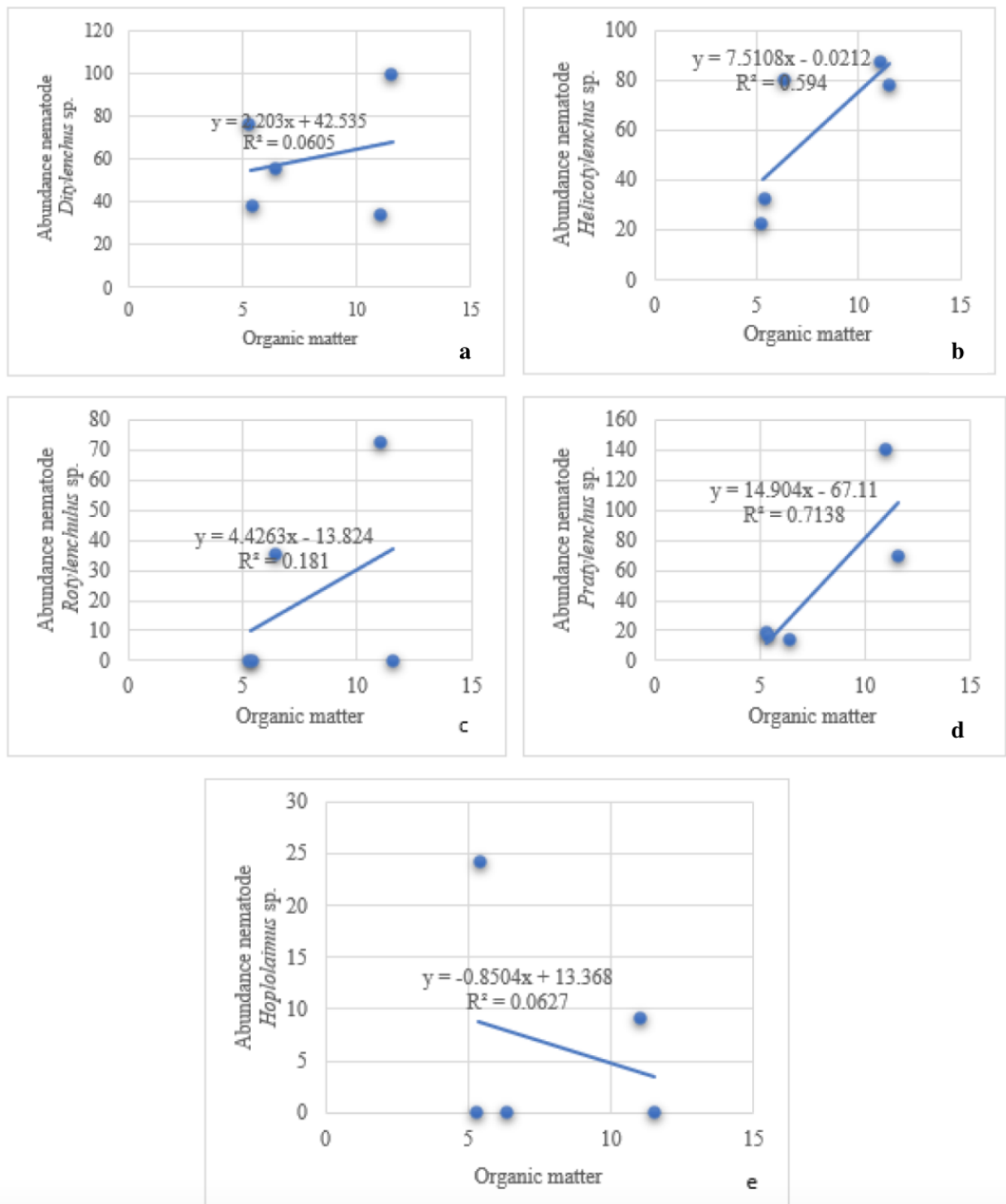


Figure 3: Organic matter influence on parasitic Nematodes abundance: (a). *Pratylenchus* sp.; (b) *Rotylenchulus* sp.; (c) *Ditylenchus* sp.; (d) *Helicotylenchus* sp.; (e) *Hoplolaimus* sp.

Parasitic nematodes diversity index on garlic at various elevations

Diversity index is information that can determine the relationship between the sample area and the number of nematodes was encountered. Parasitic

nematodes diversity index at various elevations was considered low, approximately between 0.90 to 1.36 (Table 3). According to Hariyadi *et al.* (2019), value of the diversity index is between 0 to 7 with criteria: 0 to 2 (low), 2 to 3 (medium), and >3 (high). Stable,

widespread and homogene community possesses lower diversity index than mosaic forest ecosystem or region with periodically occurred disasters.

Table 3: Parasitic nematodes Diversity Index on garlic at various elevations.

Elevation (m a.s.l)	Parasitic nematodes diversity index
> 1 600	0.90
1 401 to 1 600	1.29
1 201 to 1 400	1.34
1 000 to 1 200	1.36
<1 000	0.97

The data in Table 3 showed that at various elevations, relatively similar diversity index is found. This shows that habitat condition of garlic cropping land in survey is relatively homogene. Minor range of elevation may contribute to similar number of living parasitic nematodes found in all elevations based on the total genus of parasitic nematodes found.

