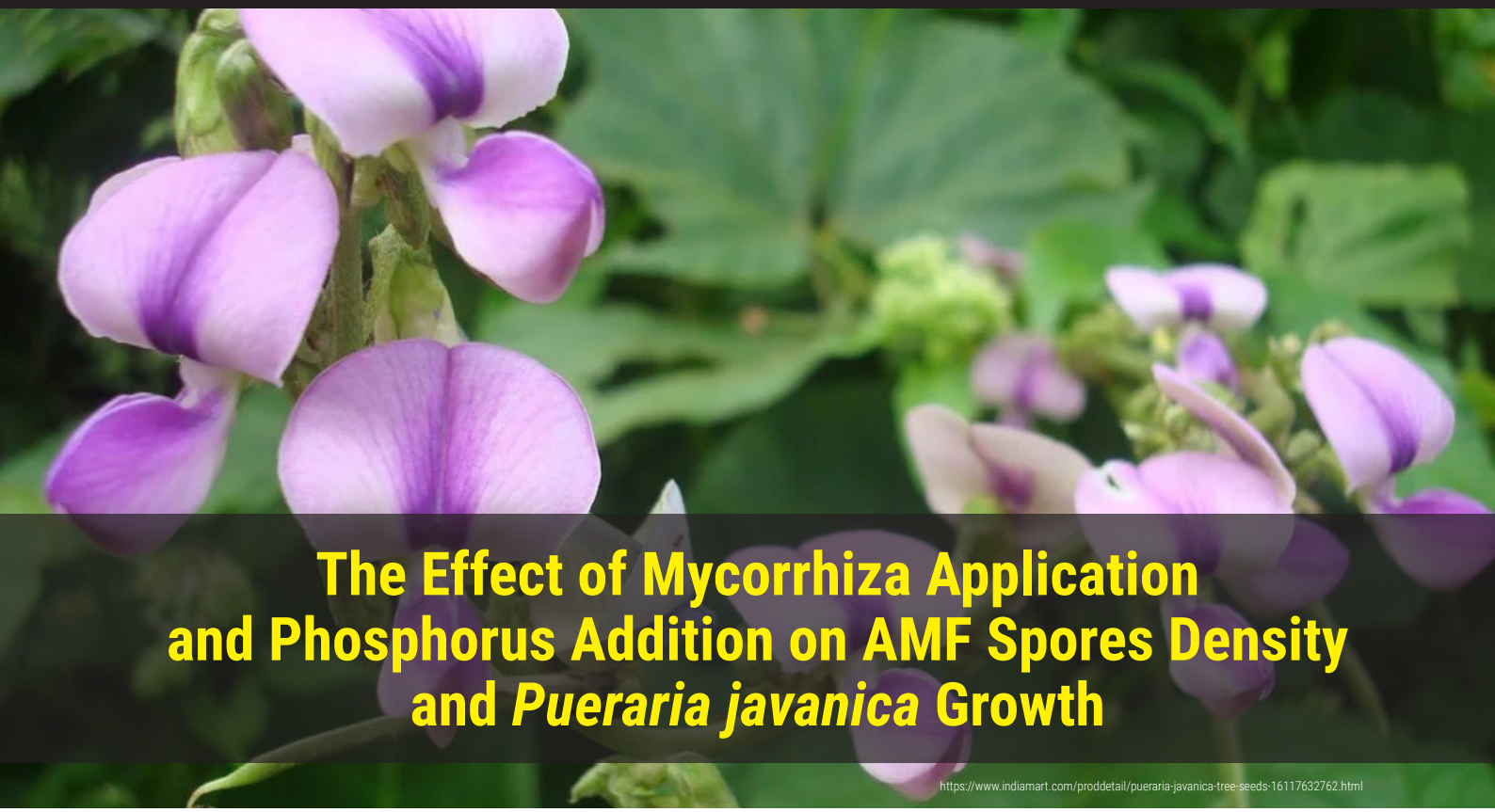
BIODIVERS
BIOTROP Science Magazine

p-ISSN: 2810-0271
DOI: 10.56060/bdv.2024.3.2.2168



The Effect of Mycorrhiza Application and Phosphorus Addition on AMF Spores Density and *Pueraria javanica* Growth

https://www.indiamart.com/proddetail/pueraria-javanica-tree-seeds-16117632762.html

Risa Rosita[1]*, Jennifer Claudia Suryadi[2], Deden Dewantara Eris[3]

