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REDUCING PRATYLENCHUS POPULATION IN COFFEE SEEDLING WITH MYCORRHIZAL FUNGI AND MYCORRHIZA HELPER BACTERIA

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ABSTRACT

The attack of *Pratylenchus coffeae* nematode on coffee seedlings can limit coffee growth. The use of beneficial microbes as biofertilizer and bioprotectant is suggested to reduce the nematode attack on coffee seedlings and improve growth. A greenhouse experiment was conducted to observe the effect of arbuscular mycorrhizal fungi (AMF) *Glomus* spp. and mycorrhiza helper bacteria (MHB) *P. diminuta* and *B. subtilis* on coffee seedling growth and *P. coffeae* populations in the soil and roots. The experiment was set up in a randomized block design with five treatments and three replications. The coffee seedlings were grown in potting soil and inoculated with *Glomus* spp. combined with 20 g or 30 g of MHB solid inoculant. Seedlings were grown in the greenhouse for 10 weeks. The experiment verified that AMF inoculation combined with MHB did not affect the plant height, leaf number, or plant dry weight but decreased the *P. coffeae* population in the soil and roots of the seedlings. Inoculation of 20 g of MHB solid inoculant with AMF was more effective in reducing nematode population in soil and roots than 30 g of MHB. This experiment verified that *Glomus* spp., as well as *P. diminuta* and *B. subtilis* enable the control of *P. coffeae* in coffee seedlings.

Contribution/Originality: This experiment is including the newest way to reduce the population of *P. coffeae* in soil as well roots of Arabica coffee by inoculating mycorrhiza helper bacteria which also has a prominent role as bioprotectant and biofertilizer.

1. INTRODUCTION

Indonesia is the third largest coffee-producing country in the world after Brazil and Vietnam. Coffee plantations generate foreign exchange and income for farmers and coffee traders at the regional and national levels. The variety of coffee cultivated in Indonesia is generally Arabica due to its adaptability to altitudes of 1,000 m above sea level. Increasing coffee production requires fertilization management since most of the soils in coffee plantations naturally contain low nitrogen (N) and phosphorus (P). Another challenge for optimizing coffee cultivation in the tropics is reducing the attack of the endoparasitic nematode *Pratylenchus coffeae*, which damages roots and inhibits nutrient uptake by the roots. *P. coffeae* attack the cortical parenchyma, limiting the water absorption and nutrient uptake, and then cause serious root lesions (Yu et al., 2012).

The application of plant growth promotor soil microbes as biofertilizer and bioprotectant is suggested for sustainable agriculture and green economy. Nowadays, biofertilizer inoculation is intended to increase a plant's productivity and reduce the use of inorganic fertilizer. The use of arbuscular mycorrhizal fungi (AMF) in coffee plantation is reported to sustain coffee production due to nutrient acquisition by the fungi. Moreover, the AMF has a potential role as a biocontrol for plant parasitic nematodes and soil-borne diseases in agriculture (Patel, Jhala, Raghunandan, & Solankia, 2022). A well-known AMF species, *Glomus* spp, is a natural soil inhabitant which colonizes the roots of coffee trees to form arbuscular mycorrhiza (Prates et al., 2019).

Novel research demonstrates the effectivity of AMF to colonize roots is enhanced by mycorrhiza helper bacteria (MHB). Researchers found that MHB stimulates mycorrhizal infection in ectomycorrhiza (Gupta & Chakraborty, 2020) and arbuscular mycorrhiza (Deveau & Labbé, 2016). Some species of MHB are rhizosphere beneficial microbes belonging to the phosphate solubilizing bacteria (PSB) group, with a prominent role in the solution of inorganic P by secreting organic acid (Serrano, Mardad, & Soukri, 2013). The bacteria also promote plant growth by excreting phytohormones-like auxin, cytokinins, and gibberellins, producing antibiotic substances, and withstanding stress conditions (Rawat, Das, Shankhdhar, & Shankhdhar, 2021).

