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# Effect of Vermicompost in Propagation of Mycorrhizal (*Glomus* sp) Post-Coal Mining Land with Cereal Host Plant

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**ABSTRACT.** The composition of host plant species and nutrient sources affects the success of mycorrhizal (*Glomus* sp) propagation in post-coal mining land. Plants that can be used as alternative hosts are cereals such as corn, millet, and sorghum. Vermicompost is an alternative source of nutrients to supply the nutrient needs of post-coal mining soil media. This study aimed to analyze the addition of vermicompost, the effect of different host plants, and the combination of both in the propagation of *Glomus* sp. The study was conducted in a greenhouse using a factorial completely randomized design with the first factor being the host plant and the second factor being the dose of vermicompost. The results showed that the addition of vermicompost increased the number of spores and the percentage of mycorrhizae colonization on the roots of each host plant with optimum yield. The combination dose of vermicompost (2 g pot-1) and millet as a host plant produced the highest number of spores of 4,880 spores pot-1 with root colonization reaching to 63.3%

***Keywords :*** *Propagation, Cereals, Glomus* sp*, Coal, Vermicompost*

## INTRODUCTION

The management of ±65 ha of post-coal mine land in Margomulyo Village, Samboja Subdistrict, Kutai Kertanegara Regency, East Kalimantan is carried out by stockpiling *overburden* material, making the land degraded and limited in utilization. Some vegetation grows wild such as *Rhodomyrtus tomentosa*, *Paspalun conjugatum*, and *Cromolaena odorata*. This fact is a presumption that these plants can grow with arbuscular mycorrhizal fungi (FMA). Prayoga and Prasetya's (2021) research in the same post-coal mine land found *Glomus* as many as 100-196 spores 100 g soil$^{-1}$ with the percentage of colonies in the three plant roots of 51-83%.

Although the presence of FMA is found in all ecosystems, it does not mean that it has no disadvantages. The existence of FMA tends to be limited, damaged, and lost along with environmental changes. *Glomus* sp has the potential to be used to help accelerate revegetation of post-coal mine land due to its adaptability to extreme environments (Margarettha, 2011). FMA from post-mining land will be more tolerant and optimal when reapplied to the original ecosystem. Conditions that contradict the needs of mycorrhizal growth make it necessary to optimize the revegetation of post-coal mine land.

The propagation of FMA spores is the key to success in revegetating post-coal mine land because the number of FMA spores that can be applied is increasing. FMA spores can be propagated through pot culture. Determination of host plants in FMA propagation must be considered because FMA is obligatively symbiotic. Without a host plant, the hyphae produced by FMA are very few and can only survive for about 1 month (Toruan-Mathius *et al.*, 2016)

Cereals including corn, millet and sorghum can be used as alternative hosts. These plants have a lot of roots and fast growth with a high level of adaptation to stress environments, making them suitable for use as hosts in FMA propagation. Research by Tahat *et al*. (2008) proved that corn and sorghum can produce the number of *G mosseae* spores as much as 111-167 spores 10 $g^{-1}$ of soil. Another study by Silva *et al.* (2005) millet plants were able to associate well with *Glomus etunicatum*, *Gigaspora margarita*, *Scutellospora heterogama* and the highest number of spores was 153 spores $g^{-1}$ soil (*G. etunicatim*) compared to the host sorghum.

Planting media is also an important factor in providing nutrients for host plants and FMA development in the propagation media. Vermicompost is an alternative nutrient source produced from the metabolic process of worms containing various macro and micro elements. Research by Cavender *et al.* (2003) showed that the addition of vermicompost can increase the availability of N, P, K and stimulate FMA colonization on the roots. FMA plays a role in converting organic P elements in vermicompost into a form available to plants. Research Nusantara *et al.* (2010 ) vermicompost can increase the number of *G. etunicatum* spores and FMA colonization on the roots compared to the use of red hyponex

This study aims to analyze the addition of vermicompost, the effect of corn, millet and sorghum as host plants, and the right combination between them in the propagation of FMA (*Glomus* sp) in soil originating from post-coal mine land. These results are expected to help accelerate the revegetation of post-coal mine land, especially in Margomulyo Village, Samboja District, Kutai Kartanegara Regency, East Kalimantan.

