DIVERSITY OF PLANT-PARASITIC NEMATODES ON MEDICINAL PLANTS IN ME LINH STATION FOR BIODIVERSITY, VINH PHUC PROVINCE, VIETNAM

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Abstract

Plant-parasitic nematodes are known as one of the most important pests attacking various plants in the world, and investigating the nematode component is very essential for management of this pest and prevent damage to plants in general. Our survey of plant-parasitic nematodes on medicinal plants in Me Linh Station for Biodiversity, a place for conservation of precious plants and animals in Vietnam, identified ten species that belong to nine genera, five families, and two orders of plant-parasitic nematodes parasitizing six medicinal plants. *Excoecaria cochinchinensis* was parasitized by the highest number of nematode genera (5 genera, including *Xiphinema*, *Discocriconemella*, *Meloidogyne*, *Helicotylenchus*, and *Hemicriconemoides*), while *Hymenocallis littoralis* was associated with the highest number of plant-parasitic nematodes (2060 nematodes/250g soil). The results also showed that *Discocriconemella limitanea* was found to be a dominant species with the highest number of individuals on 6 medicinal plants, and the genus *Helicotylenchus* had the highest frequency of appearance (5/6 plants or 83.3%). These nematodes caused symptoms such as yellowing leaves, root galls, and root lesions, which directly affect the quality and yield of medicinal plants. Based on the results, this study showed that plant-parasitic nematodes are a potential threat to the cultivation of medicinal plants in Me Linh Station for Biodiversity, and thus, control measures should be applied to ensure sustainable cultivation of medicinal plants in this place.

Keywords: *Bixa Orellana, Excoecaria cochinchinensis, Hymenocallis littoralis, Morinda officinalis, Polyscias fruticosa, Zingiber zerumbet.*

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INTRODUCTION

The Me Linh Station for Biodiversity belongs to the Institute of Ecology and Biological Resources, located at the border part of Tam Dao National Park in Vietnam. The main function of this station is to organize basic research on biodiversity, maintaining a living-collection of tropical plants and animals, taking measures for the conservation of gene pool, rehabilitation and development of endangered species of plants and animals in Vietnam, as well as develop ex-situ conservation methods by importing plants from outside. Currently, the Me Linh Station for Biodiversity is building a living-collection of medicinal plants in a botanical garden with many precious plants such as *Morinda* * officinalis* How, *Polycias* *fruticosa* (L.) Harms, *Hymenocallis littoralis* (Jacq.) Salisb, etc.

For successful cultivation of medicinal plants, pest control must be of primary concern in order to limit their outbreak, especially of plant-parasitic nematodes (PPNs) (Perry & Moens, 2013). Nematodes are highly diverse, complex, and specialized Metazoa that colonize terrestrial, marine, and freshwater habitats from the tropics to the poles (Hickman et al., 2003; De Ley, 2006; Hallem et al., 2011). Plant-parasitic nematodes are one of the most important pests that are seriously threatening the food security of the world, causing an estimated economic loss of at least 12.3% (157 billion USD) worldwide (Hassan et al., 2013). A number of studies of plant-parasitic nematodes associated with medicinal plants has been executed, and plant-parasitic nematodes are associated with almost all medicinal plants studied to date (Ngo, 2000; Nguyen & Nguyen, 2000; Khanzada et al., 2007; Nguyen et al., 2015; Abtahi & Bakooie, 2017; Eapen & Pandey, 2018). Remarkably, yield losses caused by plant-parasitic nematodes on medicinal plants were up to 30% (Eapen & Pandey, 2018).

Furthermore, monoculture of plants might provide more suitable condition for plant-parasitic nematodes and increase nematode transmission possibility which may cause serious disease on host plants. Thus, this study is aimed at investigating diversity of plant-parasitic nematodes on medicinal plants at Me Linh Station for Biodiversity, and determining the most important plant-parasitic nematode group based on symptoms, density, and frequency of plant-parasitic nematodes.

MATERIALS AND METHODS

**Sampling:** medicinal plants with symptoms such as stunting, yellow leaves, root-knot, root lesion in Me Linh Station for Biodiversity were chosen for collecting samples. After the removal of the detritus layer, about 1 kg soil and 5 g roots around the rhizosphere of medicinal plants were collected from the upper 30 cm soil layer using a core 5×30 cm (Nguyen et al., 2019b). The soil and roots were kept in a nylon bag, placed in a cool container, and transported to the Department of Nematology in the Institute of Ecology and Biological Resources.

**Extraction:** nematodes from soil and root were extracted using modified Baermann tray method (Whitehead & Hemming, 1965). Swollen nematodes were dissected directly from root tissues under a stereomicroscope using a scalpel and forceps (Hartman & Sasser, 1985).

