

The application of arbuscular mycorrhizal fungi and mycorrhizae helper bacteria combined with compost to reduce the *Pratylenchus coffeae* population in an Arabica coffee plantation

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ABSTRACT

Arbuscular mycorrhizal fungi (AMF) and mycorrhiza helper bacteria (MHB) are potential biological agents that control *Pratylenchus coffeae*, a major endoparasitic nematode in coffee plantations. An experiment was conducted to determine the role of AMF and MHB inoculants combined with compost in reducing plant damage and the number of nematodes in the soil and roots, as well as increasing the AMF infection rate in juvenile (JP) and mature (MP) Arabica coffee plants. The field experiment with a randomized block design was conducted with both juvenile (JP) and mature plants, consisting of 8 combination treatments and one control with three replications. The JP and MP plants received 1.5-50 g and 25-100 g AMF inoculant/plant and compost at doses of 5 or 7.5 and 10 or 15 kg/plant, respectively, while the MHB liquid inoculant concentrations were 10 mL and 100 mL, respectively. The control plants were not treated with AMF, MHB, or compost. The AMF and MHB inoculants combined with compost reduced leaf and root damage, but increased the degree of AMF infection in the roots of JP and MP. The nematode populations in roots and shoots of treated JP were reduced by 48.7-74.5% and 56.0-80.5%, respectively; in mature plants, the reductions ranged from 40.5 to 74.4% in roots and 44.0 to 75.4% in the soil. These results suggest that AMF, MHB, and compost effectively reduced *P. coffeae* infection in Arabica coffee plants under field conditions.

Key words: Glomus; degree of mycorrhizal infection; nematode population; plant damage.

1 INTRODUCTION

Coffee (*Coffea* spp.) is an important global agricultural commodity. Brazil, Vietnam, and Indonesia were the top coffee-producing countries in the year 2022/2023. According to the International Coffee Organization - ICO (2023), the global production of coffee in the year 2022/2023 reached 168.2 million 60-kg bags, including 94 million bags of Arabica and 74.2 million bags of Robusta, among which Indonesia's output was 1.85 million bags.

One of the most serious challenges facing coffee production is *Pratylenchus coffeae*, a root-lesion nematode that invades coffee plants under field conditions (Thiep et al., 2019; Hindersah et al., 2022; Machado et al., 2023). The yield losses of Robusta coffee due to root-lesion nematode can reach 78% with an average of 57% (Budiman et al., 2019). However, greater yield losses in Arabica coffee than in Robusta coffee have been reported since Arabica is less resistant to root-lesion nematode attack (Trinh et al., 2012). A greater incidence of *Pratylenchus* spp. has been detected in acidic, low salinity, low organic matter, and sandy soils with low contents of silt and clay (Trinh et al., 2009; Chalanska; Labanowski; Sas, 2016). The root-lesion nematode of *Pratylenchus* spp. damage coffee root tissues, which manifests as reduced water and nutrient uptake, decreased photosynthesis, and restrictions in sucrose transport from leaves. The impaired metabolism then induces severe chlorosis, leaf fall, twig dieback, and stunting,

which together cause reductions in overall plant productivity (Rahayu; Sari, 2017; Thiep et al., 2019).

Controlling root-lesion nematodes in coffee plantations encounters some difficulties since coffee is a perennial crop that provides a favorable environment for root-lesion nematode multiplication (Machado et al., 2023). To reduce the need for chemicals, biological agents and organic matter are essential for nematode control and soil improvement. Beneficial soil microbes are suggested as biological agents and biofertilizers to control parasitic nematodes. Arbuscular mycorrhizal fungi (AMF) and mycorrhiza-helper bacteria (MHB) are candidate biological agents for controlling root-lesion nematode since the MHB is closely associated with AMF and is involved in the development of arbuscular mycorrhizae.

AMF protects plants from nematode attack by competing for space and nutrients, enhancing plant tolerance, inducing plant systemic resistance, and changing the microbial composition of the rhizosphere (Schouteden et al., 2015). However, the effects of various AMF species on *Pratylenchus* spp. populations in the roots of important crops include negative, no effect, and positive effects (Gough et al., 2020). The MHBs inhabiting the rhizosphere zone promote the development of arbuscular mycorrhizal symbiosis (Nasslahsen et al., 2022). The MHB of *Bacillus* sp. and *Pseudomonas* sp. induces spore germination and mycelial formation from AM propagules (Asyiah et al., 2021).

The MHB *Bacillus* and *Pseudomonas* are P-solubilizing (Kalayu et al., 2019) and N-fixing bacteria (Li et al., 2017).

AMF increases the uptake of inorganic orthophosphate by roots through extensive mycelial growth under low phosphorus (P) availability (Feng et al., 2003; Hou et al., 2021). Moreover, fungi secrete acid phosphatases to catalyze the degradation of organic P and provide inorganic P available for uptake by roots in the soil under P-limited conditions (Sato et al., 2019). Organic matter amendment in coffee plantations is an important practice for improving soil quality and crop growth and yield (Dzung et al., 2013; Widiatmono et al., 2020). The metabolism of AMF and MHB is dependent on organic matter, e.g., they obtain their energy from organic carbon. On the other hand, organic matter has been reported to reduce the viability and populations of root-lesion nematodes (Rahayu; Sari, 2017; Cole et al., 2020).

Despite their multiple functions as biocontrol agents and biofertilizers, the management of AMF and MHB to control *P. coffeae* in coffee plantations by introducing biological agents is limited. Previous research has confirmed that single species of MHB (*P. diminuta* and *B. subtilis*) might replace synthetic nematicides to reduce the *P. coffeae* population by up to 89.7% (Asyiah et al., 2015). Mixed inoculation of *Glomus* spp. and MHB (*B. subtilis* and *P. diminuta*) increased the degree of AMF infection but reduced the infection degree of *P. coffeae* in coffee seedlings by 20-60% (Hindersah et al., 2022a). Moreover, the solid-based mixed MHB inoculant increased shoot height but decreased *P. coffeae* populations in the roots of coffee seedlings (Hindersah et al., 2022b). The objective of this field experiment was to evaluate the role of an AMF inoculant, MHB, and compost in reducing plant damage, *P. coffeae* infection, and increasing the arbuscular mycorrhizae establishment in juvenile (JP) and mature (MP) Arabica coffee plants. This study is prominent in establishing the sustainable agricultural practice of Arabica coffee plants to protect plants from severe *P. coffeae* attacks and further promote growth and yield.

