



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

 Betty Natalie Fitriatin<sup>1+</sup>

 Reginawanti Hindersah<sup>2</sup>

 Iis Nur Asyiah<sup>3</sup>

 Dwi Suci Rahayu<sup>4</sup>

<sup>1,2</sup>Department of Soil Sciences and Land Resources Management, Agriculture Faculty, Padjadjaran University, Jatinangor, West Java, Indonesia.

<sup>1</sup>Email: [betty.natalie@unpad.ac.id](mailto:betty.natalie@unpad.ac.id) Tel: +628122387122

<sup>2</sup>Email: [reginarwanti@unpad.ac.id](mailto:reginarwanti@unpad.ac.id) Tel: +62811221834

<sup>3</sup>Biology Education Program, Faculty of Teacher and Education Training, University of Jember, Jember Indonesia.

<sup>3</sup>Email: [iisnaza.fkip@unej.ac.id](mailto:iisnaza.fkip@unej.ac.id) Tel: +628123458446

<sup>4</sup>Indonesian Coffee and Cocoa Research Institute, Jember, Indonesia.

<sup>4</sup>Email: [amariawidi@gmail.com](mailto:amariawidi@gmail.com) Tel: +628133760476



(+ Corresponding author)

### ABSTRACT

#### Article History

Received: 18 May 2022

Revised: 13 July 2022

Accepted: 27 July 2022

Published: 15 August 2022

#### Keywords

Liquid

Formula

Mycorrhiza

MHB

Population

P-solubilizing.

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<sup>8</sup>+ *P. coffeae*; *Glomus* + MHB 10<sup>9</sup>+ *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<sup>8</sup> 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  $30 \pm 2$  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
<i>P. coffeae</i>	7.65a	8.28a	0.32a	0.12a
<i>Glomus</i> + <i>P. coffeae</i>	12.86b	9.00a	0.44a	0.12a
<i>Glomus</i> + $10^8$ MHB + <i>P. coffeae</i>	16.90c	9.60a	0.45a	0.20b
<i>Glomus</i> + $10^9$ MHB + <i>P. coffeae</i>	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
<i>P. coffeae</i>	212.00c	325.67c	537.67c	70ab
<i>Glomus</i> + <i>P. coffeae</i>	81.60b	115.80b	197.40b	60a
<i>Glomus</i> + $10^8$ MHB + <i>P. coffeae</i>	79.00b	111.60b	190.60b	73b
<i>Glomus</i> + $10^9$ MHB + <i>P. coffeae</i>	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 + <i>P. coffeae</i>	-	-	-
<i>Glomus</i> + <i>P. coffeae</i>	61.51a	64.44b	63.28b
<i>Glomus</i> + MHB $10^8$ + <i>P. coffeae</i>	62.76a	65.73b	64.55b
<i>Glomus</i> + MHB $10^9$ + <i>P. coffeae</i>	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	-
<i>P. coffeae</i>	12.37	13.22	20.08	16.46
<i>Glomus</i> + <i>P. coffeae</i>	13.72	21.74	20.16	16.67
<i>Glomus</i> + $10^8$ MHB + <i>P. coffeae</i>	15.12	29.01	20.87	19.61
<i>Glomus</i> + $10^9$ MHB + <i>P. coffeae</i>	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. subtilis*) 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.

**Funding:** This research is supported by Ministry of Agriculture of Indonesia (Grant number: 54.34/HM.240/I.1/3/2016.K).

