
Study on nematodes (*Pratylenchus* spp.) on Arabica coffee in the Northwestern Vietnam

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Abstract Arabica coffee is one of the most important crop grown in Northwestern Vietnam. The growing area is estimated to be 20,000 ha, accounting for approximately 40% of Vietnam's Arabica coffee area, and it mainly distributed in Son La and Dien Bien provinces. The climate and elevation of those areas are suitable for growing Arabica coffee. Especially, the coffee is recognized as one of the high quality in the world trade. However, Arabica coffee cultivation in this region has threatened by nematode disease, causing leaf fall, twig dieback, dry fruit and leading to reduce productivity. The survey results of 19 tea coffee gardens in 2 provinces in 2018 had 84.81% of the gardens infected with *Pratylenchus* spp. The nematode density ranged from 21.91 to 48.3 individuals/ 100g of soil and from 43.5 to 99.2 individuals/ 2g roots. After 6 months, infection of *Pratylenchus* spp in plants with the density of 10, 100 and 500 individuals/pot, in greenhouse conditions, disease index was 31.45%, 62.48% and 84.78%, respectively. It is the first report to detect *Pratylenchus* spp in Arabica coffee in Muong Ang (Dien Bien) and Son La.

Keywords: Arabica coffee, nematode diseases, *Pratylenchus* spp., nematode density

Introduction

Vietnam is the second-largest coffee producer in the world, accounting for nearly 20% of total global coffee production. The area of 2016 is 645,381 ha, the output of green coffee reaches 26.7 million bags (over 1.6 million tons), Arabica coffee is 1.1 million bags. In northwestern Vietnam, Arabica coffee has been growing for a long time, but only really developed after 1996 when planting new varieties of resistible rust coffee. Today, the area of coffee reached about 20,000 ha mainly distributed in Son La and Dien Bien provinces. This region has a favorable climate and height for Arabica coffee to grow and develop, especially very good quality that is highly appreciated in the world market (Tran, 2002). However, coffee growing in Vietnam has been facing many challenges due to pests and diseases. There are many diseases causing yellowing of leaves, dry branches,

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dried fruits, and pods, causing severe productivity loss, many coffee gardens are seriously ill and lose their plants (Figure 1). One of the cause of symptoms is coffee root nematode. Tran *et al.* (2001) and Loang *et al.* (2001) reported that *Pratylenchus coffeae* and *Meloidogyne* sp. are common nematodes for coffee in the Western Highlands, Vietnam. When nematode density reaches 20 individuals/ 50g soil and 70 individuals/ 5g roots, Robusta coffee plants are likely to suffer from leaf yellow rot disease. Trin *et al.* (2012) investigated nematodes in *Coffea arabica* and *C. canephora* in 15 regions of 7 coffee producing provinces in Vietnam and identified 21 plant parasitic nematode species in the coffee root zone. Dominant species such as *Rotylenchulus reniformis*, *Meloidogyne* spp., *P. coffeae* and *R. arabocoffeae*, *P. coffeae* has the highest frequency (27% compared to other species), the average density of 124 individuals / 250 cm³ of soil. Moreover, Trinh *et al.* (2018) further reported to disciver *Meloidogyne daklakensis* n. sp. (Nematoda: Meloidogynidae), a new root-knot nematode associated with Robusta coffee (*Coffea canephora*) in the Western Highlands, Vietnam. According to Vu (2012), coffee root rot disease in Arabica coffee in Son La and Dien Bien is caused by fungi and other nematodes. Campos *et al.* (1990) suggested that the nematode species *Meloidogyne* and *Pratylenchus* spp. is the main species attacking coffee plantations.

The objectives were to survey, collect samples, isolate and identify the causal agent of nematode disease in Arabica coffee, and to determine the nematode density on Arabica coffee root rot disease.