All life phases of *P. coffeae* nematodes, including male as well as female juveniles and adults, infect and damage the roots of coffee plants (Asyiah, Wiryadiputra, Fauzi, & Harni, 2015). The role of mycorrhizae in inhibiting nematode penetration and development has been well documented (Asyiah, Soekarto, & Husain, 2012; De La Peña, Echeverría, Van Der Putten, Freitas, & Moens, 2006; Serfoji, Ambo, Ethiopia, Rajeshkumar, & Selvaraj, 2010). Previous research has shown that the PSB consortium composed of *P. diminuta* (PD) and *B. subtilis* (BS) on a bagasse-based carrier has the populations of each bacteria up to 10^8 colony forming unit (CFU)/mL. On the other hand, inoculation of both bacteria decreases the population of *P. coffeae* by 64.2% and increases the growth of coffee seedlings by 34.2% - 35.4% (Asyiah. et al., 2015). In the current study, solid inoculants were applied with the *Glomus* spp. on Arabica coffee seedlings. The objective of this greenhouse experiment was to observe the effect of solid inoculants of AMF *Glomus* spp. combined with MHB on the growth of seedlings and the population of *P. coffee* in the soil and roots of coffee seedlings after nematodes infestation.

2. MATERIALS AND METHODS

The greenhouse experiment was carried out at the Coffee and Cocoa Research Center. Arabica coffee seeds were provided by the coffee plantation in Banyuwangi Regency. The AMF inoculants were produced on zeolite-based media using corn as the host plant. The density of Glomus spp in the inoculant was 100 spores/g. The PD and BS belong to the Laboratory of Soil Biology Universitas Padjadjaran and the Microbiology Laboratory of Universitas Jember, respectively. Both bacterial species were phosphate solubilizing bacterium.

2.1. Mycorrhiza Helper Bacteria Preparation

The liquid inoculant of MHB (mixed of PD and BS) was prepared by inoculating 1% of mixed pure culture bacteria (2:3; v:v) in 2% molasses-based broth. The culture then incubated for three days at room temperature on the reciprocal shaker at 115 rpm to obtain the final population of 10^8 CFU/mL. Solid inoculant of MHB was developed using bagasse-based carrier media. A total of 10% of the bacterial liquid culture described above was inoculated with the solid media and incubated for 14 days before use. The population of PD and BS in ready-use solid media was 10^8 CFU/g.

2.2. Nematode Extraction

A juvenile and mature stadia of *P. coffeae* were extracted from the roots of Arabica coffee plants that were naturally infected by nematodes in the plantation. A modified Baermann funnel method was used to extract the nematodes from infected coffee roots (Van Bezooijen, 2006). The male and female nematodes in juvenile and mature

stadia were collected unless the nematodes were dead and did not show active movement in water. All nematode stages were put in the water at 4°C for one day before being used in the experiment.

2.3. Experimental Design and Setup

The greenhouse experiment was conducted in a randomized block design with four treatments and one control. The treatments were a combination of AMF inoculation without and with 20 g or 30 g of MHB solid inoculants in coffee seedlings infected with *P. coffeae*. Control plants were seedlings without MHB and *P. coffeae* infestation. Each treatment was replicated three times, and each replication consisted of 10 plants.

In preparation for the seedlings, the coffee seeds were surface-sterilized with 70% ethanol and 0.1% sublimate ($HgCl_2$) and then soaked in sterilized water. The seeds then germinated on a seed tray containing sterilized sand for three months before transplanting. The three-month-old seedlings had two well-developed leaves. A total of 3.5 kg of sterilized soil was put in a polyethylene bag with five drainage holes underneath. The soil was Latosol with an acidity of 5.6, organic C of 2.39%, total N of 0.24%, C to N ratio of 9.95, available P of 14.65 mg/kg, and available K of 79.82 mg/kg. The mycorrhizal inoculant containing 100 spores of Glomus spp. and 20 g or 30 g of carrier-based MHB inoculant were mixed and put at the bottom of an 8-cm deep planting hole and covered with soil before the three-month-old seedlings were planted. A week later, 50 individual *P. coffeae* nematodes were spread on the circular band around the seedling stems and dressed with soil. All potted coffee seedlings were maintained in the greenhouse for 10 weeks.