## MATERIALS AND METHODS

The research was conducted in the Greenhouse of Agricultural Development Polytechnic of Malang using a Factorial Complete Randomized Design. The first factor is the host plants including corn, millet, sorghum and the second factor is the dose of vermicompost including V0 (0 g $pot^{-1}$) (control), V1 (1.7 g $pot^{(-1)}$)), V2 (2 g $pot^{(-1)}$)), V3 (2.3 g $pot(^{-1})$)). Consisting of 12 treatment combinations and repeated 3 times. Parameters observed at 7 weeks after FMA inoculation (MSI) included the number of spores 100 $g^{-1}$ of soil, the percentage of FMA colonization on roots, pH, total P, available P, plant canopy P uptake, and plant canopy dry weight and plant height 1-7 MSI (Week After Inoculation).

### *Soil Sampling*

The soil samples used were taken from post-coal mine land in Margomulyo Village, Samboja District, Kutai Kartanegara, East Kalimantan. Soil samples for FMA isolation were taken compositely around the roots of *Rhodomyrtus tomentosa*, *Paspalun conjugatum*, and *Cromolaena odorata* plants at a depth of 0-20 cm. Soil samples for planting media were taken as much as ± 100 kg in 4 different plots (20 × 20 meters).

### *Isolation and Identification of FMA from Post-Coal Mine Soil*

Isolation of FMA spores was carried out using the *wet sieving* method (Nusantara *et al*., 2012). The identification of FMA spores (*Glomus* sp.) was conducted using the *Manual for the Identification of Mychorhiza Fungi* (Brundrett *et al*., 1996).

### *Preparation of Planting Media*

Air-dried post-coal mine soil was sieved (≤ 2 mm) and homogenized while vermicompost was sieved (≤ 250 µm). Sterilization was done using a *baglog steamer* (90 °C

± 2 consecutive days). Soil was weighed as much as 2 kg pot$^{-1}$ while vermicompost was weighed according to the treatment dose. Treatment pots were arranged in a greenhouse (60 m × 12.5 m).

### *Host Plant Preparation*

Host plant seeds were surface sterilized using 5% chlorox *(bayclin)* solution for 5-10 minutes and washed thoroughly under running water. Seeds were germinated on sterile cocopeat media until 2 leaves appeared. Plants with uniform growth were selected and ready to be inoculated with FMA

### *Host Planting and FMA Inoculation*

The host plant was planted on a tissue bag in a ±2.5 cm deep planting hole and *Glomus* spore inoculation in distilled water was poured at the root of the plant as much as 20 spores pot$^{-1}$.

### *Culture Maintenance and Plant Height Observation*

Culture maintenance was carried out by watering using distilled water according to field capacity (morning and evening). Fertilization was not done and only vermicompost was used as treatment. Plant height observation was done manually using a meter every week (1-7 MSI).

### *Harvest*

Plant crowns (stems and leaves) were cut and oven dried (80°C± 48 hours) for dry weight. A portion of the root was taken with a certain weight, cleaned and temporarily stored in FAA for observation of FMA colonization. Harvesting of FMA spores was carried out by disassembling, pulverizing and homogenizing the planting media for spore isolation.

### *Soil Biology Observations*

Observation of the number of spores was carried out using the *wet sieving* method and counted using a *handcounter*. FMA colonization on the roots using the root staining method and calculated using the formula *Muryati et al*. (2016).

$$\text{Colonization (\%)} = \frac{\Sigma bidang\ pandang\ terkoloni}{\Sigma bidang\ pandang\ total}$$

### *Soil Chemistry Observations*

The pH was analyzed using $H_2O$ solution, available P using Bray-1 method, total P using 25% HCl extractor and plant P content using $HNO_3$ and $HClO_4$ wet saponification methods.