**Competing Interests:** The authors declare that they have no competing interests.

**Authors' Contributions:** All authors contributed equally to the conception and design of the study.

## REFERENCES

- [1] A. Anhar, U. A. Rasyid, A. M. Muslih, A. Baihaqi, and Y. Abubakar, "Sustainable Arabica coffee development strategies in Aceh, Indonesia," presented at the In IOP Conference Series: Earth and Environmental Science. IOP Publishing, 2021.
- [2] Mutala'liah, I. S., and Putra, "Abundance and diversity of plant parasitic nematodes associated with BP 308 and BP 42 clones of robusta coffee in Java, Indonesia," *BIODIVERSITAS*, vol. 19, pp. 67-70, 2018.
- [3] S. Wiryadiputra and Priyono, "Studies on the use of banana trees (*Musa* spp.) for coffee and cocoa shading: V. Development of *Pratylenchus coffeae* on some banana cultivars derived from tissue culture," *Plantation Lamp*, vol. 11, pp. 132–139, 1995.
- [4] Z. Khan and Y. H. Kim, "A review on the role of predatory soil nematodes in the biological control of plant parasitic nematodes," *Applied Soil Ecology*, vol. 35, pp. 370-379, 2007. Available at: <https://doi.org/10.1016/j.apsoil.2006.07.007>.
- [5] G. Vallejos-Torres, E. Espinoza, J. Marín-Díaz, R. Solis, and L. A. Arévalo, "The role of arbuscular mycorrhizal fungi against root-knot nematode infections in coffee plants," *Journal of Soil Science and Plant Nutrition*, vol. 21, pp. 364-373, 2021. Available at: <https://doi.org/10.1007/s42729-020-00366-z>.
- [6] E. De La Peña, S. R. Echeverría, W. H. Van Der Putten, H. Freitas, and M. Moens, "Mechanism of control of root-feeding nematodes by mycorrhizal fungi in the dune grass *Ammophila arenaria*," *New Phytologist*, vol. 169, pp. 829-840, 2006. Available at: <https://doi.org/10.1111/j.1469-8137.2005.01602.x>.
- [7] P. Serfoji, S. Rajeshkumar, and T. Selvaraj, "Management of root-knot nematode, *Meloidogyne incognita* on tomato cv Pusa Ruby. by using vermicompost, AM fungus, *Glomus aggregatum* and mycorrhiza helper bacterium, *Bacillus coagulans*," *Journal of Agricultural Technology*, vol. 6, pp. 37-45, 2010.
- [8] M. G. Van Der Heijden, F. M. Martin, M. A. Selosse, and I. R. Sanders, "Mycorrhizal ecology and evolution: The past, the present, and the future," *New Phytologist*, vol. 205, pp. 1406-1423, 2015. Available at: <https://doi.org/10.1111/nph.13288>.
- [9] S. Alamri, N. A. Nafady, A. M. El-Sagheer, M. A. El-Aal, Y. S. Mostafa, M. Hashem, and E. A. Hassan, "Current utility of arbuscular mycorrhizal fungi and hydroxyapatite nanoparticles in suppression of Tomato Root-Knot Nematode," *Agronomy*, vol. 12, pp. 1-16, 2022. Available at: <https://doi.org/10.3390/agronomy12030671>.
- [10] C. Vos, K. Geerinck, R. Mkandawire, B. Panis, D. De Waele, and A. Elsen, "Arbuscular mycorrhizal fungi affect both penetration and further life stage development of root-knot nematodes in tomato," *Mycorrhiza*, vol. 22, pp. 157-163, 2012.
- [11] W. Ellouze, C. Hamel, S. Bouzid, and M. St-Arnaud, *Mycorrhizal Fungi: Soil, agriculture and environmental implications. Chapter: 6*: Nova Science Publishers. Editors: Fulton Susanne M., 2011.
- [12] D. Burke and S. Carrino-Kyker, "The influence of mycorrhizal fungi on rhizosphere bacterial communities in forests in Forest Microbiology," *Tree Microbiome: Phyllosphere, Endosphere and Rhizosphere. Forest Microbiology*, vol. 1, pp. 257-275, 2021.
- [13] J. Poveda, P. Abril-Urias, and C. Escobar, "Biological control of plant-parasitic nematodes by filamentous fungi inducers of resistance: *Trichoderma*, mycorrhizal and endophytic fungi," *Frontiers in Microbiology*, vol. 11, p. 992, 2020. Available at: <https://doi.org/10.3389/fmicb.2020.00992>.
- [14] S. K. Gupta and A. P. Chakraborty, "Mycorrhiza helper bacteria: Future prospects," *International Journal of Research and Review*, vol. 7, pp. 387-391, 2020.
- [15] D. P. Bharadwaj, P.-O. Lundquist, and S. Alström, "Arbuscular mycorrhizal fungal spore-associated bacteria affect mycorrhizal colonization, plant growth and potato pathogens," *Soil Biology and Biochemistry*, vol. 40, pp. 2494-2501, 2008. Available at: <https://doi.org/10.1016/j.soilbio.2008.06.012>.