2 MATERIAL AND METHODS

The field experiments were conducted in the Arabica coffee plantation in Banyuwangi, East Java Province, Indonesia. The plantation is located in a tropical area at an altitude of 950 m, with an annual temperature and humidity between 23.0-29.9 °C and 67-86%, respectively, as well as a slope gradient of 15° under rainfed conditions. Before the experiment, the plants were naturally infected by Indigenous *P. coffeae*, which then showed minor symptoms of stunting, chlorosis, and leaf shedding (Figure 1). The roots were infected by indigenous *P. coffeae* of approximately 20 individuals per gram of soil.

2.1 Biological agents

The AMF inoculant consisting of *Glomus* sp., with 20 spores/g inoculant, was propagated by the trapping method using maize (Gopal et al., 2016) grown in a zeolite with a particle size of 3 mm. A liquid culture of the MHB consortium consisting of *P. diminuta* and *B. subtilis*, with a density of approximately 10⁸ CFU/mL and an initial volume ratio of 2:3, was prepared in molasses-based media. The culture was incubated on a reciprocal shaker at 115 rpm and room temperature (24-27 °C) for three days. The juvenile Arabica coffee cv. Kartika 2 and mature Arabica coffee cv. BP542A plants were grown for 2 years and 10 years, respectively, with a planting distance of 2.5 m × 2.5 m. The AMF and MHB were provided by the Universitas Jember.

2.2 Soil sampling and analysis, and compost characteristics

The soil in the experimental area where JP and MP grow is an association of latosol and regosol. Before the experiment, the soil samples were collected from depths



Figure 1: The juvenile (a) and mature (b) coffee plants parasitized by *P. coffeae* in the Kalibendo Plantation

of 0-30 cm and 30-60 cm and subjected to nutrient and solid fraction analyses according to the AOAC method (Association of Official Analytical Collaboration - AOAC, 2012) and the method developed by Van Reeuwijk (2002), respectively. The compost made from agricultural wastes consisting mainly of pulp (mesocarp) and outer skin (pericarp) of the coffee fruit was provided by Kalibendo Co. The compost had a pH of 7.2, ash content of 7.87%, water content of 21%, organic carbon content of 5.3%, total N content of 2.05%, total P content of 0.22%, total K content of 0.70%, and a C to N ratio (C:N) of 25.8%.

2.3 Experimental design

The field experiments were carried out in a plantation with JP and MP in a randomized block design consisting of 8 combination treatments and one control with three replications. The JP and MP were each separately treated with the combination of four doses of the *Glomus* inoculant, a single dose of the MHB liquid inoculant, and two doses of compost (Table 1). The control plant received neither biological agents nor compost. Each replicate consisted of 10 adjacent plants.

On the first day of the experiment, compost was spread in the soil at a depth of 20 cm on the drip line where most hair roots are usually found. The drip lines for JP and MP were 1 m and 2 m away from the plant stem, respectively. The inoculants were mixed with the MHB liquid inoculant, followed by an even mixture with compost before covering with the soil. The experiment lasted 16 weeks after the treatments were applied.

2.4 Parameter determination and statistical analysis

Leaf damage was scored on a 0-5 scale based on leaf damage scoring description (Table 2) at 4 to 16 weeks

after treatment (WAT). The infection degree (ID) was then calculated using a scale of five classes according to the Townsend–Heuberger formula described by Scalzo et al. (2012) as follows: Equation 1.

$$ID(\%) = \sum_{i=1}^i \frac{(n_i v_i)}{(N.V)} \quad (1)$$

where

v_i = infection class

n_i = the number of plants in each damage class

N = total number of plants

V = the highest class

i = the number of classes

The degree of root damage was determined by calculating the percentage of ten segments of damaged fibrous roots randomly collected within the drip line. The roots were washed, cut into 1 cm fragments, and then mixed evenly. Ten root pieces were placed on the slides, and damaged roots, which were brown or black, soft, or weak, were counted. The number of *P. coffeae* in the feeder root tissues and soil within the drip line was counted at 16 WAT using the Baermann funnel method (van Bezooijen, 2006). Nematodes were extracted from 100 g of soil samples and 10 g of root samples. The degree of mycorrhizal infection was determined at 8 and 16 WAT by microscopic observation. The 1-cm root segments were soaked in 2% KOH at 80 °C before being stained with acid fuchsin, and ten root segments per plant were analyzed. The degree of root damage was calculated by dividing the

Table 1: The *Glomus* and MHB inoculants and compost that were applied to juvenile and mature Arabica coffee plants.

Plant growth stages	Amounts of the <i>Glomus</i> inoculant (g/plant)	Doses of the MHB liquid inoculant (mL/plant)	Compost levels (kg/plant)
Juvenile (2-year-old)			
50 (JM ₁), 37.5 (JM ₂), 25, (JM ₃), 12.5 (JM ₄)		10	5 (O ₁) and 7.5 (O ₂)
Mature (10-year-old)	100 (MM ₁), 75 (M ₂), 50 (M ₃), 25 (M ₄)	100	10 (O ₁) and 15 (O ₂)

Table 2: Infection classes used to score leaf damage.

Infection Class	Description
0	Healthy plants; the roots are normal without color alteration; leaves are green without yellow spots
1	One or two leaves turn yellow without shedding
2	Less than 25% of leaves turn yellow without shedding
3	25-50% of leaves show yellowing symptoms and some leaves have been shed
4	More than 50% of leaves show yellowing symptoms and the majority of leaves have been shed
5	The plant is stressed and dies and leaf shedding is dominant

number of damaged root segments by 10 and is expressed as a percentage. A similar calculation was performed for the determination of the degree of mycorrhizal infection. The parameters of each sample were measured in triplicate.

All the data were subjected to analysis of variance (ANOVA) at a significance level of 0.05 ($p < 0.05$). If the effect of the treatment on the observed parameter was significant, the Duncan multiple range (DMR) test was performed to evaluate significant differences between treatments. Statistical analysis was performed by using Statistical Product and Service Solutions (SPSS) version 20.

3 RESULTS

3.1 Soil properties

Table 3 shows the properties of the soils at the two depths. The soils from both depths in the plantation with JP were highly acidic, but the pH at the 0-30 cm depth in the MP area was even lower. The soil organic C and total N contents in the JP area were high but lower than those in the MP area; nonetheless, the C:N ratio in the JP area was lower than that in the MP area. In general, the organic C and total N contents of the soil at the 0-30 cm depth were lower than those at the 30-60 cm depth; surprisingly, the C:N ratio at the 30-60 cm depth in the MP area was greater. The use of chemical fertilizer in both plantations was quite intensive, which resulted in high soil total N and available P contents. A high exchangeable K (K_{ex}) content was detected in all the soil samples, but an extremely high K_{ex} content was detected at the 0-30 cm soil depth in the MP area. The exchangeable Ca content for JP was

moderate but low for MP, and both the exchangeable Na and Mg contents were moderate in both areas. The soil has a sandy loam texture with less than 10% clay content.