[1]Science Innovation Technology Department, SEAMEO BIOTROP, Indonesia

[2]Faculty of Science and Technology, Universitas Pelita Harapan, Indonesia

[3]Plant Protection Research Group, Indonesian Oil Palm Research Institute (IOPRI), Indonesia

***Corresponding author:**
risa@biotrop.org

ABSTRACT

One of the suitable host plants for AMF (Arbuscular Mycorrhizal Fungi) is *Pueraria javanica*. Several factors that can affect the growth of *P. javanica* are the availability of nutrients and the activity of potential soil microbes, such as AMF. Applying potential soil microbes (AMF) to plants can increase plant growth, chlorophyll levels, and enzyme activity, and even improve soil quality. The ability of AMF to increase plant nutrient absorption causes plants with AMF tend to have optimum growth. The study aimed to understand the effect of AMF application and phosphorus (P) nutrients addition on AMF spore density and *P. javanica* growth. The experiment used a completely randomized design with one factor (formulation). Results of the study proved that phosphorus (P) addition was able to reduce AMF spore densities (*Glomus etunicatum* and *Glomus mosseae*) 3 weeks after application (WAA). The availability of P in a fairly high amount around the root area caused plants to reduce their dependence on AMF, which resulted in a decrease in AMF colonization and AMF spore densities. On the other hand, adding P nutrients proved to increase growth parameters (plant height and number of leaves) of *P. javanica* because AMF helped the absorption of P and received carbon from plants in return. Treatment P6 (*G. etunicatum* 10 g + without phosphorus) had the highest spore density value (400.33/10 g planting media), and treatment P9 (*G. mosseae* 10 g + phosphorus 10 ppm) showed the highest increase in plant height of 13.333 ($P < 0.05$) 3 weeks after application. Meanwhile, the maximum increase in the number of leaves occurred in plants that received 10 g of *G. mosseae* and 10 ppm of P every two days for three weeks. Studying AMF spore density can significantly improve plant growth, agronomic efficiency, and agricultural sustainability.

Keywords: AMF, colonization, *Glomus etunicatum*, *Glomus mosseae,* nutrient absorption


https://www.eworldtrade.com/pd/ptduajayagrupindonesia/pueraria-javanica-legume-cover/1015300/

INTRODUCTION

Arbuscular Mycorrhizal Fungi (AMF) plays an important role in increasing plants' resistance to various stresses, such as flooding, salinity, temperature, heavy metals, and diseases, due to AMF ability in increasing water and minerals absorption that are important in responding to stresses and in increasing antioxidant activity, thereby preventing apoptosis (Begum *et al.,* 2019). In addition, research by Klinsukon *et al.,* (2021) found that the addition of AMF to plants can increase plant growth, chlorophyll levels, enzyme activity, and even improve soil quality (Rosita, 2021). The ability of AMF to increase nutrient absorption by plants causes plants with AMF tend to have optimum growth. The formation of arbuscular mycorrhiza occurs when fungal spores attach to the roots of the host plant and form a flat structure called the hypopodium. The presence of the hypopodium encourages the formation of hyphae that can penetrate the outer root cell tissue. The hyphae then form branches and penetrate the inner cortex cells, where the hyphae will form the arbuscular structure. The arbuscule is where the exchange of nutrients between fungi and plants occurs. The arbuscular and cytoplasm of plant cells are separated by the apoplast and perifungal membranes that facilitate the exchange of nutrients (Genre *et al.,* 2020). In addition to arbuscules, AMF also has bubble-shaped structures called vesicles that function to store nutrients. AMF reproduction occurs due to the ability of AMF to produce spores that are resistant to extreme environments (Holland & Roth, 2023).

AMF can produce spores that reproduce when in suboptimal environmental conditions. Spores can enter a dormancy stage where metabolism is drastically reduced until environmental conditions improve and germination can occur. The resistance of spores in suboptimal environments causes spores to be produced in more significant quantities in stressed environments, for example, when soil moisture is low. In addition, other factors affect spore production by AMF, such as pH and nutrients contained in the soil. Previous research by Susanti *et al.,* (2021) found that the addition of NPK (Nitrogen-Phosphorus-Potassium) at a concentration of 10 ppm can increase spore density in corn host plants. In addition to NPK, another nutrient that affects AMF is phosphorus (P). Previous research found that the higher the phosphorus content in the soil, the more limited the diversity and production of spores by AMF (Arias *et al.,* 2012). Spores produced by various AMF species have different morphologies and structures. AMF spores can sporulate or germinate when environmental conditions improve so that they can colonize the roots of host plants.