### *Data Analysis*

Data analysis used *Genstat software and Microsoft Excel 2016*. The research data were subjected to *Analysis of Variance* (ANOVA) at F (5%) to determine the effect of each treatment. If significantly different, followed by *Duncan Multiple's Range Test* (DMRT) (5%) to determine differences between treatments. To determine the relationship and magnitude of influence between variables, correlation and regression tests were conducted.

## RESULTS AND DISCUSSION

### *Count*

The dose of vermicompost and the type of host plant had a significant effect ($P<0.05$) on the number of *Glomus* . The application of vermicompost increased the number of spores by 20.64-34.58% compared to the control (Figure 1). The slow release of vermicompost nutrients stimulates FMA to increase its activity in transforming organic P into inorganic P. Research by Cavender *et al*. (2003) explained that the addition of vermicompost to peat and mineral media stimulates FMA colonization on sorghum roots along with the increasing availability of nutrients N, P, and K. The increase and distribution of FMA colonies in plant roots provides an opportunity to increase the number of FMA spores produced (Prasetya and Ukhtansyah, 2020) .

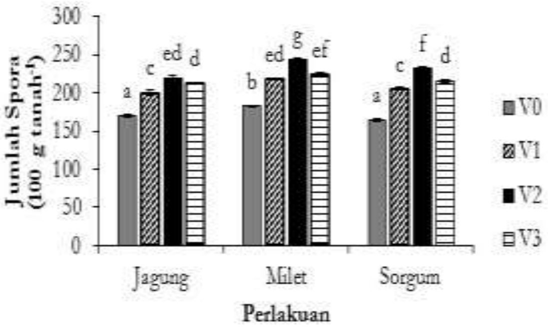


Figure 1. Effect of treatment on the number of spores

The development of FMA spores is also influenced by the type of plant used as FMA host. In general, the three host plants were able to increase the number of spores in the propagation medium, but the millet plant produced a spore count of 217 spores 100 $g^{-1}$ of soil (4,340 spores $pot^{-1}$) higher than the sorghum and corn plants (Figure 1). Research by Silva *et al.* (2005) reported that MA (*G. etunicatum*) spore production using millet as a host plant was between 19-153 spores $g^{-1}$ of soil, 93% more than using sorghum as a host plant. It is suspected that different types of host plants produce different amounts of root exudates that affect the proliferation of spores and the growth of FMA hyphae, such as the swelling and branching of hyphae (Wulandari *et al* .., 2014)

### *MA Colonization of Roots*

The dose of vermicompost and the type of host plant had a significant effect ($P<0.05$) on the colonization of *Glomus* sp on the roots. Application of vermicompost increased the percentage of colonization on the roots by 21.07-41.22% compared to the control (Figure 2). Vermicompost can provide nutrient needs, especially P for root development and growth. Massive root development has the potential to increase the capacity of FMA colonization on the roots. Supported by research *Hameeda et al.* (2007) that the application of vermicompost can increase the average root volume up to 102.32% and followed by an increase in the percentage of FMA colonization on the roots by 266.66%.

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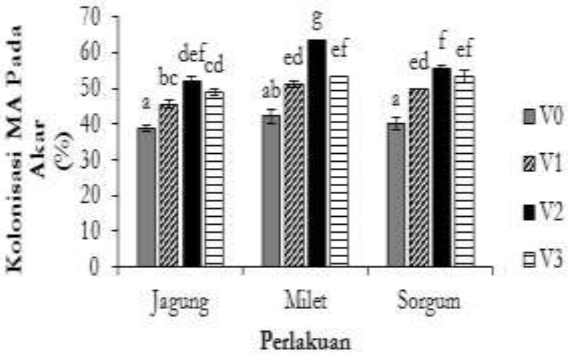