Low soil acidity induces the proliferation of *P. coffeae* (Chalanska et al., 2016). The soil contained a high sand fraction that might increase the infection of *Pratylenchus* spp. (Trinh et al., 2009). Moreover, the organic-C of soil in mature plants is lower than in juvenile plants. Low organic matter might limit the proliferation of AMF and MHB, so additional organic matter (compost) was added in this field experiment since organic matter amendment also increases the soil pH.

3.2 Leaf and root damage

3.2.1 Leaves Damages

The application of AMF and MHB inoculants and organic matter simultaneously significantly reduced leaf damage of JP and MP, which varied depending on the observation time and the level of treatment (Tables 4 and 5). On the first day of treatment (0 WAT), the scores of leaf damage caused by the endemic nematode in the JP and MP treatments were less than 1 and 2, respectively.

At 4 WAP, the effects of the treatments on leaf damage in JP were not consistent (Table 4); apparently, the AM, MHB, and manure mixture slightly increased or did not change the degree of leaf damage compared to that in the control plants.

Obvious effects were observed at 8, 12, and 16 WAP. The JP treated with the mixture of AMF inoculants and the MHB liquid inoculant exhibited less leaf damage at the end of the experiment. Regardless of the type of inoculant, higher doses of manures almost always decreased the degree of leaf

Table 3: The initial chemical and physical properties of the soil in the juvenile and mature coffee plantations before the experiment.

Soil Properties	Methods ^a	Plant growth stages at two soil depths			
		JP ^c 0-30 cm	JP 30-60 cm	MP ^d 0-30 cm	MP 30-60 cm
pH _{H2O}	Potentiometry	4.97	4.49	5.16	4.93
Organic C (%)	Walkey and Black	5.96	4.88	2.57	2.71
Total N (%)	Kjeldahl	0.58	0.48	0.24	0.18
C to N ratio	Calculation	10.27	10.16	10.71	15.05
Available P ₂ O ₅	Bray I	98.22	49.71	33.78	101.32
K _{ex}	flame photometer	0.83	0.74	1.12	0.93
Ca _{ex}		9.29	5.96	4.33	4.25
Na _{ex}		0.38	0.35	0.43	0.35
Mg _{ex}		0.52	0.52	0.40	0.30
Solid fraction (%)	Hydrometer ^b				
Silt		45	33	27	34
Clay		7	5	9	7
Sand		48	63	64	59

^a Proximate analysis using the AOAC method (2012); ^b Van Reeuwijk (2022); ^c Juvenile plants; ^d Mature plants.

damage caused by JP at 16 WAP. Inoculation of JP with 50 g (1000 spores) of AMF and 10 mL of MHB combined with 7.5 kg of compost consistently caused the lowest degree of leaf damage at 8 to 16 WAP.

The co-application of biological agents and compost did not affect the leaf damage score of MP at 4 WAT, but the responses of MP to these biological agents combined with compost were distinctively different at 8, 12, and 16 WAP (Table 5).

A few treatments influenced the leaf damage caused by MP at 8 and 12 WAT, but all treatments reduced the damage at 16 WAP. In general, higher doses of compost almost always decreased the leaf damage score of JP at 16 WAP. Inoculation with 75 g of AMF and 100 mL of MHB in combination with 15 kg of compost resulted in a lower score of leaf damage at 8 to 16 WAP (Table 5).

At the end of the experiment, the leaf damage score of JP at 16 WAP ranged from 0.67 to 1.13, with an average of 1.00, while that of the control plants was 2.74 (Table 4). The plants treated with 7.5 kg of compost had an average leaf damage score of 0.83, which was lower than that obtained for plants treated with 5 kg of compost (0.95). Compared with the treated plants, the MP that received no treatments showed more severe leaf damage at 16 WAP (score of 3.03), while the average leaf damage score in treated plants was 2.32. In general, treated juvenile plants at 16 WAT had a leaf damage score of less than 1, lower than control plants, 2.70 (Table 4). Moreover, the leaf damage score of the treated mature plants at the same time was generally lower than that of the control plants (Table 5).

Table 4: Leaf Infection degree of juvenile plants caused by *Pratylenchus coffeae* after the application of Glomus and MHB inoculants and compost.

Treatments	Leaf infection degree on different days after treatment				
	01 WAT	4 WAT	8 WAT	12 WAT	16 WAT
JM ₀ O ₀ (Control)	0.07 ±0.06a	0.87 ±0.21abc	1.90 ±0.26b	2.43 ±0.15b	2.70 ±0.10b
JM ₁ O ₁	0.17 ±0.15a	1.10 ±0.21bcd	1.73 ±0.21b	2.10 ±0.36ab	1.10 ±0.98a
JM ₁ O ₂	0.20 ±0.20a	0.67 ±0.31abc	1.13 ±0.06a	1.57 ±0.32a	0.90 ±0.44a
JM ₂ O ₁	0.50 ±0.44a	1.37 ±0.38cd	1.87 ±0.32b	1.93 ±0.40ab	0.90 ±0.40a
JM ₂ O ₂	0.37 ±0.25a	1.60 ±0.66d	1.90 ±0.71b	1.93 ±0.32ab	1.03 ±0.72a
JM ₃ O ₁	0.37 ±0.21a	0.67 ±0.12ab	1.43 ±0.21ab	1.80 ±0.35ab	1.13 ±0.40a
JM ₃ O ₂	0.27 ±0.29a	0.90 ±0.62abc	1.43 ±0.32ab	1.97 ±0.38ab	0.73 ±0.40a
JM ₄ O ₁	0.00 ±0.00a	0.43 ±0.21a	1.13 ±0.06a	1.80 ±0.50ab	1.57 ±0.64a
JM ₄ O ₂	0.33 ±0.49a	1.10 ±0.62abcd	1.70 ±0.35b	1.67 ±0.40a	0.67 ±0.55a

Values followed by the same letter within a column are not significantly different according to the DMR test at $p < 0.05$. JM₁: 50 g of AMF inoculant, M₂: 37.5 g of AMF inoculant, M₃: 25 g of AMF inoculant, M₄: 12.5 g of AMF inoculant; O₁: 5 kg compost/plant, and O₂: 7.5 kg compost/plant.