Several factors that influence this process are temperature, pH, humidity of the growing medium, nutrients in the medium, type of host plant, and even microorganisms in the environment. The sporulation time of each species varies depending on the AMF species and its environment, but generally, it takes several days to several months for the spores to germinate (Giovannetti *et al.,* 2010). One important step in identifying AMF spores is to extract and isolate AMF spores (Deveautour *et al.,* 2020). Glomus can be found in various habitats, even in polluted environments, so they show resistance to various abiotic factors (Rodrigues & Rodrigues, 2020). Some Glomus species often found are *G. etunicatum* and *G. mosseae*. Both species can increase nutrient uptake by plants so that they can be used as bio-fertilizers (Vani *et al.,* 2018). In addition, these Glomus species play a role in increasing plant resistance to salinity and drought stress (Begum *et al.,* 2019). The characteristics of *G. etunicatum* are that it has one or two layers of spore walls and is brownish-yellow, while *G. mosseae* has three layers of walls and is brownish-yellow in color (Lee *et al.,* 2006).

AMF spores in an optimal environment can germinate and colonize the host plant. One of the suitable host plants for AMF is *P. javanica*. *P. javanica* is a legume plant that can be found in Indonesia. The characteristics of this plant are that it is tolerant to various light intensities and is able to

act as a soil protector from rain. One of the properties that makes *P. javanica* often used in research is its rapid growth, which is around two months (Nursanti & Supriyanto, 2022). In addition, *P. javanica* plants have been found to be host plants for AMF colonization. *P. javanica* plants are suitable hosts for AMF because their roots are fine and strong, and their lignin content is relatively low, making the AMF colonization process easier (Lapanjang *et al.,* 2023). Factors that can affect the growth of *P. javanica* plants are the availability of water, the presence of nutrients, light intensity, and temperature. In addition, other factors that affect plant growth are internal factors of the plant, for example, hormones and genetics. One of the elements needed by plants is P, which plays an important role in plant growth and plant response to abiotic stress (Li *et al.,* 2015). Phosphorus (P) is needed in the synthesis of genetic material, the process of producing energy, and root formation. In addition, the addition of phosphorus to plants reduces damage due to abiotic stress, for example, drought and salinity (Bechtaoui *et al.,* 2021). This study aimed to study the effect of mycorrhizal application and the addition of P concentration 10 ppm on the number of spore density and growth response of *P. javanica* plants.

MATERIALS AND METHOD

Tools and Materials

The tools used in the study were sieving tools, analytical balances, scales, trays, pots, Petri dishes, pipettes, spoons, measuring cups, Erlenmeyer flasks, rulers, slides, object glasses, spore tweezers, hand counters, and stereo microscopes. The materials used in the study were zeolite media, 1000 ppm phosphate standard solution, NPK fertilizer, distilled water, Polyvinyl Lactoglycerol (PVLG) solution, aluminium foil, and filter paper. The samples in this study were *G. mosseae*, *G. etunicatum*, and *P. javanica* plants. The research procedure for the effect of adding phosphorus nutrients on the density and abundance of arbuscular mycorrhizal fungi spores was divided into four stages including *P. javanica* germination, AMF inoculation, AMF extraction and identification, and data analysis. The samples studied were two types of AMF that had been isolated and would be inoculated into *P. javanica* as the host plant. The study was conducted with a factorial randomized design and the data obtained were recorded and statistically analyzed and presented in the form of graphs and tables.

P. javanica Nursery and Seedlings

P. javanica seeds were soaked in warm water for 24 hours to facilitate the germination process. Afterwards, the seeds planted in zeolite media placed in a tray for twenty days and then transferred to sterile zeolite media as much as 300 g in a pot and left for a week (Sowmen *et al.,* 2018). During the nursery, the plants were watered with 20 ppm NPK solution every two days.

AMF Application and Phosphorus (P) Addition

The previously isolated AMF were each inoculated into the rhizosphere of the planting medium. There were ten treatments carried out, i.e., P1 (negative control) = without AMF inoculation + without phosphorus; P2 (positive control) = without AMF inoculation + 10 ppm phosphorus; P3 = *Glomus etunicatum* 5 g + without phosphorus; P4 = *Glomus mosseae* 5 g + without phosphorus; P5 = *Glomus etunicatum* 5 g + phosphorus 10 ppm; P6 = *Glomus mosseae* 5 g + phosphorus 10 ppm; P7 = *Glomus etunicatum* 10 g + without phosphorus; P8 = *Glomus mosseae* 10 g + without phosphorus; P9 = *Glomus etunicatum* 10 g + phosphorus 10 ppm; P10 = *Glomus mosseae* 10 g + phosphorus 10 ppm. Four replications were carried out for each treatment with a completely randomized design of 1 factor (formulation) so that the total treatments carried out were 40 treatments. The seedlings were maintained for three weeks and watered regularly with water or 10 ppm phosphorus fertilizer every two days based on the treatment (Nursanti & Supriyanto, 2022). Plant height, number of leaves, and leaf color were also measured and recorded before and after treatment.