Figure 2. Effect of treatment on MA colonization at aka r

Host plant suitability can also spur FMA growth and development through the formation of FMA structures in the roots. Corn, millet, and sorghum plants are fibrous-rooted with a wide rooting range and fast plant growth. These criteria are able to support the production of FMA spores effectively both from the number of spores and colonization on high roots (Auli and Kasiamdari, 2019) . Millet plants showed compatibility with *Glomus* sp which produced a colonization percentage of 52.48%, higher than the hosts of corn and sorghum. Supported by research Channabasava and Lakshman (2013) reported that the average percentage of MA colonization on millet roots grown in ex-mining soil reached 76%. This shows that differences in host plants can affect the percentage of FMA colonization in the roots. In line with research (Nurhayati, 2019) using different inoculum sources and host plant types, showed different reactions to FMA infection or colonization, where both factors had a significant effect on the observed variables.

***pH ($H_2O$)***

The dose of vermicompost and the type of host plant had a significant effect ($P<0.05$) on soil pH ($H_2O$) at 7 MSI. The addition of vermicompost resulted in a higher average pH than the control. $^+$ Vermicompost has a neutral pH so that when applied to acidic soil it can increase pH. The increase in pH is due to the dominance of H ions which can be replaced by OH ions $^-$ produced by organic acids. Organic acids are produced by vermicompost as long as the decomposition process continues. Reduced sources of active soil acidity can lead to improvements in the media environment, namely increasing pH towards the positive direction ( Aprizal *et al.* . , 2021)

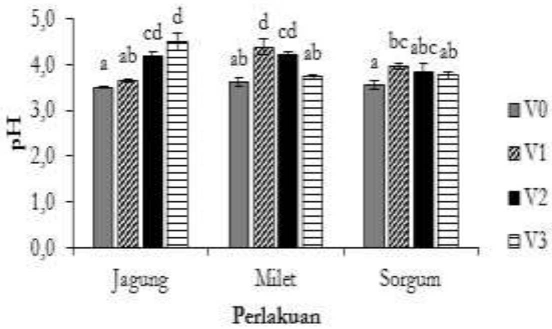


Figure 3. Effect of treatment on pH

The insignificant increase in pH and still in the very acidic category in this study was due to the very low level of post-coal mine soil acidity (Figure 3). Syawal *et al*. (2017) stated that the insignificant increase in pH was due to the level of acidity of the soil type. The involvement of FMA in the decomposition of vermicompost is thought to affect pH at the source of soil acidity indirectly. The activity and metabolism of mycorrhizal roots produce and release organic compounds that play a role in binding metal cations that cause soil acidity so that the pH increases (Nurmasyitah *et al* .., 2013)

### *P Total*

The results of variance analysis showed that the dose of vermicompost had a significant effect on total P. Total P during the vegetative peak of plants increased along with the addition of vermicompost doses in the propagation media when compared to the control. The average amount of total P increased by 0.17; 0.27; and 0.32 mg $kg^{-1}$, respectively. The application of vermicompost can increase the amount of total P in the propagation medium due to the high total P content vermicompost of 18,100 mg $kg^{-1}$. Research Cavender *et al*. (2003) showed that the addition of vermicompost in peat media by 5% was able to increase the amount of P in peat media by 174.5 mg $kg^{-1}$ which was originally only 14.5 mg $kg^{-1}$. In contrast, the symbiosis of MA in the roots of host plants cannot increase total P in the soil and plays more of a role in the process of nutrient translocation to the roots (Cozzolino *et al* .., 2013)