2.4. Parameters and Statistical Analysis

Plant biomass parameters included plant height, leaf number, and shoots and roots dry weight were measured at the end of the experiment. Plants were separated from the soil; the number of nematodes in the soil and roots were counted using the Baermann funnel method (Van Bezooijen, 2006). The decrease of the nematode population in the soil and roots and in total was calculated by comparing the population with the control plants that received neither biological agent nor nematodes. All data obtained were analyzed by analysis of variance (F test) at $p \leq 0.05$, and if any experimental treatment showed a significant effect on the parameters, it then continued with Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$.

3. RESULTS AND DISCUSSION

Analysis of variance showed that the biological agent treatments did not affect the biomass of the coffee seedlings but influenced the number of nematodes in the soil and roots. At 10 weeks after biological agent inoculation, the plant height, leaf number, and dry weight of the coffee seedlings treated with *Glomus* sp. and MHB were not different from the control plants Table 1. The growth of seedlings infected with *P. coffeae* did not decrease, even though the plants were not inoculated with MHB.

Table 1. Effect of AMF Glomus spp. and MHB solid inoculants on the growth of coffee seedlings at 10 weeks after inoculation.

Treatments	Plant Height	Leaves Number	Dry Weight (g)	
			Shoots	Roots
Control (without AMF and MHB)	15.47 a	8.00 a	0.45 a	0.25 a
Without AMF and MHB + *P coffeae*	17.33 a	8.67 a	0.50 a	0.32 a
AMF + *P coffeae*	15.80 a	9.20 a	0.41 a	0.21 a
AMF + 20 g MHB + *P coffeae*	17.33 a	8.67 a	0.40 a	0.26 a
AMF + 30 g MHB + *P coffeae*	16.33 a	9.33 a	0.36 a	0.31 a

Note: Values followed by the different letter in a column are significantly different according to DMRT at $p \leq 0.05$.

Surprisingly, the dry weights of the seedlings without nematode infestation were similar to those of the nematode-infected plants. This result indicated that 10 weeks after inoculation, the plants were not yet responsive to nematode infection.

The results showed that bioagent treatments resulted in a significant effect on nematode numbers either in the roots or soil Table 2. Without a *P. coffeae* infestation, the roots and soil were free from nematodes. In infected pots without AMF and MHB, the nematode number was significantly higher in the roots as well as in the soil. A lower dose of MHB (20 g/pot) was more effective in reducing nematode numbers than a high dose of MHB. An application of 20 g/pot of MHB solid inoculant decreased the nematode count by 86.34 % and 74.29% in roots and soil compared to the control seedlings without AMF and MHB Table 3.

Table 2. Effect of AMF Glomus spp. and MHB solid inoculants on Nematode number in roots and soils of coffee seedling at 10 weeks after inoculation.

Treatments	Nematode number		
	Roots	Soil	Total
Control (without AMF and MHB)	0	0	0
Without AMF and MHB + *P coffeae*	71.40 c	77.80 c	153.60 c
AMF + *P coffeae*	34.33 b	40.67 b	75.00 b
AMF + 20 g MHB + *P coffeae*	9.75 a	20.00 ab	29.75 ab
AMF + 30 g MHB + *P coffeae*	36.67 b	36.67 b	73.33 b

Note: Values followed by the different letter in a column are significantly different according to DMRT at $p \leq 0.05$.

Table 3. Effect of AMF Glomus spp. and MHB solid inoculants on the decline of Nematode number in roots and soils of coffee seedling at 10 weeks after inoculation.

Treatments	Nematode population reduction (%)		
	Roots	Soil	In Total
Without AMF and MHB + *P coffeae*	-	-	-
AMF + *P coffeae*	51.92 a	47.73 a	51.12 a
AMF + 20 g MHB + *P coffeae*	86.34 b	74.29 b	80.63 b
AMF + 30 g MHB + *P coffeae*	48.64 a	52.87 a	52.25 a

Note: Values followed by the different letter in a column are significantly different according to DMRT at $p \leq 0.05$.