Extraction and Identification of AMF

After three weeks, 10 g of zeolite media from the rhizosphere layer of each treatment were taken. The zeolite media was dissolved in water and poured into a spore filter. The sediment obtained on the 212 µm, 106 µm, and 63 µm sieves was taken and poured through Whatman filter paper. The filter paper was observed under a stereo microscope and the number and morphology of spores were counted and recorded. AMF spores were taken and mixed with a Polyvinyl Lactoglycerol (PVLG) solution on a glass slide. Spores were observed using a binocular microscope and identified based on morphology (Susanti *et al.,* 2023) and their abundance was calculated.

Data Analysis

The experiment of measuring parameters spore density and plant growth (plant height and number of leaves) was carried out for 9 weeks of planting (MST). Data were analyzed using SAS 9.0. If the results of the ANOVA test showed significance, further testing was carried out with the DMRT (Duncan Multiple Range Test) at an alpha (α) level of 5%.

RESULTS AND DISCUSSION

Analysis of variance showed that a single factor (formulation) had a significant effect on the parameters of AMF spore density and the increase in the height of *P. javanica* plants (Table 1). The density of AMF spores inoculated into plants after three weeks was calculated using the wet sieving technique.

The results of the analysis of variance showed that there was an interaction between the factors having a significant effect on AMF spore density ($P < 0.01$), increased plant height and number of leaves ($P < 0.05$). Furthermore, to determine the differences between the levels of interaction, a DMRT test was carried out. The results of the DMRT test are presented in Table 2.

AMF spore density decreased in samples treated with 10 ppm phosphorus (P) for three weeks (Table 2). This indicated that AMF samples without P had a significantly higher spore density compared to AMF which routinely received phosphorus. The data also showed that *G. etunicatum* had the potential to produce large numbers of spores in conditions without the addition of phosphorus (400.33 per 10 g of planting media). The results of this study are supported by previous research by Arial *et al.,* (2012) which showed that spore density tends to decrease with increasing nutrients in the planting media, because when nutrients are abundant, AMF tends to germinate and colonize roots. AMF colonization supports nutrient absorption by plants and encourage plant growth. Meanwhile, in plants that were not given P, more AMF spores were found. The reason is that in an environment with minimal nutrients, AMF produce more spores because AMF spores have higher stress resistance (Hopkins & Bennett, 2023). In addition, spores are a means of reproduction for AMF so that when environmental conditions improve, spores can germinate and colonize. However, the effect of phosphorus on spore density is also influenced by the dose of P where at certain concentrations P can encourage the production of large amounts of spores in certain host plants. Conversely, too high a concentration of P can inhibit spore germination so that further research on the effect of P doses on Glomus in *P. javanica* plants can be carried out (Giovannetti *et al.,* 2010; Dejana *et al.,* 2022)

Based on Table 2, compared to the control (without AMF), the administration of *G. etunicatum* and *G. mosseae* can increase the ability of plant nutrient absorption. The average increase in plant height for plants received AMF and phosphorus also tends to

Table 1 Results of analysis of variance on AMF spore density and *P. javanica* growth

No.	Parameter	Formulation
1	Increase in plant height	*
2	Increase in number of leaves	*
3	Spore density/10 g of planting media	**

Table 2 DMRT test results of the effect of formulation on AMF spore density and growth parameters of *Pueraria javanica*

