



## Research Article

# Plant Parasitic Nematode Abundance and Diversity in Potato (*Solanum tuberosum*) Cultivation at Various Altitudes in Wonosobo and Banjarnegara

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## ABSTRACT

Plant-parasitic nematodes are one of the causes of yield loss in potato cultivation. Currently, information on the diversity, abundance, and dominance of potato parasitic nematode genera is not available. This research aimed to determine the pattern of distribution, abundance, and dominance of parasitic nematode genera on potato plant (*Solanum tuberosum*) in Wonosobo and Banjarnegara Districts at various altitudes i.e.: 1,250–1,500; 1,500–1,750; 1,750–2,000; and 2,000–2,250 meters above sea level (m.a.s.l.). Soil rhizosphere and root samples were collected, and nematodes were extracted using the Whitehead tray modification technique. The nematodes were adjusted with Formalin Acetic acid Alcohol (FAA), mounted, and identified based on morphological characters. The diversity index was determined to distinguish plant-parasitic nematode diversity. Six genera of potato plant-parasitic nematodes were found, namely Meloidogyne, Hirschmanniella, Globodera, Criconemoides, Helicotylenchus, and Xiphinema. The highest population of plant-parasitic nematodes was found at 1,250–1,500 m.a.s.l. from both root and soil samples. The nematode populations were 56.67 nematodes/5 g root and 103.33 nematodes/100 g of soil. The abundance of parasitic nematodes did not differ significantly among different altitudes in both districts. The dominant parasitic nematodes in soil samples were Meloidogyne with 16.78%, while Globodera was 13.98%. The Shannon-Wiener index implied that the diversity of parasitic nematodes of potato plants and stability of community in Wonosobo and Banjarnegara Districts were categorized as low.

Keywords: abundance; diversity; dominance; parasitic nematodes; potato plants

## INTRODUCTION

Potato (*Solanum tuberosum* L.) is one of the most important horticultural commodity in Indonesia. Plant parasitic nematodes are major inhibiting factors for potato cultivation because of their ability to cause significant yield loss in both tropical or sub-tropical regions. Yield loss due to plant parasitic nematodes can reach 8.8%–14.6% resulting in 100–157 billion US dollar loss globally (Nicol *et al.*, 2011). Important parasitic nematodes in potato plants include *Globodera* sp., *Meloidogyne* spp., *Nacobbus aberrans*, *Ditylenchus* spp., *Pratylenchus* spp., *Belonolaimus longicaudatus*, *Xiphinema* spp., *Rotylenchus* spp., *Radopulus similis*, *Longidorus* spp.,

*Paratrichodorus* spp., *Trichodorus* spp., and *Paratylenchus* spp. which are even able to damage allelopathic plants (Luc *et al.*, 1995). Nematodes disrupt root function by hindering transport of nutrients to plant part above the ground (Dropkin, 1991).

The development of nematodes is influenced by several environmental factors such as temperature, soil moisture, soil pH, organic matter content, host performance, plant age, and soil particle size. Based on research by Wulandari and Indarti (2020), temperature has positive correlation with *D. dipsaci* nematode populations in bulb and soil. Temperature affect the development of nematodes such as egg hatching, reproduction, movement and growth rate.

In general, plant parasitic nematodes are inactive at temperatures below 15°C and temperatures above 30°C, while optimum temperatures are 25–28°C for multiplication as well as increase of puru numbers (Cook & Noel, 2002). Soil conditions including texture, aeration, humidity, pH, organic and inorganic soil properties, can also affect the development of nematodes. Soil texture is an abiotic factor that is likely to affect potential damage on coffee plants caused by plant parasitic nematode (Mutalaliah *et al.*, 2018).

Understanding the response of nematode variety to local environment is essential in developing nematode management practices. Different cropping patterns will cause different agricultural climate and soil microclimate condition that eventually affect existence of potato plant parasitic nematodes. Temperature may compromise embryonic development which is an important factor in the ecology and distribution of nematode. This temperature could be an important factor in the overall fitness of nematodes and its ability to extend its distribution range (Sikora & Fernández, 2005). Soil temperature during growing seasons affect initial energy reserves of *G. rostochiensis* larvae. *Globodera rostochiensis* grows well in all tested potato varieties between 20°C and 30°C. Temperatures of 20°C provided a better environment than 30°C. Granola grew at 20°C and was the best host *G. rostochiensis* (Nurjanah *et al.*, 2016a).

Diversity indices are commonly used to describe the types and distribution patterns of organisms, including plant parasitic nematodes at a certain site. This index can also be used to explain the effects of environmental factors, such as soil type, host type composition, or other factors, to nematode populations (Zeng *et al.*, 2012).