Figure 1. Nematodes have damaged Arabica coffee gardens in Son La province, A- Stunting and leaf become yellow, B- Coffee plants died due to root-knot

Materials and methods

Preparation of plant materials

Arabica coffee nematode, *Pratylenchus* spp. was isolated from soil and roots of coffee plants infected with nematodes. The survey was conducted on coffee gardens in Son La and Dien Bien provinces in northwestern Vietnam. The surveyed gardens in Son La at an altitude of 800 m above the sea level, the slope $\leq 5^\circ$, the coffee gardens are 10-12 years olds; the gardens in Muong Ang are about 500 m high, the slope $\leq 5^\circ$, the age of coffee is 10-12 years; The gardens in Tuan Giao have a height of about 1000 m, the slope $\geq 10^\circ$, the age of coffee gardens is 7-9 years.

Survey and collection of soil and coffee root samples

The investigation and collection of samples made in June 2018 to October 2018 on coffee gardens in Dien Bien (Muong Ang district 2 gardens, Tuan Giao district 3 gardens); In Son La province 9 gardens, the smallest garden has an area of 1 ha, the age of coffee gardens is from 7-12 years old. Sampling gardens where have planted coffee plants that exhibited symptoms of nematode disease such as yellow leaves and slow growth. Soil and root samples were taken from the young root zone, depth from 1-20 cm in 4 directions of the plant, mixed with 1 kg of soil. Each garden took 15 plants in 5 places in a diagonal corner, each position was randomly taken in three plants. Rate of disease (%) = Number of diseased plants / Total number of surveyed plants x 100.

Isolation and determination of density for *Pratylenchus* spp.

Isolation of nematodes from samples was done by using a Whitehead tray extraction and combined with Baermann funnel method (Roger and Dean, 2005). The 100 g of soil sample was spread on tissue or finely woven cloth on a coarse mesh, supported above the base of a tray to which water is added to just saturate the soil. For 3–4 days, the living nematodes were moved downward through the tissue into the water. The water was transferred to a measuring cylinder in which the nematodes settle over several hours. Water is removed and remained only 5–20 ml, and taken the great care not to resuspend the nematodes. Nematodes wasc also isolated from coffee roots by mist extraction methods. The water was collected in a tube that the nematodes settle in the tube and can be collected after 2–4 days. The volume of water was reduced in the tube by careful aspiration which modified from Bezooijen (2006) and European and Mediterranean Plant Protection Organization (2013). The numbers of nematodes were counted on a microscope. Identification of *Pratylenchus* spp. based on

morphological characteristics was done by Prof. Dr. Kasem Soyong according to Cristina and Herrera S. (2011).

Determination of the effect of nematode density on Arabica coffee root rot disease

The experiment conducted as Randomized Complete Block Design in the infected nematodes in coffee plant grown in pots with sterilized soil. The experiment consisted of 4 treatments: (1) control, no nematodes; (2) infected 10 individuals/ pots; (3) infected 100 individuals/ pots and (4) infected 500 individuals/ pots. Each treatment had 15 plant pots. Treatments were evaluated after three and six months of infection, collected five plants from each treatment as five replicates. Evaluation was observed in the following criteria:

Percentages of infected plants (%) = (number of infected plants / total observed plants) x 100. The disease index (%) = $(\sum ab) \times 100 / N \times 4$, in which a is the number of trees with root rot at all levels, b is the corresponding disease level, N is the number of observed plants, 4 is the highest disease level. The root damage area is as follows: level 1 - root of damaged of 1-25%, level 2 – damaged roots of 26-50%, level 3 - damaged roots of 51-75%, level 4 - damaged roots > 75%. The growth of plant height, root length; weight of stems and roots, nematode density in roots and soil were recorded.