**Fixation and identification:** measurements were made based on permanent slides from heat-killed nematodes with a fixation by TAF and ethanol-glycerin dehydration (Nguyen et al., 2017). For morphological examination, nematodes were observed through a Olympus BX-51 light microscope equipped with a drawing tube and digital camera. To confirm morphological identification, ITS and 28S gene regions of nematodes were amplified and sequenced (Nguyen et al., 2019a); specific primers were used for *Meloidogyne* spp. (Randig et al., 2002).

**Calculating density and frequency:** after extraction, the nematode suspension was placed in a counting disk under a microscope to count the number of nematodes for each genus.
Diversity of plant-parasitic nematodes

Density = total number of a specific genus in a sample
Frequency = (the number of a specific genus / total) × 100%

RESULTS

Species component

This study determined ten species belong to nine genera, five families, and two orders of plant-parasitic nematodes parasitizing medicinal plants in Me Linh Station for Biodiversity (table 1). Interestingly, 8 species of plant-parasitic nematodes on medicinal plants, namely Scutellonema brachyurum, Helicotylenchus cavenessi, Criconemella curvata, Hemicriconemoides mangiferae, Discocricemonella limitanea, Paratylenchus sp., Tylenchulus semipenetrans, and Xiphinema brevicolle, were recorded as new records on medicinal plants in Vietnam (Ngo, 2000; Nguyen & Nguyen, 2000; Nguyen et al., 2015).

| TABLE 1. List of plant-parasitic nematodes parasitizing medicinal plants in Me Linh Station for Biodiversity |
|---------------------------------------------------------------|
| TYLENCHIDA THORNE, 1949                                      |
| Hoplolaimidae Filipjev, 1934                                  |
| Scutellonema Andrassy, 1958                                   |
| 1. S. brachyurum (Steiner, 1938) Andrassy, 1958              |
| Helicotylenchus Steiner, 1945                                 |
| 2. H. cavenessi Sher, 1966                                    |
| 3. H. dihystera (Cobb, 1893) Sher, 1961                      |
| Criconematidae Thorne, 1949                                   |
| 4. C. curvata (Raski, 1952) de Grise & Loof, 1965            |
| Hemicriconemoides Chitwood & Birchfield, 1957                 |
| 5. H. mangiferae Siddiqi, 1961                                |
| Discocricemonella De Grisse & Loof, 1965                     |
| 6. D. limitanea (Luc, 1959) De Grisse & Loof, 1965           |
| Tylenchulidae Skarbilovich, 1974                             |
| Paratylenchus Micoletzki, 1922                                |
| 7. Paratylenchus sp.                                          |
| Tylenchulus Cobb, 1913                                        |
| 8. T. semipenetrans Cobb, 1913                               |
| Meloidogynidae Filipjev, 1934                                |
| Meloidogynae Goeldi, 1892                                     |
| 9. M. incognita (Kofoid & White, 1919) Chitwood, 1949        |
| DORYLAIMIDA PEARSE, 1942                                      |
| Longidoridae Thorne, 1935                                     |
| Xiphinema Cobb, 1913                                          |
| 10. X. brevicolle Lordello & Da Costa, 1961                   |

On all medicinal plants, Discocricemonella limitanea was the most abundant species and occupied 75.9% of total, followed by Paratylenchus sp. (occupied 14.6% of total) (Fig. 1).
Density, frequency, and effects of plant-parasitic nematodes on medicinal plants in Me Linh Station for Biodiversity

Results of this study showed that plant-parasitic nematode composition on each medicinal plant species is different, implying that plant-parasitic nematodes affected differently to each medicinal plant (table 2).

There were three genera of plant-parasitic nematodes parasitizing Polyscias fruticosa (L.) Harms, including Discocriconemella, Helicotylenchus, and Hemicriconemoides. However, densities of plant-parasitic nematodes on Polyscias fruticosa were relatively low, while the density of the genus Helicotylenchus was highest, occupied 88.9% of the total (24 individuals/250 g soil).

Only two genera, Paratylenchus and Helicotylenchus, were associated with Morinda officinalis, of which the genus Paratylenchus was dominant with 450 individuals/250g soil (91.8% of total). The relatively high density of plant-parasitic nematodes on this plant shows that plant-parasitic nematodes may be a potential threat to yield and quality of Morinda officinalis.

Table 2. Densities of plant-parasitic nematodes in the rhizosphere of medicinal plants in Me Linh Station for Biodiversity