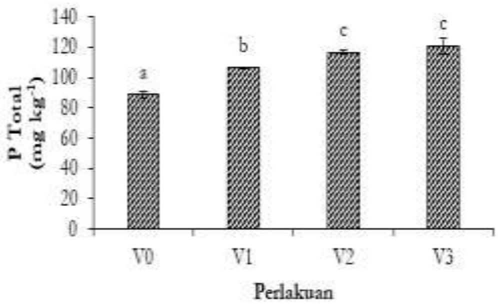


Figure 4. Effect of treatment on total P

### *P Available*

The application of vermicompost with the dose and type of host plant had a significant effect ($P<0.05$) on available P. Increasing the dose of vermicompost can increase the available P in the soil (Figure 5). Vermicompost not only functions as an inorganic fertilizer but also as a soil amendment produced from the metabolic process of earthworms, making it rich in available nutrients and organic matter. The increase in P availability can occur because the organic acid content in vermicompost such as humic and fulvic acids is able to release the bonds of metal ions so that it has an impact on reducing the concentration of active Al and P nutrients are more available to plants ( Khairuna *et al* . ., 2015)

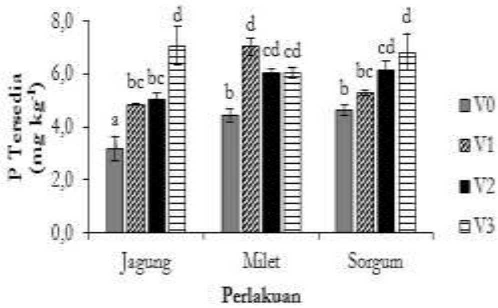


Figure 5. Effect of treatment on available P

FMA spores produced during FMA propagation play an important role in converting organic P into inorganic form. The activity of FMA in mineralizing organic P in vermicompost and post-coal mine soil can increase the availability of P in the propagation media. Research Bi *et al*. (2018) showed that the availability of P in the soil increased in FMA-inoculated soil compared to those not inoculated by FMA. FMA can encourage the increased production and activity of acid phosphatase in the soil and convert organic P into inorganic (available) P. Phosphatase enzyme is not only produced by FMA but also by plant roots. Root exudates affect microbial activity in the soil and will also have an impact on phosphatase activity and phosphate mineralization dynamics (Cabugao *et al* .., 2017)

***Plant Crown P Uptake***

The application of vermicompost and host plant species had a significant effect ($P < 0.05$) on plant canopy P uptake. The application of vermicompost resulted in higher plant canopy P uptake compared to the control (Figure 6). The content of P nutrients in vermicompost allows plants to obtain sufficient nutrients and directly absorb P elements through the roots. The addition of organic matter into the soil will increase the maximum P uptake due to the decomposition and mineralization process (Rosliani *et al*., 2009) . Each plant produces a different amount of canopy P uptake. Corn and sorghum plants are able to absorb P in the media higher than millet plants which are influenced by the ability of roots to reach nutrients to obtain the nutrients needed,. The results of the study *Hussain et al*. (2016) reported that the addition of vermicompost with FMA inoculation resulted in maximum P uptake of 375.6% to 402.9% compared to only the provision of vermicompost or mycorrhiza alone.

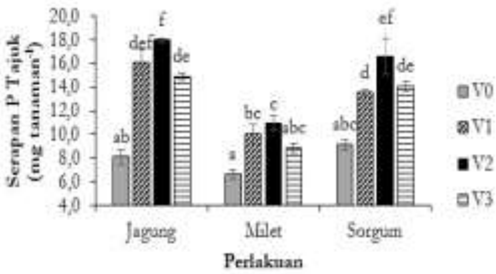


Figure 6. Effect of treatment on host plant crown P uptake

The low nutrient uptake in the control was due to the very low availability of P in the post-coal mine soil. FMA symbiosis affected P uptake directly in each plant crown, especially in the control treatment (Figure 6). The role of FMA in assisting the absorption of nutrients P contained in the soil and vermicompost input in the media has an impact on increasing plant canopy P uptake . According to Widiastuti et al. (2002) , the mechanism of P absorption with the help of FMA includes FMA mycelia that absorb nutrients from the soil, translocation of nutrients from extraradical hyphae to intraradical hyphae of FMA in the root and transfer of nutrients from FMA to internal root cells. Hyphae produced by FMA can expand the range of nutrient uptake of plant roots so that nutrient uptake is high even though media nutrient availability is low. In addition, the small diameter of FMA hyphae (2-5 µm), makes it easy to penetrate soil pores that cannot be penetrated by plant roots (Talanca, 2010) .