Results

Investigation of root rot disease on Arabica coffee

The results found root rot disease caused by *Pratylenchus* spp on 10 Arabica coffee gardens in Son La (12 gardens) and Dien Bien (7 gardens) province as shown in Table 1. It is indicated that an average of 84.81% of the gardens was seriously infected. In Son La province, the surveyed gardens was 94.45% of the gardens are infected, and 39.72% of plants in gardens were infected. In Muong Ang district, Dien Bien province was surveyed two orchards with root rot disease with the rate of infected plant is 42.34%. Meanwhile, in Tuan Giao district found 60% of the surveyed gardens which infected with the rate of 31.5%. Arabica coffee plants are mainly infected by *Pratylenchus* spp or some secondary invaders of parasitic fungi in the roots, leading to dead roots, the roots had less uptake nutrients and then leading to yellow leaves and retarded plants. The plants were surveyed the symptoms of yellow leaves, root rot and observed the rate of root rot trees was quite high in Son La which was 91.64%, in Muong Ang was 83.30 %, in Tuan Giao was 38.62 %. The percentage of coffee gardens and plants with root rot disease infected by *Pratylenchus* spp in Son La and Muong Ang were high due to the low slopes. The coffee

gardens faced water log and wet created favorable condition for disease incidence in Tuan Giao district which was a significant slope and good water drainage.

Isolation and determination for nematode density

Results of nematode isolation from soil samples which collected from 19 coffee gardens in Son La and Dien Bien provinces were determined to be infected with *Pratylenchus* spp. The density was considerably high (Table 2 and Figure 2). In the surveyed coffee gardens, the density of *Pratylenchus* spp. fluctuated with different locations. The highest density was belongs to coffee gardens in Son La, the average density was 48.3 individuals/ 100g soil and 99.2 individuals/2 g roots. The gardens in Muong Ang was averaged density of 42.64 individuals/ 100g of soil and 92.38 individuals/ 2 g roots. The gardens in Tuan Giao district found a density of *Pratylenchus* spp which lower density which averaged of 21.91 individuals/ 100g soil and 43.5 individuals/ 2g roots. The surveyed gardens in Son La and Muong Ang were a high density of *Pratylenchus* spp. because the topography of the gardens is quite flat, the ability to remain moisture. On the other hand, the coffee garden is older than the other locations. While the surveyed gardens in Tuan Giao district are located in the mountainside, high slope, good drainage, coffee gardens are young, and found less infected.

Table 1. The investigation of Arabica coffee root rot disease in the Northwestern Vietnam

| Locations | Number of garden | Area (Ha) | Year olds (year) | Infected garden (%) | Infected plant/ garden (%) | Root rot plant/ infected plant (%) |
|--------------------|------------------|-----------|------------------|---------------------|----------------------------|------------------------------------|
| Son La province | 12 | 14 | 8 - 10 | 94.45 | 39.72 | 91.64 |
| Dien Bien province | 7 | 23 | | | | |
| Muong Ang district | 2 | 15 | 10-12 | 100.00 | 42.34 | 83.30 |
| Tuan giao district | 5 | 8 | 8-10 | 60.00 | 31.50 | 38.62 |
| Average | | | | 84.81 | 36.85 | 71.18 |

Table 2. Nematode density of Arabica coffee in Northwestern Vietnam

| Locations | Number of gardens | Nematode density (individuals/ 100g of soil) | Nematode density (individuals/ 2g of root) |
|-----------|-------------------|----------------------------------------------|--------------------------------------------|
| Son La | 12 | 48.30 | 99.20 |
| Mường Ảng | 2 | 42.64 | 92.38 |
| Tuần Giáo | 5 | 21.91 | 43.50 |