| Genus              | Polyscias fruticosa (L.) Harms | Morinda officinalis How | Hymenocallis littoralis (Jacq.) | Zingiber zerumbet (L.) Sm | Bixa orellana L. | Excoecaria cochinchinensis Lour. | Frequency (%) |
|--------------------|--------------------------------|-------------------------|--------------------------------|--------------------------|------------------|----------------------------------|---------------|
| Paratylenchus      | 0                              | 450                     | 0                              | 0                        | 0                | 0                                | 16.7          |
| Xiphinema          | 0                              | 0                       | 0                              | 0                        | 0                | 0                                | 50.0          |
| Discocriconemella | 2                              | 0                       | 2,000                          | 0                        | 330              | 1                                | 66.7          |
| Criconemella       | 0                              | 0                       | 0                              | 1                        | 0                | 0                                | 16.7          |
| Meloidogyne        | 0                              | 0                       | 0                              | 50                       | 0                | 30                              | 33.3          |
| Tylenchulus        | 0                              | 0                       | 0                              | 0                        | 0                | 0                                | 16.7          |
| Helicotylenchus    | 24                             | 40                      | 0                              | 8                        | 4                | 8                               | 83.3          |
| Scutellonema       | 0                              | 0                       | 5                              | 0                        | 0                | 0                                | 16.7          |
| Hemicriconemoides  | 1                              | 0                       | 0                              | 0                        | 0                | 14                               | 33.3          |

The total number of plant-parasitic nematodes on Hymenocallis littoralis was highest among investigated medicinal plants, and four genera, including Xiphinema, Discocriconemella, Tylenchulus, and Scutellonema, were found in the rhizosphere of this plant, of which the genus Discocriconemella was the most abundant genus with 2,000 individuals/250 g soil and occupied 97.1% of the total. Densities of all other genera were lower than 50 individuals/250g soil (2.4% of total). A high number of Discocriconemella could be the main reason for many lesions on roots of Hymenocallis littoralis.

Three genera of plant-parasitic nematodes were found on Zingiber zerumbet, namely Criconemella, Meloidogyne, and Helicotylenchus, of which density of the genus Meloidogyne was highest, occupied 87.7% of the total (50 individuals/250 g soil). During this survey, symptoms such as yellowing leaves and root galls were recorded on Zingiber zerumbet, representing the
pathogenicity of genus *Meloidogyne* on this plant. Furthermore, *Meloidogyne* spp. is one of the most damaging pathogens in the world; this nematode group needs to be monitored carefully.

For *Bixa orellana*, three genera, including *Xiphinema*, *Discocrenomella*, and *Helicotylenchus*, were found, of which density of the genus *Discocrenomella* was highest with 330 individuals/250 g soil (87.3% of total). Roots of *Bixa orellana* also showed lesions similar as for *Hymenocallis littoralis* that might be caused by nematodes.

*Excoecaria cochinchinensis* was the medicinal plant parasitized by the highest number of plant-parasitic nematodes with five genera, namely *Xiphinema*, *Discocrenomella*, *Meloidogyne*, *Helicotylenchus*, and *Hemicriconemoides*. However, densities of these nematodes were relatively low, of which density of the genus *Meloidogyne* was highest with 30 individuals/250 g soil (46.9% of total). Roots of *Excoecaria cochinchinensis* also showed small galls, caused by genus *Meloidogyne*.

When it comes to frequency of nematodes on medicinal plants, genus *Helicotylenchus* was found on all medicinal plants except *Hymenocallis littoralis* (5/6 plants or 83.3%), and four genera, including *Paratylenchus*, *Crenomella*, *Tylenuclus*, and *Scutellonema*, were found on only one medicinal plant (16.7%).

**DISCUSSION**

Ten species belonging to nine genera, five families, and two orders of plant-parasitic nematodes were found in the rhizosphere of six medicinal plants in Me Linh Station for Biodiversity, and among them, eight species are new records on medicinal plants in Vietnam (Ngo, 2000; Nguyen & Nguyen, 2000; Nguyen et al., 2015). Although plant-parasitic nematodes have been reported from many medicinal plants, this study provided nematode component associated with six new hosts in Vietnam (five hosts are new to the world) (Ngo, 2000; Nguyen & Nguyen, 2000; Khanzada et al., 2007; Nguyen et al., 2015; Abtahi & Bakooie, 2017; Eapen & Pandey, 2018).

Studies on medicinal plants previously reported that the root-knot nematode (*M. incognita* and *M. javanica*), the lesion nematode (*Pratylenchus thornei*), and the stunt nematode (*Tylenuclus vulgaris*) were reported to be the most economically important nematodes on medicinal plants (Eapen & Pandey, 2018). However, based on the density and frequency of nematodes in this study, *Discocrenomella limitanea* and *Helicotylenchus* spp. can be considered as potential pathogens to medicinal plants, because they were found as nematodes with the highest density and frequency. Therefore, based on this study, we recommend further studies on the occurrence and possible impact of *Discocrenomella limitanea*, *Helicotylenchus* spp., and *Meloidogyne incognita* on medicinal plants in Me Linh Station for Biodiversity, as well as other places, for the sustainable development of medicinal plants.

This study investigated plant-parasitic nematodes from suspected host plants with symptom such as stunting, yellow leaves, root knot, root lesion, and all studied medicinal plants in this study have been found to be associated with at least two plant-parasitic nematode genera. This result indicated that plant-parasitic nematodes are highly correlated to these symptoms, and therefore, plant-parasitic nematodes are probably the main reason for these damages.

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