Treatment	Description	AMF spore density/10 g planting media	Plant height (cm)	Number of leaves
P0	Without AMF inoculation + without phosphorus	6.67 ^{g}	7.468 ^{b}	3 ^{bc}
P1	Without AMF inoculation + 10 ppm phosphorus	6.67 ^{g}	7.633 ^{b}	6 ^{ab}
P2	*G. etunicatum* 5 g + without phosphorus	258.67 ^{b}	5.268 ^{bc}	4 ^{bc}
P3	*G. mosseae* 5 g + without phosphorus	108.33 ^{de}	1.768 ^{c}	2 ^{c}
P4	*G. etunicatum* 5 g + 10 ppm phosphorus	185.67 ^{c}	8.768 ^{ab}	5 ^{ab}
P5	*G. mosseae* 5 g + 10 ppm phosphorus	65.67 ^{f}	9.400 ^{ab}	7 ^{a}
P6	*G. etunicatum* 10 g + without phosphorus	400.33 ^{a}	8.033 ^{b}	4 ^{bc}
P7	*G. mosseae* 10 g + without phosphorus	163.00 ^{c}	9.300 ^{ab}	6 ^{ab}
P8	*G. etunicatum* 10 g + 10 ppm phosphorus	131.33 ^{d}	6.233 ^{bc}	6 ^{ab}
P9	*G. mosseae* 10 g + 10 ppm phosphorus	81.33 ^{ef}	13.333 ^{a}	10 ^{a}

be higher than the control with P (Table 2). Factors affecting plant height are the nutrients available in the planting medium. AMF addition can also increase nutrient absorption by plants. Therefore, the addition of nutrients and AMF is very important for increasing plant height (Higo *et al.,* 2020), because AMF can help increase the provision of nutrients for plants by colonizing the roots. When associated with plant roots, AMF increases plant mineral nutrient absorption (P) (Rosita *et al.,* 2020). The concentration and absorption of macro and micronutrients are higher in plants inoculated with AMF compared to plants without AMF inoculation (Rosita, 2021). Meanwhile, based on Table 2, the maximum increase in plant growth (number of leaves) compared to the two controls were the plant given AMF spores and 10 ppm P (Table 2). The maximum increase in the number of leaves occurred in plants given 10 g of *G. mosseae* and given 10 ppm P every two days for three weeks. These data support the spore density data in Table 2 because plants that tend to have a lower spore density, namely plants given 10 ppm P, experienced more maximum growth than plants given AMF and not given P. This is because P obtained can be absorbed by plants more optimally with the help of AMF which colonizes the plant roots.

AMF is able to produce several organic acid compounds and even phosphatase enzymes that help the absorption of phosphate, especially inorganic phosphate, by plants (Shi *et al.,* 2023). AMF form a symbiotic relationship with plants, where AMF helps the absorption of nutrients P and receives carbon from plants in return. If plants receive sufficient nutrients from the environment, their need for mycorrhizae decreases, which can decrease mycorrhizal activity and spore production.

CONCLUSION

The addition of nutrients and P can reduce the density of mycorrhizal spores (*G. etunicatum* and *G. mosseae*) after 3 weeks after application (WAA). The decrease in AMF colonization and spore densities occur when nutrients, especially phosphorus, is sufficiently available around the root area. The addition of phosphorus nutrients can increase growth parameters (plant height). AMF helps in nutrients P absorption and receive carbon from plants in return. Treatment P6 (*G. etunicatum* 10 g + without phosphorus) had the highest spore density value (400.33/10 g of planting media) and treatment P9 (*G. mosseae* 10 g + phosphorus 10 ppm) resulted in the highest plant height increase of 13.333 ($P < 0.05$) 3 weeks after application. The maximum increase in the number of leaves occurred in plants received 10 g of *G. mosseae* and 10 ppm of P every two days for three weeks. Studying the density of mycorrhizal spores provides significant benefits in improving plant growth, agronomic efficiency, and agricultural sustainability.

REFERENCES

Arias, R. M., Heredia-Abarca, G., Sosa, V. J., & Fuentes-Ramírez, L. E. (2012). Diversity and abundance of arbuscular mycorrhizal fungi spores under different coffee production systems and in a tropical montane cloud forest patch in Veracruz, Mexico. Agroforestry Systems, 85, 179-193.

Bechtaoui, N., Rabiu, M. K., Raklami, A., Oufdou, K., Hafidi, M., & Jemo, M. (2021). Phosphate-dependent regulation of growth and stresses management in plants. Frontiers in Plant Science, 12, 679916.

Begum, N., Qin, C., Ahanger, M. A., Raza, S., Khan, M. I., Ashraf, M., Ahmed, N., & Zhang, L. (2019). Role of arbuscular mycorrhizal fungi in plant growth regulation: Implications in abiotic stress tolerance. Frontiers in Plant Science, 10, 1068.

Dejana, L., Ramírez-Serrano, B., Rivero, J., Gamir, J., López-Ráez, J. A., & Pozo, M. J. (2022). Phosphorus availability drives mycorrhiza induced resistance in tomato. Frontiers in Plant Science, 13.

