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Diversity of plant-parasitic nematodes in potato fields at different altitudes in Probolinggo District, East Java, Indonesia

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Abstract. *Prada AP, Pratiwi GNC, Wagiyana. 2022. Diversity of plant-parasitic nematodes in potato fields at different altitudes in Probolinggo District, East Java, Indonesia. Cell Biol Dev 6: 61-67.* Plant parasitic nematodes (PPNs) are microorganisms sensitive to environmental conditions. Therefore, plant parasitic nematode diversity in a field may affect the decision of effective management tactics. This study aimed to assess the diversity of plant parasitic nematodes in potato fields at three different altitudes. The study was carried out in Probolinggo District, East Java, Indonesia. The sampling area was divided into three groups based on altitude variations, namely 1,008 m asl., 1,413 m asl., and 1,875 m asl. Soil samples were collected randomly in a zigzag pattern. Samples were collected from 20 points across each field at a depth of 20-30 cm below the soil surface. Each sub-sample included up to 500 g of soil. The white head tray method extracted nematodes from 500 g of soil from each subsample. After characterizing the extracted nematodes, the absolute density and dominance values were determined. The results revealed variations in the three areas with different altitudes. The total number of plant-parasitic nematodes (PPN) discovered on the field at an altitude of 1,008 m asl. was 57.00 ± 16.43. Furthermore, at an altitude of 1,413 m asl., the total number of PPNs discovered is 41.67 ± 10.98. The total number of nematodes on the field at an altitude of 1,875 m asl. was 50.50 ± 12.60. The findings of this study also indicated that *Meloidogyne* sp. was the genus with the highest population on the field, at an altitude of 1,008 m asl. On the other hand, *Pratylenchus* sp. is the most abundant nematode in the field between 1,413 and 1,875 m asl. An identical situation also occurred with the nematode dominance values in the three fields studied.

Keywords: Abiotic, dominance, elevation, environment, population

INTRODUCTION

Potato is one of the most important commodities produced in Indonesia, and it now holds the fourth spot in terms of the top agricultural value in the horticulture industry. Probolinggo District, located in East Java Province, is one of the major production hubs for potatoes in Indonesia. However, the production of potatoes in numerous highland areas is suffering due to several issues. According to research data, fungal, bacterial, and nematode diseases are significant challenges in potato cultivation. Fungi such as *Phytophthora infestans* have caused massive losses in potato production worldwide. This fungal infection can swiftly induce blight symptoms and plant mortality (Nowicki et al. 2012). Pathogenic bacteria, such as *Ralstonia solanacearum*, have also been linked to yield losses in the field and post-harvest potatoes (Kurabachew and Ayana 2017). According to the data, infection with plant-parasitic nematodes (PPNs) is also a severe threat to potato production. Infections with nematodes in potato plants have been recorded globally. Even countries with modern agricultural technologies, such as the United States, the Netherlands, Germany, Japan, and Australia, are exposed to nematode infection in their potato fields (Jones et al. 2013). That demonstrates that PPNs are a significant threat to potato production globally (İmren 2018; Maleita et al. 2018; Orlando et al. 2020).

Infection by plant parasitic nematodes in various horticulture products results in common symptoms like wilting, reduced plant development, and lower yield (Coyne et al. 2018). Certain nematodes, such as those belonging to the genus *Meloidogyne*, can produce particular symptoms, such as the development of gall on the roots. It has been found that potato cyst nematodes develop syncytium in root tissue (Collange et al. 2011). In addition, nematodes of the genera *Pratylenchus* and *Radopholus* are endoparasitic migratory nematodes (Sarah 1989). The feeding activity of *Pratylenchus* and *Radopholus* on root tissue may directly destroy root tissue (Riley and Wouts 2001). Infection with ectoparasitic nematodes such as *Xiphinema* can reduce the length of root hairs (Van Zyl et al. 2012). These symptoms have a direct impact on the capacity of plants to absorb soil nutrients and water. As a result, the plant will eventually lack nutrients and undergo stunted development. In addition, a reduction in a plant's ability to absorb nutrients and water makes it more vulnerable to infection by other pathogens (Chitwood 2003). *Globodera* sp. and *Meloidogyne* sp. have been identified as the nematodes that pose the most issue in potato production worldwide. Depending on the plant variety, the environmental conditions, and population density, both nematodes can reduce yields by 30% to 75% (Collange et al. 2011; Trudgill et al. 2014).

Nematode infestation in potato fields has reached a serious point and must be dealt with immediately.