Table 5: Leaf damage score of mature plants caused by *P. coffeae* after the application of Glomus and MHB inoculants and compost.

Treatments	Leaf damage scores on different days after treatment				
	0 ¹ WAT	4 WAT	8 WAT	12 WAT	16 WAT
MM ₀ O ₀ (Control)	1.47 ±0.83a	1.80 ±0.79a	2.50 ±0.36bc	2.33 ±0.50ab	3.03 ±0.57b
MM ₁ O ₁	1.33 ±0.45a	2.17 ±0.67a	2.40 ±0.52bc	1.97 ±0.72ab	2.50 ±0.44ab
MM ₁ O ₂	1.17 ±0.45a	1.97 ±0.35a	2.43 ±0.15bc	1.93 ±0.29ab	2.37 ±0.06ab
MM ₂ O ₁	1.87 ±0.93a	2.20 ±0.98a	2.57 ±0.76c	2.20 ±0.36ab	2.50 ±0.10ab
MM ₂ O ₂	1.13 ±0.21a	1.23 ±0.31a	1.37 ±0.25a	1.60 ±0.36a	1.97 ±0.50a
MM ₃ O ₁	1.23 ±0.15a	1.80 ±0.30a	1.87 ±0.25abc	1.87 ±0.06ab	2.43 ±0.31ab
MM ₃ O ₂	1.53 ±0.55a	2.17 ±0.67a	2.17 ±0.67abc	2.60 ±0.44b	2.83 ±0.35b
MM ₄ O ₁	1.27 ±0.76a	1.80 ±0.95a	1.80 ±0.95abc	1.93 ±0.40ab	2.10 ±0.46ab
MM ₄ O ₂	1.00 ±0.60a	1.60 ±0.35a	1.60 ±0.35ab	1.63 ±0.58a	1.87 ±0.55a

Values followed by the same letter within a column are not significantly different according to the DMR test at $p < 0.05$. M₁: 100 g of AMF inoculant, M₂: 75 g of AMF inoculant, M₃: 50 g of AMF inoculant, M₄: 25 g of AMF inoculant; O₁: 10 kg compost/plant, O₂: 15 kg compost/plant; 0¹ WAT: day of treatment.

3.3 Root damage

Based on the ANOVA results, the effect of different treatments on root damage in response to JP and MP was significant at 16 WAT. The average reduction in root damage in MP was 18.5%, which was greater than that in JP (14.8%). The co-application of 50 g of AMF and 10 mL of MHB inoculant and 5 kg of compost to JP resulted in a greater reduction in root damage than the control treatment, while the other treatments did not affect root damage in JP (Figure 2a). Most treatments significantly reduced root damage in response to MP (Figure 2b), and a reduction of more than 25% was recorded in plants that received 50, 75, or 100 g of AMF. Surprisingly, a smaller amount of inoculant (25 g) co-applied with 15 kg of compost slightly increased the root damage score of MP (Figure 2b). The application of a higher dose of compost (15 kg/plant) reduced root damage in MP-treated plants, but at a lower dose (5 kg/plant), a greater reduction in root damage in JP-treated plants than in high-dose plants possibly occurred (Figure 2b).

According to the DMR test, the lowest root damage score of JP (44.44%) was recorded with the application of 50 g of AMF inoculant and 5 kg of compost (Figure 2a), but the lowest root damage score of MP (48.89%) was obtained when plants received of AMF inoculant (75 g) combined with 15 g of compost (Figure 2b).

3.4 Number of *P. coffeae* in roots and the soil

In this study, the soil and roots were already infested by nematodes before the experiment (Figure 3). The population of *P. coffeae* in the soil of JP and MP was lower than that in roots. The degree of infection in the soil was low, but this endoparasites nematode immediately migrated from the soil to the roots in search of nutrients. In the JP and MP plantations, no severe leaf shedding was detected, but leaf yellowing occurred.

Biological treatments and compost significantly reduced the populations of nematodes in the roots and soil of

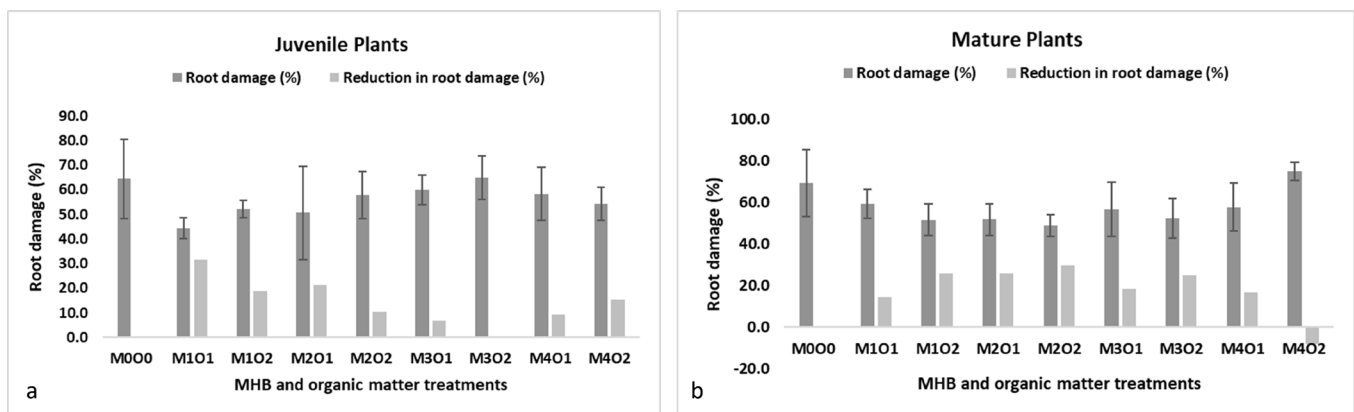


Figure 2: Root damage score of juvenile (a) and mature (b) plants after the application of *Glomus* and MHB inoculants and compost. Values followed by the same letter within a column are not significantly different according to the DMR test at $p < 0.05$.