Wonosobo and Banjarnegara have been long recognized as potato cultivation regions in Indonesia, but the study of potential disturbance by plant parasitic nematodes is still limited. Therefore, this research was conducted to determine the pattern of distribution, abundance and diversity of plant parasitic nematodes on potatoes at various altitudes. Based on this information, the economic important of nematodes can determine, while attacks can be prevented to reduce economic loss.

## MATERIALS AND METHODS

### Study Sites

The survey was conducted at the center of potato cultivation at Wonosobo and Banjarnegara Districts. Locations of land samplings were marked with a geographical positioning system (GPS) to measure geographical position, soil altitude, soil temperature. Research was conducted between September to November 2016. Soil sampling was taken from fields, while nematode extraction and identification was done at Nematology Laboratory, Department of Plant Protection, Faculty of Agriculture, Universitas Gadjah Mada, Yogyakarta.

### Soil and Root Sampling

Sampling was conducted on >50 days-old potato plants suspected of being infected with parasitic nematodes. These plants showed yellowing, wilting, dwarf growth symptoms were found at several locations in Wonosobo and Banjarnegara Districts. Sampling locations were divided into 4 areas based on the altitude i.e. 1,250–1,500; 1,500–1,750; 1,750–2,000; 2,000–2,250 meters above sea level (m.a.s.l.).

### The Geographical Conditions of Study Sites

Wonosobo Regency is a mountainous area with altitudes of 250–2,250 m.a.s.l. Average air temperature is 14.3–26.5°C during the day, dropping to 20°C at night, in July–August down to 12–15°C at night and 15–20°C in the afternoon. Wonosobo Regency is located at 7°43'13" and 7°04'40" Latitude South (LS) as well as 109°43'19" and 110°04'40" Longitude East (BT). Average rainy day is 196 days and rainfall rates are 3,400 mm (Bappeda Wonosobo, 2016). Air humidity in Banjarnegara District is 80% (BPS Provinsi Jawa Tengah, 2016).

Banjarnegara Regency is located between 40–2,300 m.a.s.l. The average air temperature is 20–26°C, the coldest temperature is 3–18°C with coldest temperatures recorded in the Dieng. Air humidity 80–85% with average rain rates of 3,000 mm/year (BPS Provinsi Jawa Tengah, 2016).

### Isolation-Extraction of Nematodes from Soil and Roots

Isolation and extraction of nematodes were done from 100 g of soil samples using a modified Whitehead Tray method (Hooper, 1985). The filtration was

carried out by installing a nylon screen over the buffer tray (base of the perforated tray) and above it, filter paper was placed until surface of buffer tray were closed. Incubation was done for 24 hours at room temperature (21–29°C). The suspension of the nematodes were poured into a beaker glass. Excess water was reduced by pipettes until the lagging suspension of nematodes was approximately 50 ml. The nematode suspension were stored in a black bottle and placed in the refrigerator.

Nematode isolation and extractions from the root tissues (100 g of root per sample) were performed using mist extraction technique (Hooper, 1986). Furthermore, the supernatant is discarded leaving nematode containing suspension. The suspension was stored in the same way as nematode suspension from soil samples.

### Identification of Nematodes

Nematodes were indentified to genera based on morphological characters using identification keys in print or electronic form (software). In general, the identification of nematodes can be done by their appearance of the whole body (e.g. posture of rest, stiletal shape and size, cuticle morphology, body size or morphometric comparison), anterior portion (mouth, head shape and skeleton, stylet, esophagus, median bulb, overlapping esophagus, body wall, male and female reproductive organs, tail form (Southey, 1986). Identification was also done by observing the morphological and anatomical features of the nematodes directly under a microscope or through photographs. Identification was based on *Plant Parasitic Nematodes: A Pictorial Key to Genera* (Mai, 1996).

### Analysis of Nematodes Abundance and Diversity

#### Abundance of potato plant parasite nematodes.

Abundance (A) of nematodes genera is the average density which nematode were found per sample and calculated by using the modified method of Adamou *et al.* (2013):

$$A = \frac{\sum X_i}{e}$$

Where  $X_i$  was the number of nematodes per liter of soil or gram of dried root,  $e$  = number of samples in which the given nematode was present. The A values were log transformed. A genera was

considered abundant when it was present in at least 30% of samples with at least 300 individuals per liter of soil or 20 individuals per gram of dry root. Abundance data were analyzed by using one way ANOVA and tested using LSD test at  $\alpha=0.05$ .