Nematode infections may even be responsible for a potential loss of up to 80 billion dollars worldwide (Kumar et al. 2020). The *Meloidogyne* species is responsible for root gall symptoms in most horticultural plants and most commonly infects potato plants (Onkendi et al. 2014; Rusinque et al. 2021). Potato tubers infected with these nematodes reportedly indicate symptoms similar to scabies (Majeed and Muhammad, 2018). Aside from *Meloidogyne* sp., *Globodera* sp. is another common nematode affecting potato crops (Trudgill et al. 2014; Mwangi et al. 2015).

A variety of biotic and abiotic variables regulates the PPNs diversity. The kind of plant, the age of the plant, and the presence of other microorganisms in the rhizosphere are all biotic variables that influence the diversity and abundance of nematodes in a field (Coyne et al. 2018; Silva et al. 2018). Furthermore, the diversity of nematodes in the soil is influenced by soil type, humidity, temperature, altitude, and other physical and chemical conditions surrounding plant roots (Mateille et al. 2014). For example, potato fields in the low-temperature highlands have a greater *Globodera* sp. infection rate than those in the high-temperature lowlands (Phillips et al. 2015). In addition, *Meloidogyne graminicola* is typically more prevalent in rice fields with sandy soils. Nematodes are typically found in lower population density in loamy soils, another phenomenon supporting the claim that environmental factors influence the diversity of nematodes (Bouwman and Arts 2000; Kandji et al. 2001).

The presence of PPNs on potato plants in Indonesia has been documented in some prior studies; these studies indicated that major potato-growing regions across the country, including Wonosobo, Batu City, and several locations in West Java, were all likely to be infected by nematodes (Aprilyani et al. 2015; Syafii et al. 2018). However, in Indonesia, the diversity of PPNs that infect potato plants and their diversity at various altitudes still needs further study.

Nematodes are highly sensitive to environmental changes. Therefore, the nematodes that predominate at various altitudes may be of distinct species. Considering the unique lifespan of nematodes, it is important to observe information on the diversity at various field altitudes. Different species of nematodes that infest a field may necessitate distinct management strategies (Ralmi et al. 2016). For example, ectoparasitic and endoparasitic nematodes would require specific management measures. In addition, the prevalent nematode genus or species will influence the selection of nematicide. For instance, certain active nematicide components are effective against *Meloidogyne* and *Globodera* species but less against *Pratylenchus* species (Ebone et al. 2019). In contrast, trap crops and crop rotation significantly impact the nematode genus to be managed. Some nematode species resist control with trap plants (Vestergård 2019). Also, crop rotation does not affect some nematodes if the plant used in crop rotation is still the nematode's host plant (Nusbaum and Ferris 1973).

In light of the information presented above, it is necessary to research the inventory of plant-parasitic nematodes in potato fields located at different elevations. This study is important since there has yet to be a study done on the identification and inventory of nematodes in the Probolinggo District at the various field elevations before this investigation.

MATERIALS AND METHODS

Study area

The research was conducted between December 2020 and June 2021. Three potato fields in Probolinggo District, East Java Province, Indonesia, were sampled for this study. The first land was located at 7° 55' 37.0"S 113° 07' 27.0"E and was 1,008 meters above sea level. The second land was located at 7°57'22.0"S 113°03'36.0" E, at an altitude of 1,413 m asl. Furthermore, the third land has coordinates of 7°58'02.0"S 113°00'52.0" E and an 1,875 m asl. altitude. Nematodes were extracted and observed at the Plant Pest Organism Control Technology Laboratory, Plant Protection Study Program, Faculty of Agriculture, Universitas Jember, Jember, East Java, Indonesia.

Soil sampling

The sampling was done on potato crops 60 days after planting. The sampling area was divided into three groups based on altitude variations, namely 1,008 m asl., 1,413 m asl., and 1,875 m asl. Following soil sampling protocols for regular nematode examination, samples were taken in a zigzag pattern. Samples were collected with a shovel from 20 points across each field at a depth of 20–30 cm below the soil surface. Each sub-sample included up to 500 g of soil. The samples were labeled and placed in a cool box before being transported to the laboratory for observation. In addition to taking samples, we also made a record of the general situation of the land at the time. The most widely used active ingredients in pesticides, the average temperature and humidity, the area of land, crop varieties, crop rotation systems, crop intercropping systems, fertilization, and temperature and humidity averages were recorded. The information was gathered by discussing with the farmers in charge of the land surveyed.

Nematodes extraction

The white head tray method extracted nematodes from 500 g of soil from each subsample for 48 hours (Bell and Watson 2001). The nematodes were then filtered using a 400-mesh sieve. Then, the filtered nematodes were kept in DESS solution, with a pH of 8, 0.25 M EDTA, 20% DMSO, and saturated with NaCl (Yoder et al. 2006). After that, the nematode samples were kept at room temperature until observed.