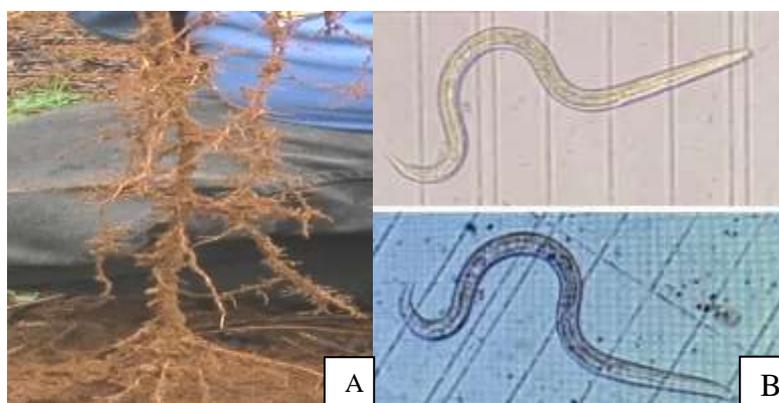


Figure 2. Collection, isolation and identification of nematode, A=Root symptom, B=Nematode

Effect of density of *Pratylenchus* spp. on young Arabica coffee plants in greenhouse conditions

The plants were about 20 cm high, planted in pots which placed in a greenhouse, away from natural sources of infection. After 2 weeks inoculation, roots were stabilized and started to infect by *Pratylenchus* spp. The growth of Arabica coffee plants after three and six months was exhibited in Table 3 and 4. After three months of infection with *Pratylenchus* spp., the root growth of the plants in treatments was not significantly different from controls. The length of the stem and roots in the 100 and 500 individuals/pot treatments were not significantly different, but there was a difference with the treatment of 10 individuals/pot at 95% probability. The infected plants with nematodes continued to reproduce, and increasing their density. However, the level of density increased in different treatment that were differed from each treatment. After three months, the nematode density in the treatments found infection of 100 and 500

individuals/pot, which were equivalent (64.12 and 66.42 individuals/100g soil, and 102.06 and 110.2 individuals/2g roots, respectively). The infected plants with nematodes was stunted, leaves were small in size and turned yellow. The higher density of nematodes resulted to high obviously symptoms on the roots and leaves. It was due to the root system is harmed, showed that the root volume was smaller than the control treatment. The damaged root system did not provide enough nutrients for the plant, leading to stunting, small leaves and gradually turning to yellow. The infection rates from treatments of 10, 100 and 500 individuals/pot resulted to increased significantly of 15.40%, 26.42% and 34.8%, respectively.

Table 3. Effect of *Pratylenchus* spp. density on young Arabica coffee plants in greenhouse conditions after three months of infection

| | Infected root (%) | Disease index (%) | Length (cm) | | Weight (g) | | Nematode density (individuals) | |
|---------------------|-------------------|-------------------|-------------|-------|-------------|-------|--------------------------------|------------|
| | | | Stem | Root | Stem + leaf | Root | 100g of soil | 2g of root |
| | | | | | | | | |
| Control | 0 | 0 | 28.38 | 27.22 | 14.34 | 3.15 | 0 | 0 |
| 10 | 100 | 15.40 | 23.72 | 21.15 | 10.22 | 2.20 | 15.50 | 198.20 |
| 100 | 100 | 26.42 | 21.20 | 20.00 | 8.72 | 1.92 | 24.32 | 322.00 |
| 500 | 100 | 34.80 | 20.10 | 18.64 | 7.56 | 1.66 | 42.60 | 380.72 |
| CV(%) | | | 22.64 | 26.15 | 32.00 | 36.27 | 30.66 | 40.25 |
| LSD _{0.05} | | | 3.27 | 3.57 | 2.16 | 0.89 | 12.70 | 52.27 |

Table 4. Effect of *Pratylenchus* spp. density on young Arabica coffee plants in green house conditions after six months of infection