## Plant Height

Vermicompost and host plant species significantly affected plant height as a single factor. The dose of vermicompost had a significant effect (P < 0.05) on plant height at 2 to 5 MSI while the type of host plant had a significant effect (P < 0.05) on plant height at 1, 2, 3, 6 and 7 MSI (Table 1).

Table 1. Effect of vermicompost and host plants on plant height at 1 to 7 MSI

| Perlakuan | Tinggi Tanaman (cm), MSI | | | | | | |
|---|---|---|---|---|---|---|---|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Tanaman Inang | | | | | | | |
| J | 9,5 b | 13,92 b | 16,54 b | 18,33 | 21,54 | 24,88 a | 32,83 a |
| M | 8,3 a | 10,92 a | 13,38 a | 18,21 | 20,50 | 32,88 b | 33,12 a |
| S | 10,4 b | 15,17 b | 18,38 b | 20,92 | 23,21 | 30 b | 46,92 b |
| Media | | | | | | | |
| V0 | 9,2 | 12,06 a | 13,67 a | 16,22 a | 18,39 a | 22,22 | 29,94 |
| V1 | 9,2 | 12,72 a | 15,72 a | 18,72 a | 20,5 a | 26,61 | 39,67 |
| V2 | 10,0 | 15,61 b | 19,44 b | 23,5 b | 27,11 b | 33,22 | 43,89 |
| V3 | 9,3 | 12,94 a | 15,56 a | 18,17 a | 21 a | 25,72 | 37,00 |

Plant height growth is influenced by the continuity of the plant's photosynthesis process. Photosynthesis converts absorbed nutrients into carbohydrates and is used to form leaves, stems, and other parts. Vermicompost has a high humus content and is rich in essential nutrients for plants such as nitrogen, phosphorus, and potassium (NPK). According to Irawan *et al.* (2015) P element plays a role in cell division, especially in the development of meristem tissues and affects the growth of plant height. Vermicompost is known to encourage plant vegetative growth and stimulate the development of plant shoots and roots significantly than its mere ability to provide nutrients in a form available to plants (Adhikary, 2012) .

Plant growth is also influenced by the adaptability of plants to new environments and FMA symbiosis. FMA symbiosis can help plants to grow more stable in unfavorable conditions such as low pH and P availability. FMA symbiosis in the host plant root will have an impact on the expansion of the root zone so that the uptake of nutrients and water as basic ingredients in the photosynthesis process becomes more optimum (Chalimah *et al.*, 2007) . Supported by the statement of Hartoyo *et al.* (2018) , that FMA symbiosis with plants is able to increase photosynthesis by 14% higher than that of non FMA symbiotic plants and express plant genetics on plant height growth.

## *Plant Crown Dry Weight*

The addition of vermicompost and the type of host plant had a significant effect (P<0.05) on the dry weight of the host plant crown. The average crown dry weight of corn, millet, and sorghum hosts were 3.58 g; 1.53 g; and 3.65 g, respectively (Figure 7). The addition of vermicompost resulted in a higher average crown dry weight than the control. An increase in crown dry weight was also reported in the results of research (Nusantara *et al.*, 2010) which showed that the addition of vermicompost of 150-172 mg resulted in a crown dry weight with an average of 1.02 g, 18% higher than the control using artificial fertilizer of 0.86 g. The dry weight of the plant reflects the amount of plant nutrients in the plant. Plant dry weight reflects the amount of nutrient content that can be absorbed in the process of plant photosynthesis. The heavier the plant dry weight indicates the process of plant development and growth is good and more elements are absorbed.