Nematodes analysis

The plant-parasitic nematodes were observed under a light microscope and classified by genus. A Nikon Eclipse E200 stereo microscope and a calibrated camera were used to identify nematodes. The absolute population density of nematodes and their dominance values on land were calculated using the formula below (Mirsam et al. 2020):

$$PD = \frac{n}{500\ g\ soil}$$

$$D = \frac{PD \times \sqrt{absolute\ frequency}}{100} \times 100\%$$

Where: PD: population density; n: nematodes number; D: dominance value

RESULTS AND DISCUSSION

Field condition

In this study, we collected data from farmers on the common agricultural methods employed in the area. Based on the findings of interviews, it is known that the cultivation techniques of the three fields employed in this study have both similarities and variations. In this study, all fields were fertilized using organic and inorganic fertilizers. Organic fertilizer from fermented animal dung, such as cow and horse manure, is the average organic fertilizer utilized in the three assessed fields. Furthermore, it is known that the potato plants planted in the three assessed fields are Granola varieties, frequently cultivated in the Probolinggo District because of their suitability to the district's microclimate and general weather conditions. Moreover, according to farmers, the yields of the Granola varieties are easier to sell than those of other kinds of potato varieties.

The three fields investigated had distinctively diverse patterns of crop rotation and intercropping. For example, leeks are grown in certain areas as part of a rotational cropping system. On the other hand, some farms did not engage in crop rotation and instead planted potatoes throughout the year. In addition, the species of pests that attacked each of the three fields were not substantially different. Nevertheless, the farmers reported that all three fields were infected with nematodes. Data related to cultivation techniques in the field surveyed in this study are presented in Table 1.

Differences in farming practices have an impact on soil nematode populations. Nematodes are sensitive to environmental changes such as temperature fluctuations, pH, and soil structure changes (Bongers and Ferris 1999; Sun et al. 2013). Fertilizer use will also influence plant development and, in turn, the root exudate secreted by plants. Differences in the quantity and variety of root exudate secreted by plants will impact the nematode population in the soil, either directly or indirectly. Root exudates have a direct part in nematode-plant communication signals. Because the nematodes have a chemotaxis response to root exudates emitted by plants, variations in root exudates will influence the type of nematode attracted to the plant root area (Huang et al. 2014; Williams and De Vries 2020). Changes in root exudates will indirectly affect bacterial and fungal communities in the soil, and changes in bacterial and fungal communities will indirectly affect nematode communities in the soil. It was noted in several earlier studies that variations in cultivation methods would influence nematode diversity in the soil (Narula et al. 2012; Lareen et al. 2016). Even in some studies, it is mentioned that cultivating methods are one approach to controlling plant-parasitic nematodes (Atandi et al. 2017).

Table 1. General field condition of the research area

Altitude	Variable		Description
1,008 m asl.	Land area	:	0.25 Ha
	Plant variety	:	Granola
	Crop rotation system	:	Crop rotation with leeks
	Fertilization system	:	Organic fertilizers (bran and animal manure), inorganic fertilizers (Phonska)
	Commonly used active pesticide ingredients	:	Mancozeb
	Average air temperature	:	26°C
	Average air humidity	:	84%
1,413 m asl.	Land area	:	0.25 Ha
	Plant variety	:	Granola
	Crop rotation system	:	No crop rotation
	Fertilization system	:	Organic fertilizer (animal manure), inorganic fertilizer (ZA, SP, Phonska)
	Commonly used active pesticide ingredients	:	Mancozeb
	Average air temperature	:	21°C
	Average air humidity	:	83%
1,875 m asl.	Land area	:	0.25 Ha
	Plant variety	:	Granola
	Crop rotation system	:	There is no crop rotation, but intercropping with leeks
	Fertilization system	:	Organic fertilizers (animal manure) and inorganic fertilizers (Phonska, NPK, ZA)
	Commonly used active pesticide ingredients	:	Mancozeb
	Average air temperature	:	15°C
	Average air humidity	:	76%

The kinds of weeds present will be greatly influenced by both the average temperature and the different altitudes (Nowak et al. 2015). In addition, the species of plants that grow above ground influence the diversity and quantity of root exudates found directly below the ground's surface. Therefore, it is possible that nematodes also be discovered in some weeds, which leads one to speculate that these plants could act as alternative hosts (Munif et al. 2022). For example, *Cyperus rotundus* is a kind of weed with an impressive level of resistance to infection by *Meloidogyne* sp. On the other hand, the root systems of some weeds, such as *Centrosema pubescens*, *Cyperus kyllingia*, and *Cyperus irria*, may provide an ideal environment for the growth of *Meloidogyne* sp. (Widiyanto 2016). This finding also explains how and why nematode populations are impacted by the environment in which they are located.

