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INFLUENCE OF BIOLOGICAL AGENTS IN CONTROLLING NEMATODES AND IMPROVING GROWTH OF COFFEE SEEDLING AND SOIL P-AVAILABILITY

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ABSTRACT

Pests and diseases cause significant yield losses in coffee cultivation in Indonesia. In particular, *Pratylenchus coffeae* is the most common nematode in Indonesia known to harm coffee plants. In dealing with the threat of *P. coffeae*, mycorrhiza is used as a biological agent to increase the growth of coffee plants as well as control the nematode. This experiment was conducted to determine the formulation of biological agents in the form of mycorrhizae enriched with mycorrhizal helper bacteria (MHB) to control nematodes and increase the growth of coffee seedlings and soil P-availability. The mycorrhizae used in this experiment were *Glomus* spp., while the liquid MHB formula used *Pseudomonas diminuta* and *Bacillus subtilis*. The randomized block design was used with treatments consisting of control (without biological agents); inoculation of *P. coffeae*; Glomus + *P coffeae*; Glomus + MHB 10^8+ *P coffeae*; Glomus + MHB 10^9+ *P coffeae*. Observations were made on the growth of coffee seedlings and the nematode population of *P. coffeae* 10 weeks after administered treatments. Results revealed that inoculation of biological agents *Glomus* spp. + the liquid MHB formula increased the growth of coffee seedlings, soil P-availability, and reduced the population of *P. coffeae*. The best treatment was *Glomus* spp. + MHB 10^8 which can reduce the population of *P. coffeae* by 65%.

Contribution/Originality: This research has been conducted to determine the formulation of biological agents in the form of mycorrhizae enriched with mycorrhizal helper bacteria (MHB) to control nematodes and increase the growth of coffee seedlings and soil P-availability.

1. INTRODUCTION

One commodity widely cultivated by farmers is coffee. Coffee plants require special attention in their cultivation, taking into account the problem and prevalence of pest and disease attacks. The main problem in smallholder coffee plantations is low productivity and quality, most often caused by pests and diseases [1].

Pratylenchus coffeae is the most common and harmful nematode of coffee in Indonesia [2] as they are found in almost all coffee-producing areas, even at altitudes between zero and more than 1,000 m above sea level. Decreased production by *P. coffeae* in robusta coffee ranged from 28.7% to 78.4%, while Arabica coffee, especially the type of coffee that is susceptible to damage, suffered even more damage and the plant is typically observed to only last for 2 years [3]. Controlling and minimizing the damages of *P. coffeae* is absolutely necessary and must direct the coffee

agribusiness forward towards a national green economy in order to meet the demands of the international market in the instances of food security, environmental preservation, and improving the welfare of farmers. One way to control plant pest organisms in line with the green economy concept is through biological control. Some soil bacteria and fungi are natural enemies of nematodes, so much so that they are categorized as parasite and nematode predators [4], and as such, the application of these natural enemies can reduce nematode populations in the field. According to Vallejos-Torres, et al. [5], the application of arbuscular mycorrhizal fungi (AMF) reduces damages caused by nematodes in coffee plants and there are further publications regarding the inhibition of penetration and nematode development due to mycorrhizal inoculation [6-10]. Mycorrhizal symbiosis is considered an interaction between plants and fungi, but this definition must also include supporting organisms known to exert mutual influence on each other, resulting in the so-called "mycorrhizosphere" [11]. The mycorrhizosphere is composed of mycorrhizae, external mycelium, and supporting organisms [12], and its effect has been shown to increase plant nutrition, growth, and disease resistance [13].Bacteria that are able to increase the development of mycorrhizae are named Mycorrhiza Helper Bacteria (MHB) [14], and AMF and its supporting organisms (bacteria) have shown potential to be applied as such a biofertilizer. Several researchers have found that bacteria isolated from mycorrhizal fungi can stimulate mycorrhizal infection, spore production, and also resistance to plant pathogens [15].