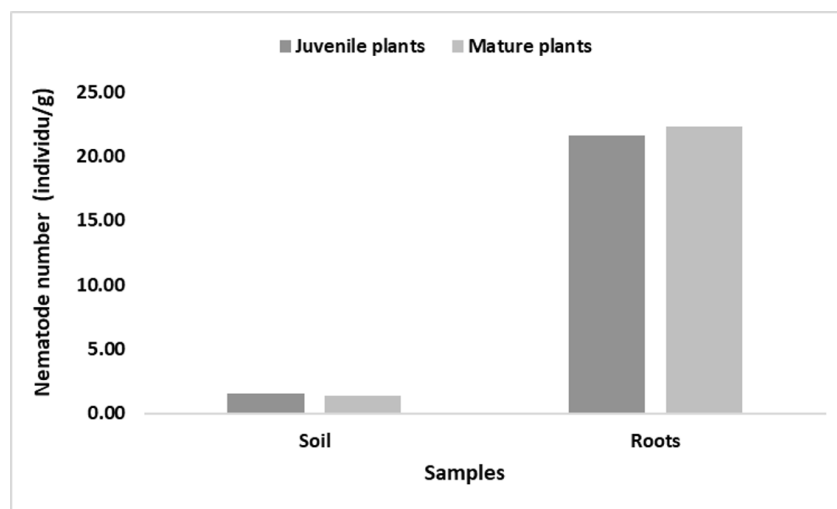


Figure 3: Populations of *Pratylenchus coffeae* in the soil and roots of juvenile and mature plants before the experiment.

both JP and MP at 16 WAT (Figures 4a and 4b). The populations of nematodes in roots at both plant growth stages (juvility and maturity) were greater than those in the soil. The lowest number of *P. coffeae* in the roots of JP was found when the plants received 25 and 50 g of the AMF inoculant combined with 5 kg of compost (Figure 4b), but the smallest population in the soil was recorded in plants treated with 50 g of AMF inoculant and 5 kg of compost (Figure 4b).

The MP treated with 75 g of *Glomus* inoculant, 100 mL of MHB, and 15 kg of compost (M_2O_2) and with 50 g of the AMF inoculant spores, 100 mL of MHB, and 10 kg of compost (M_3O_1) had the lowest numbers of *P. coffeae* in the roots (Figure 5a); moreover, the plants in the M_2O_2 treatment had the lowest number of nematodes in the soil (Figure 5b). The application of low and high doses of compost decreased the nematode populations in both roots and the soil compared to those in the control (Figures 4 and 5). To suppress the nematode populations in both the roots and soil, a lower dose of

compost was effective; however, a higher compost level might be needed for a greater reduction in the nematode population.

3.4 Degree of root infection by arbuscular mycorrhizal fungi

The experiment confirmed that the exogenous propagules of *Glomus* spp. effectively colonized the roots of JP and MP. Before the experiment, the degree of AMF infection was 46% and 32% in JP and MP, respectively. The combination treatments significantly increased the degree of AMF infection (Figure 6). The average infection rate of the JP-treated plants at 8 WAT was 70.89%, which was lower than that at 16 WAT (81.92%) (Figures 6a and 6b), while the treated MP showed a lower AMF infection rate at 8 WAT (70.40%) than at 16 WAT (75.61%) (Figures 6c and 6d).

The lowest nematode population in juvenile and mature plants was observed with the combined treatments of 50 g of AMF inoculant + 5 kg of compost and 75 g of AMF inoculant

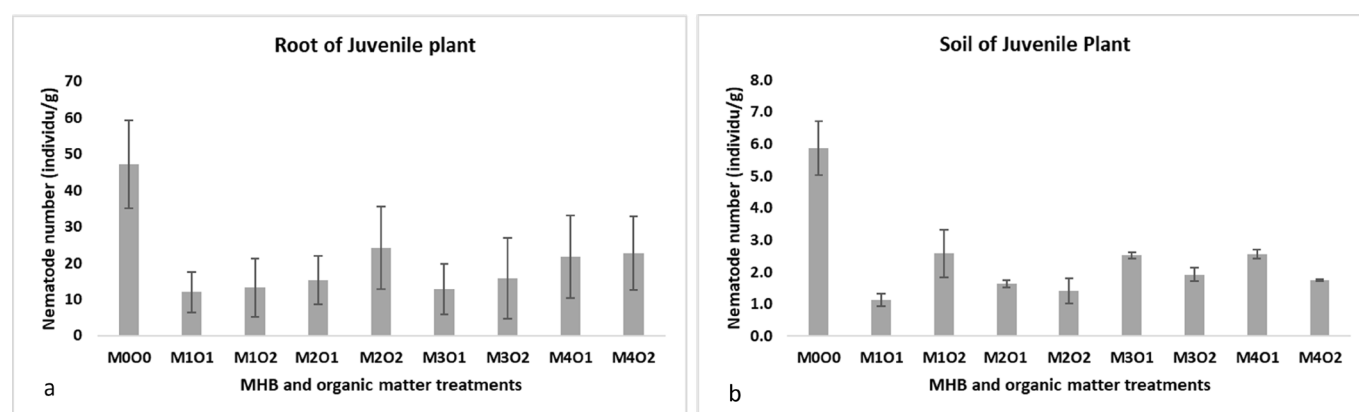


Figure 4: The number of *Pratylenchus coffeae* in the roots (a) and soil (b) of juvenile plants after the application of AMF and MHB inoculants and compost. Values followed by the same letter within a column are not significantly different according to the DMR test at $p < 0.05$.

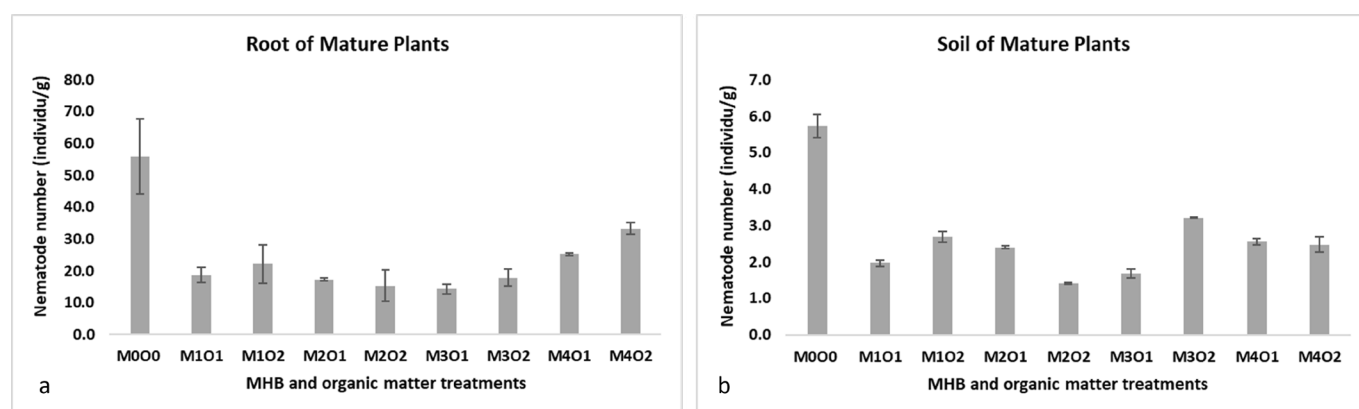


Figure 5: The number of *Pratylenchus coffeae* in the roots and soil of mature plants after the application of AMF and MHB inoculants and compost. Values followed by the same letter within a column are not significantly different according to the DMR test at $p < 0.05$.