Parasitic nematodes dominance on garlic plants at various elevation

This research showed that at >1 600 m a.s.l elevation, the dominant parasitic nematode was *Ditylenchus* sp. with 41.55 % (Figure 4). *Ditylenchus* sp. was also dominant at 1 201 m to 1 400 m a.s.l with 11.83 % appearance and at < 1 000 m a.s.l with 24.61 % appearance. Yavuzaslanoglu *et al.* (2015) finds that *Ditylenchus* sp., activity depends on soil texture and pH. Nematodes activity is found to be higher in sandy soil with pH 7 than in clay soil with pH 5. Beside, *D. dipsaci* is mostly found at 22 °C than at other temperatures (17 °C and 27° C). This research found that clay loam texture with pH 5.9 and at 22.5 °C was dominant at > 1 600 m a.s.l elevation while at < 1 000 m a.s.l, sandy loam texture with pH 5.4 and at 25.5 °C temperature was dominant. Nematodes are commonly found in soil with sandy texture. However, it is possible for the nematodes to be found in other type of soils. The condition is affected by nematodes migration ability through soil particles. At 1 401 to 1 600 m a.s.l, the dominant parasitic nematode was *Helicotylenchus* sp. with 16.97 % (Figure 4). *H. multicinctus* was found in banana plants at all elevations, especially, higher elevations (< 1 600 m a.s.l). It was found with quite low dominance at lower elevations (< 646 m a.s.l). *Helicotylenchus dihystera* which was dominant at higher elevations (Kamira *et al.*, 2013). This nematodes distribution is strongly

influenced by elevation and temperature. According to Pinochet and Cisneros (1986), *Helicotylenchus dihystera* population reaches its highest population in summer months with high temperature and population abundance reaching < 320 individuals in 500 cm³ soil. The dominant parasitic nematode at 1 000 m to 1 200 m a.s.l was *Pratylenchus* sp. with 16.83 %. Kamira *et al.* (2013) finds that *Pratylenchus goodeyi* is a dominant species at higher elevations (> 1 200 m a.s.l) yet *Pratylenchus* spp. distribution becomes uncertain at lower elevations (9 to 646) m a.s.l.

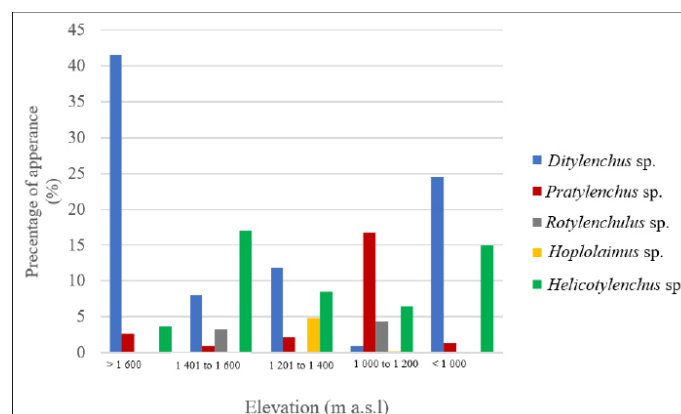


Figure 4: Parasitic nematodes dominance on garlic plants at various elevation.

Conclusions and Recommendations

In conclusion, the parasitic nematodes found were *Ditylenchus* sp., *Rotylenchulus* sp., *Hoplolaimus* sp., *Helicotylenchus* sp., and *Pratylenchus* sp. Abiotic factors such as temperature has positive influence to *Helicotylenchus* sp abundance. Hoeweever pH has negative the significant effect to *Hoplolaimus* sp. nematodes abundance. Organic matter has positive influence to *Pratylenchus* sp. nematodes in garlic. Diversity index of parasitic nematodes in garlic at various elevation was considered low, ranging between 0.90 to 1.36. Based on >1 600 m a.s.l, 1 201 m to 1 400 m a.s.l and < 1 000 m a.s.l elevations, the dominant parasitic nematode in garlic was *Ditylenchus* sp., while at 1 401 m to 1 600 m a.s.l, the dominant parasitic nematode was *Helicotylenchus* sp., and at 1 000 m to 1 200 m a.s.l elevation, the dominant parasitic nematode was *Pratylenchus* sp.

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Novelty Statement

Plant-parasitic nematodes identification in garlic is extremely necessary since there is very little information about plant-parasitic nematodes of garlic in Indonesia. Based on analysis process, the authors found five parasitic nematodes genera in garlic, such as *Ditylenchus* sp., *Helicotylenchus* sp., *Hoplolaimus* sp., *Rotylenchulus* sp., and *Pratylenchus* sp.

Author's Contribution

ASW conceptualized and designed the study, elaborated the intellectual content, performed literature search, carried out experimental studies, data acquisition, data analysis, statistical analysis, manuscript preparation. SI defined the intellectual content, carried out literature search, manuscript review/revision, and guarantor. MIM and NVM elaborated the intellectual content, performed literature search, performed manuscript review/revision, and guarantor.

Conflict of interest

The authors declares that there is no conflict of interests regarding the publication of this article.

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