The use of PGPR-based biofertilizers, especially phosphate solubilizing microbes (PSM), can replace the use of inorganic fertilizers [16]. In particular, PSM can also be used to control plant parasitic nematodes [17] as well as make plants more resistant to pathogen attacks Campos-Soriano, et al. [18]. Vázquez, et al. [19] showed that there was a significant link between inoculation of microorganisms (bacteria and fungi) with PSM and mycorrhizae on mycorrhizal colonization. Based on the research results that have been reported, the three biological agents have the potential to synergize with the benefiting effect of controlling P. coffeae by more than 80% while increasing plant growth and increasing soil P-availability. However, further studies on the effect of mycorrhizal and MHB in reducing *P. coffeae* in coffee plants and soil P-availability still need to be investigated. This experiment was conducted to determine the formulation of biological agents using mycorrhizae enriched with mycorrhizal helper bacteria (MHB) to control nematode population and increase the growth of coffee seedlings and soil P-availability.

2. MATERIALS AND METHOD

The bacteria used as MHB were *Pseudomonas diminuta*, belonging to the Department of Biology Faculty of Teacher and Education Training, University of Jember, and *Bacillus subtilis*, belonging to the Laboratory of Soil Biology Faculty of Agriculture Universitas Padjadjaran. The mycorrhiza used was *Glomus aggregatum* (belonging to the Agriculture Faculty of Gajah Mada University). The coffee seeds used were arabica coffee from the Banyuwangi Region, East Java coffee plantations. The *P. coffeae* used as research material was obtained from the extraction of the roots of coffee plants that had been attacked by *P. coffeae*. Extraction of *P. coffeae* was carried out using a modified Baermann method [20].Pure cultures of *B. subtilis* and *P. diminuta* were grown on nutrient agar media. After being incubated at a temperature of 30 ± 2 C for 24 hours, 3 full loops were taken and suspended in 10 ml of sterile water and shaken using a vortex to homogenize to form a suspension with a density of 10^{12} cfu/ml. 1 ml of the isolate suspension was poured into 500 ml of Nutrient Broth (NB) media in an Erlenmeyer flask with a capacity of 750 ml then put into a water bath at 30 C while shaking for 24 hours. Bacterial cells were then harvested and suspended in sterile distilled water. 10% of the bacterial suspension solution comparison of *P. diminuta* and *B. subtilis* 2:3 was then inoculated into molasses liquid medium 2%.

Planting media for testing biological agents was produced in the form of soil and sand with a ratio of 1:1. Soil analysis showed that the growing media used in this study contained a moderate total of C-org (2,39%) and N (0,24%), high available P (14,65 ppm) and K (79,82 ppm). The treatments tested included control (without biological agents); *P coffeae*; *Glomus* + *P coffeae*; *Glomus* + MHB 10^{8}+ *P coffeae* and *Glomus* + MHB 10^{9}+ *P coffeae*. Growth of coffee, number of nematodes, and soil P-availability were observed and studied 10 weeks after treatment.

3. RESULT AND DISCUSSION

Table 1 shows that inoculation of AMF with enriched MHB increased plant height. Glomus + MHB 10^8 + *P coffeae* had the highest plant height (16,90 cm), although this was not significantly different from the treatment with 10^9 MHB (14,96 cm). In this experiment, the application of AMF with MHB enriched was not able to increase significantly the number of leaves, shoot dry weight, and root dry weight. However, *Glomus* and MHB inoculation have the potential to increase the number of leaves and shoot dry weight.

Table 1. Effect of Glomus and MHB on the growth of coffee seedlings at 10 weeks after treatment.

Treatments	Plant Height (cm)	Number of leaves	Shoot dry weight (g)	Root dry weight (g)
Without biological agents	10.85b	8.00a	0.36a	0.23b
P coffeae	7.65a	8.28a	0.32a	0.12a
Glomus + *P coffeae*	12.86b	9.00a	0.44a	0.12a
Glomus + 10^8 MHB + *P coffeae*	16.90c	9.60a	0.45a	0.20b
Glomus +109 MHB + *P coffeae*	14.96bc	9.45a	0.44a	0.17a

Note: The mean number followed by the same letter is not significantly different based on Duncan's test at 5%.

Table 2 showed that the application of biological agents was able to reduce the population of *P. coffeae*, both in roots, soil, and the total population. The lowest population of *P. coffeae* was in the Glomus + MHB 10^8+ *P. coffeae.*

Table 2. Effect of *Glomus* spp. and MHB on the nematode population at 10 weeks after treatment.