+ 15 kg of compost, respectively (Tables 9 and 10). The appropriate doses of AMF inoculant and compost that were effective at reducing root damage in juvenile plants were 50 g and 5 kg, respectively. However, a higher compost dose (15 kg) was ideal for reducing both leaf and root damage and the number of *P. coffeae* in mature plants; which is the important parameters to indicate the severity of nematode attack in coffee.

4 DISCUSSIONS

Soil provides the essential macronutrients N, P, and K, but soil acidity (low pH) limits P availability (Antoniadis; Koutroubas, 2015). Under low pH conditions, essential micronutrients (metals) are generally available for root uptake (Adamczyk-Szabela; Wolf, 2022). Despite the adaptability of coffee plants to various types of soils, nutrient sufficiency is essential for plant growth and yield (Amponsah-Doku et al., 2022). Tropical soils, which are a mixture of latosol and regosol, have a solum depth of more than 1.5 m to ensure the root growth of coffee plants, and soils with a sandy loam texture and a clay content less than 10% are suitable for coffee cultivation (Snoeck et al., 2016).

The root-lesion nematodes are root pathogens that live in soil and attack roots when they are under stress. (Davis; MacGuidwin, 2000). Soil is a natural habitat of RLNs, whose life cycle includes six stages, namely, the egg state, juvenile

(first-stage larva; J1, second-stage larva; J2, third-stage larva; J3, and fourth-stage larva; J4), and adult stages. The eggs can be laid in the soil or roots, and second-stage juveniles (J2) to adults can infect the plant roots (Jackson-Ziems, 2016). The soil used in this research has a sandy loam and loam texture that is suitable for endoparasitic proliferation (Chalańska; Labanowski; Sas, 2016).

All combination treatments significantly reduced the nematode population (Figure 5), and most treatments significantly reduced root damage either in JP or MP (Figure 2). Various mechanisms by which AMF can reduce nematode infection have been suggested. Fungi can enhance the resistance or tolerance of a plant to *Pratylenchus* (Schouteden et al., 2015). Various studies have shown that mycorrhizae can inhibit the penetration and development of parasitic nematodes in plant roots. During their growth and the establishment of symbiotic relationships with host plants, AMF can increase water, N, and P uptake (Baum et al., 2015). Gough et al. (2020) noted that AMF could compensate for root damage caused by *Pratylenchus* spp. by enhancing the uptake of P and micronutrients such as Fe, Mn, Zn, and Cu.

The endoparasitic nematodes *Pratylenchus* spp. feed on cells in the root cortex; thus, causing root damage (Jones et al., 2013). The root-lesion nematode and AMF inhabit the same ecological niche in the roots of many crops (Gough et al., 2020). The reduction in leaf and root damage observed in the present study is related to the reduction in nematode

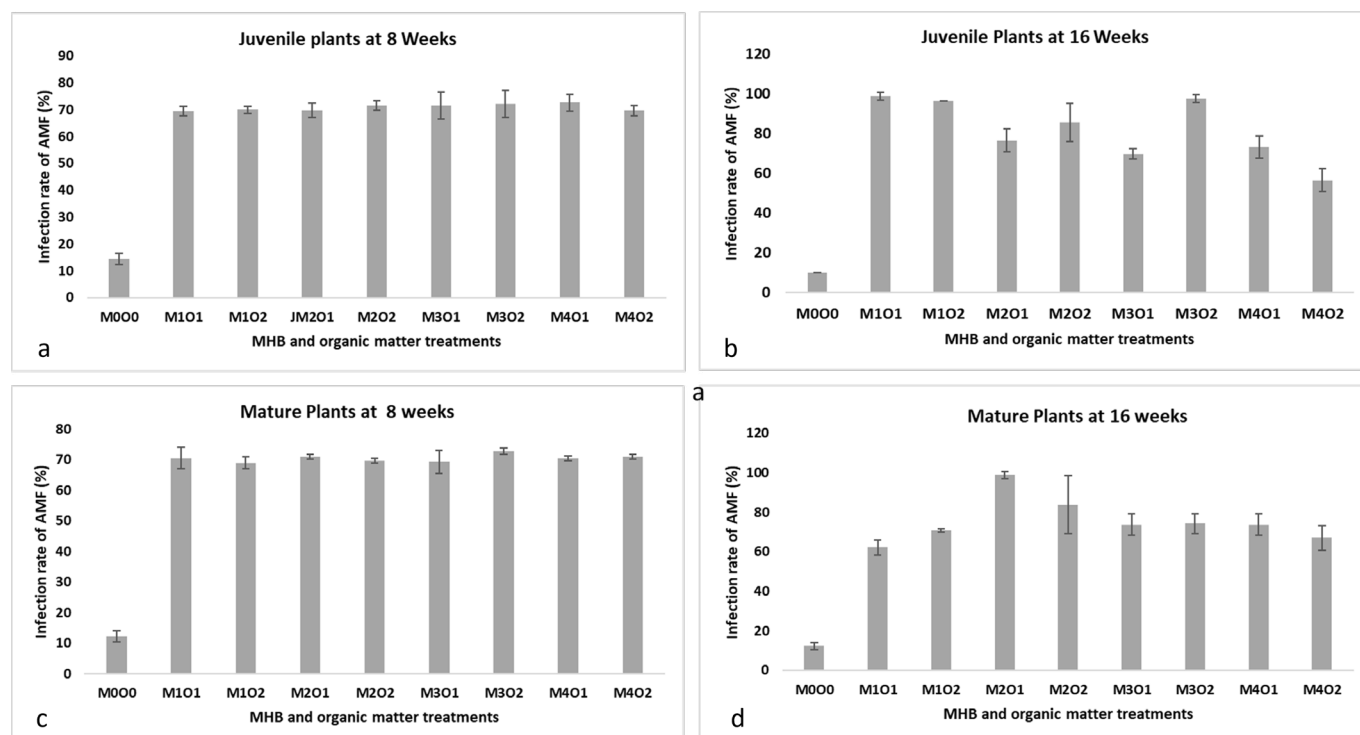


Figure 6: Infection rates of AMF in juvenile and mature plants at 8 (a and b) and 16 weeks (c and d) after treatment, respectively. Values followed by the same letter within a column are not significantly different based on the DMR test at $p < 0.05$.

populations in roots and the soil. In this study, the nematode populations in roots and shoots of JP treated with AMF, MHB, and compost were reduced by 48.7-74.5% and 56.0-80.5%, respectively; in mature plants; however, the reductions ranged from 40.5 to 74.4% in roots and 44.0 to 75.4% in the soil.