Deveautour, C. A., Chieppa, J., Nielsen, U. N., Boer, M. M., Mitchell, C. A., Horn, S., & Powell, J. R. (2020). Biogeography of arbuscular mycorrhizal fungal spore traits along an aridity gradient, and responses to experimental rainfall manipulation. Fungal Ecology, 46, 100899.

Genre, A., Lanfranco, L., Perotto, S., & Bonfante, P. (2020). Unique and common traits in mycorrhizal symbioses. Nature Reviews Microbiology , 18, 649-660.

Giovannetti, M., Avio, L., & Sbrana, C. (2010). Spore germination and pre-symbiotic mycelial growth. In H. Koltai & Y. Kapulnik (Editors), Arbuscular mycorrhizas: Physiology and function. Dordrecht (NL): Springer. p. 47-68.

Higo, M., Azuma, M., Kamiyoshihara, Y., Kanda, A., Tatewaki, Y., & Isobe, K. (2020). Impact of phosphorus fertilization on tomato growth and arbuscular mycorrhizal fungal communities. Microorganisms, 8, 178.

Holland, S., & Roth, R. (2023). Extracellular vesicles in the arbuscular mycorrhizal symbiosis: Current understanding and future Perspectives. Molecular Plant-microbe Interactions, 36, 235–244.

Hopkins, J. R., & Bennett, A. E. (2023). Spore traits mediate disturbance effects on arbuscular mycorrhizal fungal community composition and mutualisms. Ecology, 104, e4016.

Klinsukon, C., Lumyong, S., Kuyper, T. W., & Boonlue, S. (2021). Colonization by arbuscular mycorrhizal fungi improves salinity tolerance of eucalyptus (*Eucalyptus camaldulensis*) seedlings. Scientific Reports, 11, 4362.

Lapanjang, I., Zakaria, E., Edy, N., & Barus, H. N. (2023). Effectiveness of multiple culture of arbuscular mycorrhizal fungi (AMF) from the rhizosphere of cocoa on host plant *Pueraria javanica* . IOP Conference Series: Earth and Environmental Science, 1253, 1-6.

Lee, J., Park, S. H., & Eom, A. H. (2006). Molecular identification of arbuscular mycorrhizal fungal spores collected in Korea. Mycobiology, 34, 7–13.

Li, X., Li, Y., Zhang, Z., & Li, X. (2015). Influences of environmental factors on leaf morphology of Chinese jujubes. PloS one , 10: e0127825.

Nursanti, I., & Supriyanto, R. (2022). Pertumbuhan legume cover crops (*Puararia javanica*) pada tanah pasca penambangan batubara plus zeolit [Growth of legume cover crops (*Pueraria javanica*) on post coal-mining soil plus zeolite]. Jurnal Media Pertanian, 7, 7-10.

Rodrigues, K. M., & Rodrigues, B. F. (2020). Glomus. Beneficial Microbes in Agro-Ecology, 561–569.

Rosita, R., Widiastuti, R., Mansur, I., & Faulina, S. A. (2020). Potential use of *Claroideoglomus etunicatum* to enrich signal grass (*Brachiaria decumbens* Stapf.) for silvopasture preparation. Menara Perkebunan, 88(1).

Rosita, R. (2021). Pertumbuhan dan kemampuan fitoremediasi *Brachiaria decumbens* Stapf. yang diperkaya *Claroideoglomus etunicatum* dan *Bacillus* sp. pada tanah bekas tambang batu bara [Growth and phytoremediation ability of *Brachiaria decumbens* Stapf. enriched with ***Claroideoglomus etunicatum*** and *Bacillus* sp. On post coal-mining soil]. [Master Thesis]. Bogor (ID): IPB University.

Susanti, R., Suryanti, E., & Rosita, R. (2023). Efektivitas pemberian berbagai konsentrasi pupuk NPK dalam kultur trapping fungi mikoriza arbuskula terhadap pertumbuhan tanaman jagung (*Zea mays* L.) [The effectiveness of applying various concentrations of NPK fertilizer in trapping cultures of arbuscular mycorrhizal fungi on the growth of corn plants (*Zea mays* L.)]. Prosiding Semnas Biologi XI Tahun 2023 FMIPA Universitas Negeri Semarang, 1, 51-57.

Vani, M. S., Hindumathi, A., & Reddy, B. N. (2018). Beneficial effect of arbuscular mycorrhizal fungus, ***Glomus fasciculatum*** on plant growth and nutrient uptake in tomato. Indian Phytopathology, 71, 115–122.