PPNs population density at various altitudes

Plant-parasitic nematodes have variable population densities at different altitudes. For example, the PPN population per 500 g of soil was 57.00 ± 16.43 at 1,008 m asl., which is also 36.7% greater than the number of parasitic nematodes observed at 1,413 m asl. (41.67 ± 10.98). However, there are 21% higher parasitic nematodes at an altitude of 1,875 m asl. than at 1,413 m asl. (50.50 ± 12.60). Details of the PPNs population density in this study are presented in Table 2.

A broad variety of variables, such as temperature and the soil's physical qualities, might stimulate differences in nematode densities in a particular region. The amount of fertilizer typically applied by farmers and the type of fertilizer directly impact the soil's physical characteristics. According to several research findings, the number of Globodera species has been observed to grow with altitude (Jones et al. 2017). The difference in altitude has a direct bearing on the temperature differential that exists between the three locations. In this research, the temperature at an altitude of 1,008 m asl. was much higher than at an altitude of 1,875 m asl. It is known that the difference between 26°C and 15°C is considerably important. In addition, the air pressure in regions that are higher in height tends to be lower than the air pressure in areas that are lower in altitude. Because nematodes are sensitive microorganisms, it is considered that these factors explain why there is a disparity in the total number of nematodes found at various elevations (Nyang'au et al. 2021; Zhang et al. 2021).

There have been several studies done in the past that have indicated that the density of nematodes varies in response to different environmental conditions (Barker and Olthof, 1976). In general, the differences in altitude will influence the farming practices that farmers adopt at each different altitude. For instance, farmers usually utilize mulch to reduce the amount of water present at higher altitudes (Liu and Siddique 2015). In addition, the likelihood of a plant pathogen infection being present at higher elevations is often increased. Because of this condition, farmers are pressured to use more pesticides to reduce the number of plant diseases that arise (Shunthirasingham et al. 2011). However, the use of agrochemicals such as fungicides and bactericides will, in an indirect way, change the biological and chemical composition of the soil. These changes will cause a shift in the basic features of the soil's microbial ecology, including a change in the number of nematodes (Ney et al. 2019).

PPNs dominance index at various altitudes

There were six different parasitic nematode genera at an altitude of 1,008 m asl., with *Meloidogyne* sp. having the most members. The other genera were *Helicotylenchus* sp., *Longidorus* sp., *Pratylenchus* sp., *Rotylenchus* sp., and *Trichodorus* sp. Furthermore, there were six different parasitic nematode genera at an altitude of 1,413 m asl.: *Criconema* sp., *Globodera* sp., *Longidorus* sp., *Meloidogyne* sp., *Pratylenchus* sp., and *Trichodorus* sp. The genus with the greatest population was *Pratylenchus* sp. While there were four different genera at an altitude of 1,875 m asl., including *Longidorus* sp., *Pratylenchus* sp., *Trichodorus* sp., and *Tylenchulus* sp., the genus with the largest number was from the genus *Pratylenchus* sp. Detail on the nematode population found in this study is presented in Table 3.

This study shows that changes in altitude considerably impact PPN population density and dominance; however, this is mainly acceptable because of the varying environmental conditions present in each area. For example, table 3 shows that the total number of parasitic nematodes at an altitude of 1,008 m asl. is 36.7% greater than the total number of parasitic nematodes at 1,413 m asl. and that at 1,875 m asl., with the total number of parasitic nematodes is 21.1% higher than the entire population.

In this study, it was shown that *Meloidogyne* sp. had the maximum dominance value at 1,008 m asl. *Meloidogyne* sp. was the most dominant nematode, followed by *Pratylenchus* sp. Even more remarkably, *Pratylenchus* sp. was discovered to be the most predominant nematode in both fields at 1,413 m asl. and 1,875 m asl. This study's findings are consistent with previous research indicating that *Meloidogyne* nematodes are more common at intermediate and low altitudes and that *Meloidogyne* does well in warmer areas (Whitehead 1969). Moreover, *Pratylenchus* was more prevalent in two areas with greater altitudes. Several investigations corroborate this finding, showing that *Pratylenchus* sp. is abundant in higher elevations (Gaidashova et al. 2004).