The MHB of *Bacillus* sp. and *Pseudomonas* sp. are effective PGPRs that provide nutrients and phytohormones to plants to ensure their growth. Maintaining the optimal health status of coffee plants is essential for preventing severe disease. The reduction in *P. coffeae* infection by both bacterial species present in the MHB consortium might be related to the increase in AMF colonization of coffee roots since MHB can facilitate the induction of mycelial proliferation from AMF propagules (Asyiah et al., 2021). This study clearly showed an increase in mycorrhizal colonization of the roots of MBH-treated juvenile and mature coffee plants. The effect of compost on reducing the population of the endo-parasitic nematode *Pratylenchus* spp. in roots and the soil of patchouli has been reported by Subarjah et al. (2016), which is in line with the results indicating the role of the MHB liquid inoculant in reducing the *P. coffeae* population in the roots of coffee seedlings obtained by Hindersah et al. (2022a; 2022b).

Figure 6 shows that the combination treatments significantly increased the degree of AMF infection. An increase in AM infection in coffee to decrease the nematode attack was reported. Arbuscular mycorrhizal establishment in Vietnam is effective in reducing the population of *P. coffeae* in soil (Pham et al., 2020). The results of this field experiment agree with the increase of mycorrhizal infection degree and decrease of *P. coffeae* attack in Arabica coffee seedlings after the application of AMF *Glomus* spp. and MHB *B. subtilis* and *P. diminuta* (Hindersah et al., 2022b). AMF can directly synthesize or induce the production of phytohormones, including auxin, cytokinin, and gibberellin, during their association with host plants (Schmitz; Harrison, 2015). Phytohormones regulate various physiological processes and plant responses to biotic and abiotic stresses (Asami; Nakagawa, 2018).

The positive effect of organic matter on the increase in the colonization of roots of juvenile and mature coffee plants with mycorrhizae was confirmed. Organic matter serves as an energy and carbon source and is thus essential for the heterotrophic metabolism of both MHB and AMF. The role of organic matter in increasing soil pore spaces and particle density, as well as the contents of essential macronutrients such as N, P, and K, has been reported (Dzung et al., 2013). The improvement in soil fertility resulted in better root and shoot growth in the coffee plants.

5 CONCLUSIONS

In both juvenile plants (JP) and mature plants (MP) of Arabica coffee, the application of an arbuscular mycorrhizal fungi (AMF) inoculant containing *Glomus* sp., and mycorrhiza helper bacteria (MHB) consortium liquid inoculant consisting

of *B. subtilis* and *P. diminuta* sp., and compost alleviated leaf and root damage and reduced nematode populations in roots and the soil. The increase in the degree of root infection caused by AMF was demonstrated following the co-application of AMF, MHB, and compost. Mature plants (MP) were more susceptible to the endoparasitic nematode *P. coffeae* than were juvenile plants (JP). At the end of the experiment, the average leaf damage score of treated JP and MP was lower than that of the control plants. Moreover, the average reduction in leaf damage in the MP treatment group was significant compared to that in the control group. Overall, the leaf and root damage caused by JP and MP was significantly reduced by the AMF and MHB inoculants and compost. The nematode populations in the roots and in the soil of treated JP and MP were reduced, compared to those in untreated plants. Field experiments revealed that AMF, MHB, and compost were effective at reducing *P. coffeae* infection in Arabica coffee plants under field conditions.

The increase in infection rates of both JP and MP by AMF (*Glomus* sp.) was remarkable, and the infection degrees in the 16th week were greater than those in the 8th week. These findings are essential for the effective management of endoparasitic nematodes using biological agents and compost, which are mainly required for reducing the yield losses of Arabica coffee plants due to *P. coffeae* attack.

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7 AUTHOR CONTRIBUTION STATEMENT

Conceptual Idea: Asyiah, I.N.; Hindersah, R; Methodology design: Asyiah, I.N.; Data collection: Asyiah, I.N.; Data analysis and interpretation: Asyiah, I.N.; Hindersah, R., and Writing and editing: Hindersah, R.

8 REFERENCES

- ADAMCZYK-SZABELA, D.; WOLF, W. M. The impact of soil pH on heavy metals uptake and photosynthesis efficiency in *Melissa officinalis*, *Taraxacum officinalis*, *Ocimum basilicum*. **Molecules**, 27(15):4671, 2022.
- AMPONSAH-DOKU, B. et al. Improving soil health and closing the yield gap of cocoa production in Ghana-A review. **Scientific African**, 15:e01075, 2022
- ANTONIADIS, V.; KOUTROUBAS, S. D. Phosphorus availability in low-P and acidic soils as affected by liming and P addition. **Communications In Soil Science and Plant Analysis**, 46(46):1288-1298, 2015.