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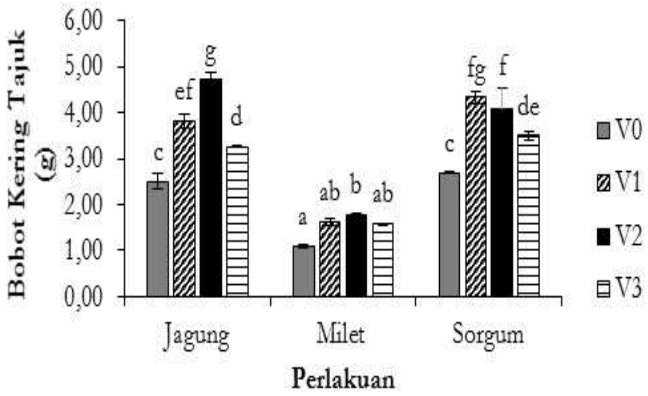


Figure 7: Effect of plant crown dry weight

Differences in crown dry weight in each host plant are influenced by several factors such as nutrient availability in the soil, plant nutrient uptake, and FMA symbiosis. The low crown dry weight in the control was due to low P uptake in the post-coal mine soil media. However, FMA can maximize nutrient uptake in post-coal mine soil media that have very low nutrient availability, for example P. FMA symbiosis can assist plant roots in reaching nutrient uptake that cannot be reached by root hairs through external hyphae that spread widely into the media (Indriani *et al*., 2011) . FMA symbiosis can also increase the surface area of contact between roots and soil up to 47 times (Suharno and Sancayaningsih, 2013) . FMA plays an optimal role in nutrient translocation and accumulation by the host plant when a suitable host is available and the addition of nutrients such as vermicompost.

***Relationship between FMA Colonization in Roots and the Number of Spores***

FMA colonization on roots showed a positive linear relationship to the number of spores (r= 0.92) and influenced 84% of the number of spores and 16% influenced by other factors ($R^2$=0.84) (Figure 8). FMA sporulation begins with spores that germinate and infect (colonize) plant roots through hyphae that will develop to form new FMA spores. Spore germination is influenced by a number of factors, including host suitability, root exudates, inoculum type, and environmental factors. The low available P and pH in this study stimulated the plants to release exudates in response to these conditions. Exudates will affect the surrounding conditions of the plant, stimulating FMA to germinate and colonize the host plant roots through the hyphae formed. FMA has a mechanism for forming structures to survive in the form of spores that can function as reproductive tools (Irawan *et al*., 2015).

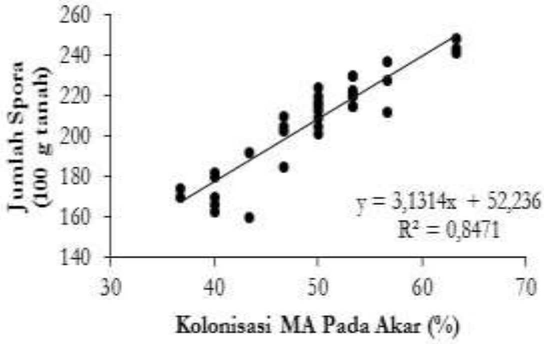


Figure 8. Relationship between MA colonization of roots and number of MA spores

The suitability of host plant species such as root type has the potential to increase the total root surface that can be colonized by FMA. The increasing colonization of FMA on the roots will produce more spores. Supported by the results of research Nusantara *et al.* (2010) which showed that the increase in the number of FMA spores produced in G etunicatum propagation media was directly proportional to the percentage of FMA colonization on the roots that increased. Increased FMA colonization capacity on the roots has the potential to increase the number of FMA spores through extraradical hyphae that form and spread on the host plant roots.