| | Infected root (%) | Disease index (%) | Length (cm) | | Weight (g) | | Nematode density (individuals) | |
|---------------------|-------------------|-------------------|-------------|-------|-------------|-------|--------------------------------|------------|
| | | | Stem | Root | Stem + leaf | Root | 100g of soil | 2g of root |
| | | | | | | | | |
| Control | 0 | 0 | 52.83 | 52.20 | 26.14 | 5.70 | 0 | 0 |
| 10 | 100 | 31.45 | 30.12 | 25.00 | 15.20 | 3.12 | 72.05 | 86.12 |
| 100 | 100 | 62.48 | 27.22 | 22.10 | 9.82 | 1.96 | 64.12 | 102.06 |
| 500 | 100 | 84.78 | 24.42 | 20.04 | 7.05 | 1.48 | 66.42 | 110.12 |
| CV(%) | | | 24.21 | 36.25 | 28.30 | 33.62 | 31.02 | 38.40 |
| LSD _{0.05} | | | 4.32 | 3.05 | 2.16 | 0.89 | 5.12 | 12.20 |

After 6 months, the infection rate was significantly different to increase in each treatment. The disease index increased, the root system is destroyed. The stem height and rhizosphere root decreased significantly when compared to the control. Result showed no or without normal roots in the infected plants of 100 and 500 individuals/pot. The root weight was 1.96 and 1.48 g in the infected plants of 100 and 500 individuals/pot. Nematode density at this stage was no longer available as in three months after infection. At this time, the density of nematodes in the soil increased, while the density of the roots was less. This phenomenon is caused by a rapid increase in nematode density, focusing on nutrient uptake was less too after the nematode destroyed the root system, causing stunted trees and dead.

Discussion

As result, the survey of 19 Arabica coffee gardens with an area of 37 ha in Dien Bien and Son La provinces averaged 84.81% of the gardens were infected with root lesion nematode, *Pratylenchus* spp. The infection rate was 36.85%; 71.18% in Dien Bien and Son La provinces. However, *Pratylenchus* spp have not been recorded from coffee gardens in Muong Ang (Dien Bien) and Son La. However, Vu (2012) stated that coffee root rot disease in Arabica coffee in Son La and Dien Bien is caused by fungi and other nematodes. Moreover, Campos *et al.* (1990) reported that *Pratylenchus* spp. is one of the main nematode species attacking coffee plantations in Vietnam. The research finding found that *Pratylenchus* spp with density of 48.3 individuals/ 100g of soil and 99.2 individuals/ 2g of roots in Son La province, and in Muong Ang district, the density was 42.64 individuals/ 100g of soil and 92.38 individuals/ 2 g of roots. In Tuan Giao district was 21.91 individuals/ 100g of soil and 43.5 individuals/ 2g of roots. Withn this, Tran and Nguyen (2001) and Loang *et al.* (2001) stated that *Pratylenchus coffeae* is one species of root lesion nematode that infected coffee trees and when nematode density reached 20 individuals/ 50g soil and 70 individuals/ 5g roots, it destroyed the Robusta coffee plants to become yellow leaves and root rot disease. However, Tran and Nguyen (2001) and Loang *et al.* (2001) reported that to confirm that *Pratylenchus coffeae* and is one of common nematode for coffee in the Western Highlands, Vietnam.

Infection of nematodes on young Arabica coffee plants has significantly affected the growth of coffee plants. In the formulas infected with the density of 10, 100 and 500 individuals/pot, after six months the plants stunted, leaves small, turns yellow, the disease index is 31.45%, 62.48% and 84.78%, respectively. Plant height and root mass were very low compared to controls, the treatment infected 100 and 500 individuals/pot, root weight was only 1.96 and 1.48g, respectively. Campos *et al.* (1990)

suggested that the nematode species *Meloidogyne* and *Pratylenchus* spp., is the main species attacking coffee plantations.

Prevention of coffee nematodes is difficult and complicated. Farmers often use a combination of control, but the effect is still not satisfactory. This research finding presented the causal agent of root rot diseases of Arabica coffee which caused by *Pratylenchus* spp. in Son La and Dien Bien provinces. *Pratylenchus* spp is found to be infected Arabica coffee in Muong Ang (Dien Bien) and Son La for the first time in Vietnam. Further research investigation is to find the biological control agent against this plant parasitic nematode to be applied in Arabica coffee.

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