#### Density of potato plant parasite nematodes.

Calculation of nematode population density was done using the following formula (Rosya & Winarto 2013):

$$\text{Population density of nematodes (K)} = \frac{\text{The number of individuals of a genus}}{\text{Volume of Unit Sample}}$$

#### Diversity of nematode genera based on altitude.

The diversity of parasitic nematodes was calculated by the Shannon-Winner Index and Simpson Index. Meanwhile, the abundance of nematodes was the average density of the nematodes per sample found at a site. The total number of nematodes was expressed as the number of individuals per 100 g of dry soil using the Simpson diversity index (D) calculated as a measure of nematode diversity (Oksanen *et al.*, 2015).

## RESULTS AND DISCUSSION

### Abundance of Plant Parasitic Nematodes

Abundance of parasitic nematodes at various altitudes was not significantly different. However, highest nematode abundances were recorded in elevation 1,250–1,500 m.a.s.l. for soil samples and in elevation 1,500–1,750 m.a.s.l. for root samples (Table 1). Population abundance of plant parasitic nematodes from soil were 25.33 individuals, while from plant root was 13.34 individuals. The abundance of plant parasitic nematode populations both root or soil samples from Wonosobo and Banjarnegara Districts in detail can be seen in Table 1. Plant parasitic nematodes abundance was correlated with altitude. Similar results were found in research by Dong *et al.* (2017) where nematode populations were highly correlated with altitude and elevation.

Plant parasitic nematodes are found in the rhizosphere of host plants due to nematode diet and suitable temperature. Upper soil (ground surface) generally contained little numbers of parasitic nematodes because temperatures are relatively high during the day due to the influence of ultraviolet light.

Table 1. Abundance of plant parasitic nematodes in potato cultivation with various altitudes in Wonosobo and Banjarnegara Districts

Altitude Place (m.a.s.l.)	Population/ 5 g Root	Population/ 100 g Soil
1,250–1,500	11.33 ns	25.33 ns
1,500–1,750	13.34 ns	9.33 ns
1,750–2,000	6.67 ns	18.66 ns
2,000–2,250	10.66 ns	22.00 ns

Description: “ns” were not significantly different at  $\alpha=0.05$

Temperature affects biological activities of nematodes, such as their egg hatching, mobility, invasion, and growth. The level of nematode activity varies according to the environment temperature and are able to stay active between 5°C–40°C. Activity tends to increase at a temperature of 25°C, drop at 30°C, and become inactive at 40°C (Mulyadi, 2009).

Abundance and distribution of nematode populations in various ecosystems shows that nematodes have high adaptability due to their morphological variations, such as their head structure, and oral devices that allows nematodes to have a wide variety of food; feeding behavior and responses to environmental conditions. In addition, body length, egg stadia, larvae (juvenile) and adults each have different morphological and adaptation characteristics causing nematodes to have the ability to utilize different food sources and live in various environments. Each species varies among geographic distribution (Mulyadi, 2009).

Observation of parasitic nematodes from potato roots and soil in Wonosobo and Banjarnegara Districts discovered 6 genera of parasitic nematodes

among all altitudes such as *Meloidogyne* sp., *Hirschmanniella* sp., *Globodera* sp., *Criconomoides* sp., *Helicotylenchus* sp., *Xiphinema* sp (Table 2). Dong *et al.* (2017) and Zang *et al.* (2012) stated that nematode community compositions showed strong correlation with altitude zonation and altitudes. Results showed that high density of nematodes from the genus *Helicotylenchus* and *Globodera* (Table 2). The *Helicotylenchus* genus is spiral-like nematodes that enters plant root. Frequently all the nematodes enter the roots to feed inside the plant tissue. These nematodes are common parasitic nematodes found in plants, including hardwood trees species, soybeans, olives, cotton, millet, tomatoes (Dropkin, 1991). In addition, *Helicotylenchus* are found at soil depths <30 cm and 50 cm (Mutalaliah *et al.*, 2018). High population of *Globodera* sp. in potatoes can lead to a decrease in potato production (Mulyadi, 2009). *G. rostochiensis* cysts were distributed at regions with temperatures ranged from 18–24°C and the altitude ranging from 1,540 to 2,073 m.a.s.l. (Nurjanah *et al.*, 2016b).

#### Diversity and Abundance of Nematode Species

According to Shannon-Wiener index, the diversity of parasitic nematodes on potato plants from both roots and soil samples were categorized as low and the stability of the community was also low due to low H' value. The diversity and abundance of nematode populations at root and soil in Wonosobo and Banjarnegara Districts can be seen in Table 3.