In the nursery, the cotyledon leaves emerge six weeks after sowing. During germination, roots actually begin to interact with beneficial microbes in the soil, but in this experiment, the germination media were sterilized and not inoculated with AMF or MHB. Therefore, at the germination stage, the roots were not infected by any beneficial microbes. In this experiment, AMF and MHB inoculation was carried out when the seedlings were transplanted to the potted soil. The absence of bioagent effect on the coffee seedlings' growth might be due to the delay in the mycorrhizal colonization and the rhizosphere-MHB interaction in promoting seedling growth. Nonetheless, the degree of root infection and the MHB population (in this case, a PSB) were not measured at the end of the greenhouse experiment.

The inoculation of bioagents at the early seed germination stage is highly recommended so that root–microbial interactions occur as early as possible. However, the results of this study were different from the positive effects of mycorrhizae on coffee seedlings reported by some researchers. Arabica coffee seedlings had taller shoots in soils amendment with organic matter and inoculated with several species of mycorrhizal fungi (Osorio, Alzate, & Ramirez, 2002). In Indonesia, the AMF inoculation increased the height of coffee seedlings up to 27.29% and shoot dry-weight up to 121.21% compared to the control (Suparno, Prabawardani, Yahya, & Taroreh, 2015). However, the absence of the effect of P solubilizing bacteria on coffee seedling development was demonstrated (Cisneros-Rojas, Sánchez-de Prager, & Menjivar-Flores, 2017).

Nematode *P. coffee* infestation with AMF and MHB inoculation decreased the nematode population in roots and soil. The mycorrhizae establishment was able to suppress the population of *P. coffeae* due to various factors, such as increased plant nutritional status, microbial changes in the rhizosphere, competition for nutrients and penetration

sites, as well as anatomical and biochemical changes in roots (Lindeman, 1994). The Mycorrhiza increased the synthesis of phytoalexin (phenolic compounds) and induced lignification in root endodermal cells (Elsen, Declerck, & De Waele, 2001). The phenolic compounds create a toxic environment for nematode proliferation; this involves plant defense mechanisms and causes plant resistance against nematode attack (Ohri & Pannu, 2010).

The MHB was reported to secrete certain extracellular enzymes to intensify the interactions of mycorrhizae, soil, plants, and pathogens in the soil. Chitinase produced by *Pseudomonas* and *B. Subtilis* (Senol, Nadaroglu, Dikbas, & Kotan, 2014; Zhong, Ding, & Guo, 2015) catalyzes the hydrolytic degradation of chitin, which is widely distributed in nature including in the nematode body wall. Both *Pseudomonas* and *Bacillus* have peroxidase activity (Abbasi, Ahmed, Zaki, Shuakat, & Khan, 2014; Nikoo, Sahebani, Aminian, Mokhtarnejad, & Ghaderi, 2014); the peroxidase has a significant role in preventing the infection process and inducing moderate plant defense mechanisms in response to nematodes (Harni, Supramana, Sinaga, Giyanto, & Supriadi, 2012; Nikoo et al., 2014).

4. CONCLUSION

The greenhouse experiment revealed that inoculation of AMF *Glomus* spp. combined with MHB *P. diminuta* and *B. subtilis* on coffee seedlings infested with *P. coffeae* nematodes did not affect the plant growth parameter, i.e., plant height, leaf number, and biomass. Nonetheless, both MHB clearly decreased the *P. coffeae* population in the soil and roots of coffee seedlings. Applying 20 g of MHB combined with AMF was more effective in reducing nematode populations than 30 g of MHB. This experiment found that AMF *Glomus* spp., as well as MHB *P. diminuta* and *B. subtilis*, have a prominent role in controlling the endoparasitic nematode *P. coffeae* in Arabica coffee seedlings.

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