Table 2. PPNs population density found in different potato field altitudes

Altitudes	Population density 500 g^{-1} soil
1,008 m asl.	57.00 ± 16.43
1,413 m asl.	41.67 ± 10.98
1,875 m asl.	50.50 ± 12.60

Table 3. PPNs genera and their absolute population found from different potato field altitudes

Altitude	Genera	Ordo	Sub ordo	Family	PPNs absolute population
1,008	*Helicotylenchus* sp.	Tylenchida	Tylenchina	Hoplolaimidae	1.68
	Longidorus sp.	Dorylaimida	Dorylaimina	Longidoridae	8.38
	Meloidogyne sp.	Tylenchida	Tylenchina	Meloidogynidae	20.12
	Pratylenchus sp.	Tylenchida	Tylenchina	Pratylenchidae	16.76
	Rotylenchus sp.	Tylenchida	Tylenchina	Hoplolaimidae	3.35
	Trichodorus sp.	Dorylaimida	Diphtheroporina	Trichodoridae	6.71
1,413	*Criconema* sp.	Tylenchida	Tylenchina	Criconematidae	1.49
	Globodera sp.	Tylenchida	Tylenchina	Heteroderidae	1.49
	Longidorus sp.	Dorylaimida	Dorylaimina	Longidoridae	8.93
	Meloidogyne sp.	Tylenchida	Tylenchina	Meloidogynidae	5.95
	Pratylenchus sp.	Tylenchida	Tylenchina	Pratylenchidae	14.88
	Trichodorus sp.	Dorylaimida	Diphtheroporina	Trichodoridae	8.93
1,875	*Longidorus* sp.	Dorylaimida	Dorylaimina	Longidoridae	17.57
	Pratylenchus sp.	Tylenchida	Tylenchina	Pratylenchidae	21.96
	Trichodorus sp.	Dorylaimida	Diphtheroporina	Trichodoridae	4.39
	Tylenchulus sp.	Tylenchida	Tylenchina	Tylenchulidae	6.59

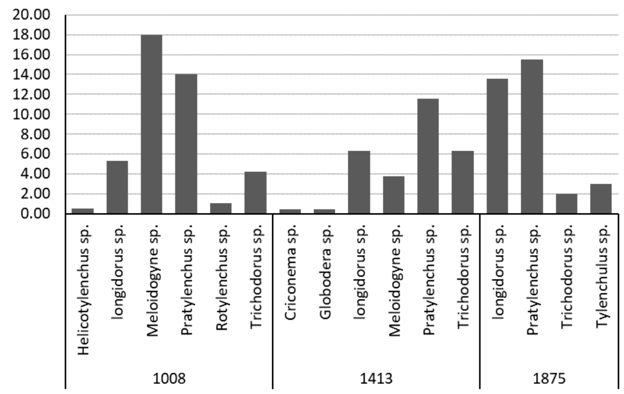

Figure 1. The dominance index of PPNs based on altitude area

PPNs dominance index found in this study is presented in Figure 1. The nematode species, however, have a major impact on the diversity of nematodes in a field. Different species of nematodes within the same genus may have different environmental preferences. Since we did not investigate at the species level in this study, future research is needed to uncover the unresolved issues with its limitations.

Meloidogyne sp. was present and had a dominating value that tends to be high at various elevations, particularly on land at the height of 1,008 m asl., where the PV value is 17.99. According to Elling (2013), *Meloidogyne* sp. is a nematode widely found in tropical countries with warm, humid climate zones, including the lowlands and the highlands. Additionally, this nematode has a broad host range and the potential to disseminate globally. According to Tapia-Vázquez et al. (2022), this nematode has more than 2,000 hosts and is classified as cosmopolitan. Therefore, this nematode species may live and thrive in various soil conditions, cropping techniques, crop rotations, and multiple commodities (Singh et al. 2013).

Pratylenchus sp. is a parasitic nematode with various hosts, particularly in agricultural crops. The nematodes of the species *Pratylenchus* sp. induce root lesions, which can result in yield loss and crop failure if the infection is severe (Jones and Fosu-Nyarko 2014). The habitat of the nematode *Pratylenchus* sp. tends to be dense in plant rhizosphere regions where a sufficient number of hosts are present (Bucki et al. 2020). According to calculations and analyses, this nematode has the largest population and dominance value at altitudes of 1,413 m asl. and 1,875 m asl. It is in line with previous research that the habitat of this nematode is plant root areas with a range of hosts. Therefore, it is suspectible that these nematodes are abundant in potato plants and other types of host plants in the crop rotation system (Jones and Fosu-Nyarko 2014).

The value of the dominance difference between the two nematodes is often impacted by changes in the characteristics of the environment in which they live. This research reveals that the average temperatures of the air in each of the three areas that were investigated had significant differences from one another. Due to the land being located at lower altitudes, the average air temperature is more critical.

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