- ASSOCIATION OF OFFICIAL ANALYTICAL COLLABORATION - AOAC. **Official method of analysis**: Association of analytical chemists. 19th Edition, Washington DC, 2012.
- ASAMI, T.; NAKAGAWA, Y. Preface to the special issue: Brief review of plant hormones and their utilization in agriculture. **Journal of pesticide science**, 43(3):154-158, 2018.
- ASYIAH, I. N. et al. Population of *Pratylenchus coffeae* (Z.) and growth of arabica coffee seedlings inoculated by *Pseudomonas diminuta* L., and *Bacillus subtilis* (C.). **Pelita Perkebunan**, 31(1):30-40, 2015.
- ASYIAH, I. N. et al. Mycorrhizal fungi *Glomus* spp. propagation in zeolite enriched with mycorrhiza helper bacteria for controlling nematode in coffee. **IOP Conference Series Earth and Environmental Science**, 883:012021, 2021.
- BAUM, C.; EL-TOHAMY, W.; GRUDA, N. Increasing the productivity and product quality of vegetable crops using arbuscular mycorrhizal fungi: A review. **Scientia Horticulturae**, 187:131-141, 2015.
- BUDIMAN, A. et al. Morphological and molecular characteristics of *pratylenchus coffeae* from the origin of robusta coffee plantation in Malang, East Java. **Jurnal Perlindungan Tanaman Indonesia**, 23(2):211-218, 2019.
- CHALANSKA, A.; LABANOWSKI, G.; SAS, D. Root-lesion nematodes (*Pratylenchus* spp.) in ornamental plant nurseries - Influence of soil texture, acidity, salinity and organic matter content. **Communications in Biometry and Crop Science**, 11(2):98-104, 2016.
- COLE, E. et al. Impacts of manures and manure-based composts on root lesion nematodes and *Verticillium dahliae* in Michigan potatoes. **Phytopathology**, 110(6):1226-1234, 2020.
- DAVIS, E. L.; MacGUIDWIN, A. E. Lesion nematode disease. **The Plant Health Instructor**. 2000.
- DZUNG, N. A. et al. Evaluation of coffee husk compost for improving soil fertility and sustainable coffee production in rural central highland of Vietnam. **Resources and Environment**, 3(4):77-82, 2013.
- FENG, G. et al. Contribution of arbuscular mycorrhizal fungi to utilization of organic sources of phosphorus by red clover in a calcareous soil. **Applied Soil Ecology**, 22(2):139-148, 2003.
- GOPAL, S. et al. Trap culture technique for propagation of arbuscular mycorrhizal fungi using different host plants. **Korean Journal of Soil Science and Fertilizer**, 49(5):608-613, 2016.
- GOUGH, E. E. et al. 2020. A systematic review of the effects of arbuscular mycorrhizal fungi on root-lesion nematodes, *Pratylenchus* spp. **Frontier in Plant Science**, 11:923, 2020.
- HINDERSAH, R. et al. Role of mycorrhiza helper bacteria on mycorrhizal colonization and nematode *Pratylenchus coffeae* infection. **Biotropia**, 29(3):244-253, 2022a.
- HINDERSAH, R. et al. Reducing *Pratylenchus* population in coffee seedling with mycorrhizal fungi and mycorrhiza helper bacteria. **International Journal of Sustainable Agricultural Research**, 9(3):146-151, 2022b.
- HOU, L. et al. Arbuscular mycorrhizal enhancement of phosphorus uptake and yields of maize under high planting density in the black soil region of China. **Scientific Reports**, 11:1100, 2021.
- INTERNATIONAL COFFEE ORGANIZATION - ICO. Coffee Report and Outlook. **International Coffee Organization**. 2023. Available in: https://icocoffee.org/documents/cy2022-23/Coffee_Report_and_Outlook_April_2023_-_ICO.pdf. Access in: January, 20, 2024.
- JACKSON-ZIEMS, T. A. Root-lesion nematodes. In: HARVESON, R. M. et al. **Compendium of sunflower diseases and pest**. St. Paul, MN: APS Press. p. 71-73, 2016.
- JONES, J. T. et al. Top 10 plant-parasitic nematodes in molecular plant pathology. **Molecular Plant Pathology**, 14(9):946-961, 2013.
- KALAYU, G. Phosphate solubilizing microorganisms: Promising approach as biofertilizers. **International Journal of Agronomy**, 2019:4917256, 2019.
- LI, H-B. et al. Genetic diversity of nitrogen-fixing and plant growth promoting *pseudomonas* species isolated from sugarcane rhizosphere. **Frontier in Microbiology**, 8:1268, 2017.
- MACHADO, A. C. Z. et al. Nematode problems in coffee and their sustainable management. In: KHAN, M. R.; QUINTANILLA, M. **Nematode diseases of crops and their sustainable management**, Academic Press, p. 641-651, 2023.
- NASSLAHSEN, B. et al. Mycorrhizae helper bacteria for managing the mycorrhizal soil infectivity. **Frontier in Soil Science**, 2:979246, 2022.
- PHAM, T. T. et al. Combination of mycorrhizal symbiosis and root grafting effectively controls nematode in replanted coffee soil. **Plants (Basel)**, 9(5):555, 2020.

- RAHAYU, D. S.; SARI, N. P. Development of *Pratylenchus coffeae* in biochar applied soil, coffee roots and its effect on plant growth. **Pelita Perkebunan**, 33(1):24-32, 2017.
- SATO, T. et al. Secretion of acid phosphatase from extraradical hyphae of the arbuscular mycorrhizal fungus *Rhizophagus clarus* is regulated in response to phosphate availability. **Mycorrhiza**, 29(6):599-605, 2019.
- SCALZO, R. et al. Effects of different fungicide treatments on grape, must and wine quality. **Communication in Agriculture and Applied Biological Science**, 77(3):151-161, 2012.
- SCHOUTEDEN, N. et al. Arbuscular mycorrhizal fungi for the biocontrol of plant-parasitic nematodes: A review of the mechanisms involved. **Frontier in Microbiology**, 6:1280, 2015.
- SCHMITZ A. M.; HARRISON M. J. Signaling events during initiation of arbuscular mycorrhizal symbiosis. **Journal of Integrative Plant Biology**, 56:250-261, 2014.
- SNOECK, D. et al. Cacao nutrition and fertilization. In: LICHTFOUSE, E. **Sustainable agriculture reviews**. Cham: Springer, vol 19. p. 155-202, 2016.
- SUBARJAH, C.; HIMAWAN, T., PUSPITARINI; R. D. Effects of compost on nematode *Pratylenchus* sp. (Tylenchida: Pratylenchidae) population in Patchouli. **Journal of tropical life science**, 6(2):101-106, 2016.
- THIEP, N. V. et al. Study on nematodes (*Pratylenchus* spp.) on arabica coffee in the Northwestern Vietnam. **International Journal of Agricultural Technology**, 15(4):675-684, 2019.
- TRINH P. Q. et al. Plant-parasitic nematodes associated with coffee in Vietnam. **Russian Journal of Nematology**, 17(1):73-82, 2009.
- TRINH, P. Q. et al. Resistance screening of *Coffea* spp. accessions for *Pratylenchus coffeae* and *Radopholus arabocoffeae* in Vietnam. **Euphytica**, 185:233-241, 2012.
- VAN BEZOOIJEN, J. **Methods and techniques for nematology**. 2006. Available in: <http://www.nematologia.com.br/files/tematicos/5.pdf> Access in: August 23, 2024.
- VAN REEUWIJK, L. P. **Procedures for soil analysis**. 6th edition. International Soil Reference and Information Centre. FAO of UN: Wageningen The Netherlands, 2002. 119p.
- WIDIATMONO, B. R.; LUSIANA, N.; SRIWULANDARI, C. The effects of livestock's manure utilization as fertilizer on coffee plant's growth. **Advances in Food Science, Sustainable Agriculture and Agroindustrial Engineering**, 3(2):53-67, 2020.