- [16] A. Pradhan, A. Pahari, S. Mohapatra, and B. B. Mishra, "Phosphate-solubilizing microorganisms in sustainable agriculture: Genetic mechanism and application. Chapter 5 in Advances in Soil Microbiology: Recent Trends and Future Prospects, Microorganisms for Sustainability 4. T. K. Adhya et al. (eds.)," ed: Springer Nature Singapore Pte Ltd, 2017, pp. 81-99.
- [17] E. Gamalero and B. R. Glick, "The use of plant growth-promoting bacteria to prevent nematode damage to plants," *Biology*, vol. 9, p. 381, 2020. Available at: <https://doi.org/10.3390/biology9110381>.
- [18] L. Campos-Soriano, M. Bundó, M. Bach-Pages, S.-F. Chiang, T.-J. Chiou, and B. San Segundo, "Phosphate excess increases susceptibility to pathogen infection in rice," *Molecular Plant Pathology*, vol. 21, pp. 555-570, 2020. Available at: <https://doi.org/10.1111/mpp.12916>.
- [19] M. Vázquez, S. César, and R. Azcon, "Interactions between arbuscular mycorrhizal fungi and other microbial inoculants (Azospirillum, Pseudomonas, Trichoderma) and their effects on microbial population and enzyme activities in the rhizosphere of maize plants," *Applied Soil Ecology*, vol. 15, pp. 261-272, 2000.
- [20] D. J. Hooper, J. Hallmann, and S. A. Subbotin, "Methods for extraction, processing and detection of plant and soil nematodes," *Plant Parasitic Nematodes in Subtropical and Tropical Agriculture*, vol. 2, pp. 53-86, 2005.
- [21] H. Patel, Y. Jhala, B. Raghunandan, and J. Solanki, *Role of mycorrhizae in plant-parasitic nematodes management. In Trends of Applied Microbiology for Sustainable Economy: A volume in Developments in Applied Microbiology and Biotechnology. Editor: Ravindra Soni et al: Elsevier Inc, 2022.*
- [22] H. Etesami and B. R. Jeong, "Contribution of arbuscular mycorrhizal fungi, phosphate-solubilizing bacteria, and silicon to P uptake by plant: a review," *Frontiers in Plant Science*, vol. 12, p. 1355, 2021. Available at: <https://doi.org/10.3389/fpls.2021.699618>.
- [23] K. Mohammadi, S. Kholesro, Y. Sohrabi, and G. Heidari, "A review: Beneficial effects of the mycorrhizal fungi for plant growth," *Journal of Applied Environmental and Biological Sciences*, vol. 1, pp. 310-319, 2011.
- [24] C. J. Huey, S. C. Gopinath, M. Uda, H. I. Zulhaimi, M. N. Jaafar, F. H. Kasim, and A. R. W. Yaakub, "Mycorrhiza: A natural resource assists plant growth under varied soil conditions," *3 Biotech*, vol. 10, pp. 1-9, 2020. Available at: <https://doi.org/10.1007/s13205-020-02188-3>.

*Views and opinions expressed in this article are the views and opinions of the author(s), International Journal of Sustainable Agricultural Research shall not be responsible or answerable for any loss, damage or liability etc. caused in relation to/arising out of the use of the content.*