Magurran (1988) explained that the value of the diversity index (H') is related to the species richness at a particular location and also influenced

Table 2. Density of plant parasitic nematodes in potato crops cultivation at various altitudes in Wonosobo and Banjarnegara Districts

Altitude (m.a.s.l.)	Population/5 g roots			Population/100 g Soil				
	Hel	Glo	Hir	Glo	Cri	Xip	Hel	Mel
1,250–1,500	30	20	6.67	20	13.33	16.67	30	23.33
1,500–1,750	16.66	13.33	10	26.67	6.67	6.67	3.33	10
1,750–2,000	10	13.33	10	16.67	6.67	6.67	26.67	36.67
2,000–2,250	6.66	20	10	13.33	16.67	16.67	13.33	10
Amount	63.33	66.67	36.67	76.67	33.33	46.67	73.33	80
Average	15.83	16.67	9.167	19.17	8.33	11.67	18.33	20

Description : Hel: *Helicotylenchus*, Glo: *Globodera*, Hir: *Hirschmaniella*, Cri: *Criconomoides*, Xip: *Xiphinema*, Mel: *Meloidogyne*.

Table 3. Diversity of plant parasite nematodes on potatoes cultivation at various altitudes in Wonosobo and Banjarnegara Regencies

No.	Altitude (m.a.s.l.)	Sum		Population of Plant Parasitic nematodes								Sum
		Root	Soil	Root				Soil				
				Hel	Glo	Hir	Glo	Cri	Xip	Hel	Mel	
1	1,250–1,500	56.67	103.33	30	20	6.67	20	13.33	16.67	30	23.33	140
2	1,500–1,750	40	53.33	16.67	13.33	10	26.67	6.67	6.67	3.33	10	6.67
3	1,750–2,000	33.33	96.67	10	13.33	10	16.67	10	6.67	26.67	36.67	80
4	2,000–2,250	36.67	56.67	6.67	20	10	13.33	3.33	16.67	13.33	10	116.67
Total		166.67	310	63.33	66.67	36.67	76.67	33.33	46.67	73.33	80	403.33
Average		8.33	5.5	3.167	3.33	1.83	3.83	1.667	2.33	3.67	4	20.16
Proportion				0.13	0.1398	0.07	0.16	0.06	0.09	0.15	0.1678	1
Shannon-Wiener Diversity Index				0.26	0.27	0.19	0.29	0.18	0.22	0.28	0.29	2.03
Species Richness												1.99
Index's of Domination				0.02	0.02	0.008	0.03	0.006	0.01	0.03	0.03	1

Description : Hel: *Helicotylenchus*, Glo: *Globodera*, Hir: *Hirschmanniella*, Cri: *Criconomoides*, Xip: *Xiphinema*, Mel: *Meloidogyne*.

by the distribution of species abundance. If assuming the distribution is normally spread, then in the range of 100 species will get the value of  $H' \approx 3$ , and to obtain  $H' > 5$  required 105 species. According to Magurran (1988),  $H' \leq 1$  values implied low diversity and  $1 \leq H' \leq 3$  values were medium and medium-sized communities.

Diversity of *Globodera* sp. nematodes may be caused by the ability of this parasitic nematode to survived 1,000–2,000 m.a.s.l. and temperatures between 15°C–22°C (Mulyadi, 2009). The population level of nematodes in soils are affected by the soil type. Generally, in relatively mild soil types nematode populations developed better in the same plant species that have similar resistance level. Development and multiplication of nematodes depend on the initial population and vulnerability of plants. The intensity of the damage usually increases slowly over time, when compared with the rapid increase found on recording plant (Sikora & Fernandez, 2005).

## CONCLUSION

Based on parasitic nematode surveys on potato plants in Wonosobo and Banjarnegara Districts, there were six genera of plant parasitic nematodes in Wonosobo and Banjarnegara namely *Meloidogyne* sp.,

*Criconomoides* sp., *Helicotylenchus* sp., *Hirschmanniella* sp., *Globodera* sp., and *Xiphinema* sp. The most dominant nematode genera found on both root and soil was at 1,250–1,500 m.a.s.l. with abundance of 56.67 nematodes/5 g of root and 103.33 nematodes/100 g of soil. The total abundance of parasitic nematodes in Wonosobo and Banjarnegara Districts both from root or soil based on altitude were not significantly different. Dominant parasitic nematodes within soil samples were *Meloidogyne* consisting 16.78% of the population, while *Globodera* was 13.98%. According to Shannon-Wiener index, diversity of potato parasitic nematodes in Wonosobo and Banjarnegara was categorized as low and stability of this community was also low. Genus *Globodera* still exist and is widely distributed at various altitudes in Wonosobo and Banjarnegara. This genus is categorized as a A2 Class II quarantine pest in Indonesia.

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