Treatments	Number of root nematodes	Number of soil nematodes	Number of total nematodes	Root damage score (%)
without biological agents	0a	0a	0a	66a
P coffeae	212.00c	325.67c	537.67c	70ab
Glomus + *P coffeae*	81.60b	115.80b	197.40b	60a
Glomus + 108 MHB + *P coffeae*	79.00b	111.60b	190.60b	73b
Glomus +109 MHB + P coffeae	84.00b	158.75b	242.75b	74b

Note: the mean number followed by the same letter is not significantly different based on Duncan's test at 5%.

Table 3 details the percentage decrease in the population of *P. coffeae* caused by the inoculation of biological agents. In it, we see that Glomus + MHB 10^8 + *P coffeae* had the highest percentage of total *P. coffeae* nematode population decline at 64.55%. When compared with the treatment of giving MHB that has not been formulated, the percentage of this population decline decreased by 20-25%. The formulation process that has been carried out has not been able to maintain the ability of *P. diminuta* and *B. subtilis* in inhibiting the development of the nematode *P. coffeae.*

Table 3. Effect of *Glomus* spp. and MHB on the percentage nematode population decline at 10 weeks after treatment.

Treatments	Root nematode population decline (%)	Soil nematode population decline (%)	Total nematode population decline (%)
Without biological agent + *P coffeae*	-	-	-
Glomus + *P coffeae*	61.51a	64.44b	63.28b
Glomus + MHB 10^8+ *P coffeae*	62.76a	65.73b	64.55b
Glomus + MHB 10^9+ *P coffeae*	60.38a	51.25a	54.85a

Note: The mean number followed by the same letter is not significantly different based on Duncan's test at 5%.

Figure 1. Roots of coffee seedlings at 10 weeks after treatment (A: without biological agents; B: without biological agents + *P coffeae*; C: Glomus + *P coffeae*; D: Glomus + MHB 10^8+ *P coffeae*; E: Glomus + MHB 10^9+ *P coffeae*).

Table 4 shows that mycorrhiza and MHB increased the soil P-availability. The application of Glomus + MHB 10^8+ *P coffeae* was able to increase the available P content in the soil by up to 29%. According to Patel, et al. [21] increased nutrient absorption occurs due to the thick hyphal sheath. Increased root metabolism is due to increased oxygen consumption and phosphatase enzymes. Mycorrhizae can secrete a phosphatase enzyme that can decompose nutrients from an unavailable state to be available for absorption by plants, particularly in the instance of phosphates in low concentrations in the soil solution [22]. Mohammadi, et al. further state that mycorrhizae in the presence of a thick hyphal sheath can increase the surface area of the root system, thereby increasing the absorption area [23]. According to Huey, et al. [24] the presence of fungal hyphae, which can easily penetrate the soil, provides an advantage in nutrient uptake by providing a wider cruising space due to having a smaller diameter, thereby providing a wider field of nutrient absorption.

Table 4. Effect of *Glomus* spp. and MHB on soil P at 10 weeks after treatment.

Treatments	P-available (ppm)	Increasing of P (%)	P-Total (me/100 g)	Increasing of P- total (%)
Without biological agents	10.74	-	16.78	-
P coffeae	12.37	13.22	20.08	16.46
Glomus + *P coffeae*	13.72	21.74	20.16	16.67
Glomus + 10^8 MHB + *P coffeae*	15.12	29.01	20.87	19.61
Glomus +10^9 MHB + *P coffeae*	14.74	27.14	19.71	14.84

Note: The mean number followed by the same letter is not significantly different based on Duncan's test at 5%.

The inoculation of MHB helps to increase the effectiveness of mycorrhizal infection against plant roots through several mechanisms, one of which is the bacteria initiating the formation of IAA to form short roots to allow increased interaction possibilities. Furthermore, the bacteria are able to produce enzymes that are able to soften cell walls so that the arbuscular endomycorrhizae can interact better with the roots as indicated by the increasing degree of mycorrhizal infection. An increase in the degree of mycorrhizal infection will increase the phosphatase enzyme produced so that the soil P-availability will also increase.

4. CONCLUSIONS

In this study, we conclude that mycorrhiza and mycorrhiza helper bacteria (*P. diminuta* and *B. sublitis*) increased the growth of coffee seedlings, soil P-availability, and reduced the population of *P. coffeae.* The inoculation of *Glomus* spp. + MHB 10^8 reduced the population of *P. coffeae* by 65% and increased the soil P-availability by up to 29% and the total P in the soil by 19%. Mycorrhizae enriched by MHB have the potential to be developed as biological fertilizers and biocontrol.

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