***Relationship between pH and Spore Count***

pH shows a positive correlation to the number of spores (r= 0.54) and forms a quadratic nonlinear line to the number of FMA spores ($R^2$= 0.41). pH affects 41% of the number of spores and 59% is influenced by other factors (Figure 9). Based on the quadratic equation, the maximum number of spores that can be produced is 225 spores at pH 4.37. Propagation media with pH <4.37 or >4.37 will produce fewer spores.

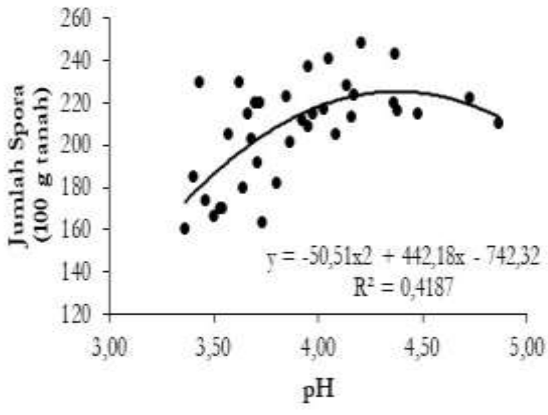


Figure 9. Relationship between pH and the number of FMA spores

Germination and development of FMA spores in the propagation media are influenced by media acidity. Research by Setiadi (2002) explained that FMA propagation using planting media with soil acidity levels of 4.3-6.4 resulted in more optimum FMA colonization on the roots compared to propagation media with alkaline conditions of 7.2-8.2. According to the statement of Yusriadi *et al*. (2018) this is thought to be due to the nature of

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"*arcidophylis*" owned by FMA, allowing spores to develop more and more in acidic conditions

***Relationship between Available P and Spore Count***

Available P showed a positive correlation to the number of spores (r= 0.66) and formed a quadratic non-linear line to the number of MA spores ($R^2$= 0.51). Available P has a 51% effect on the number of spores and 49% is influenced by other factors (Figure 10). Based on the quadratic equation, the maximum number of spores that can be produced is 225 spores at an available P of 7.76 mg $kg^{-1}$. Propagation media with P available< 7.76 mg $kg^{-1}$ or >7.76 mg $kg^{-1}$ will produce fewer spores.

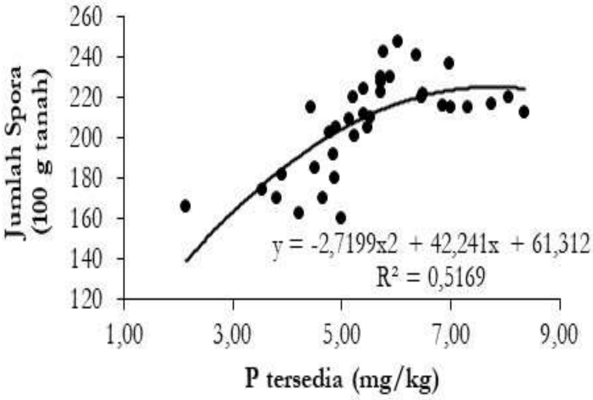


Figure 10. Relationship of available P to MA spore count

The availability of P influences MA colonization of host plant roots, which can have both positive and negative effects on MA formation. MA actively assists plants in absorbing P in conditions of low P nutrient availability through hyphae. Conversely, at high P availability MA is not needed by plants in assisting the absorption of P nutrients. Research Balzergue *et al.* (2013) explains that at high P availability will inhibit the formation of symbiotic MA with host plant roots due to plant roots can directly absorb P elements so that it has an impact on inhibiting the formation of MA structures such as arbuscules and vesicles. This is supported by the statement of Yusriadi *et al*. (2018) that in conditions of high P availability, MA does not develop actively and only lives by utilizing the results of plant photosynthesis due to the rejection of the response to colonize plant roots that affect plant metabolism.

**CONCLUSION**

The addition of vermicompost increased the number of Glomus spores on post-coal mining land by 20.64% to 34.58% and the percentage of colonization on roots by 21.07% to 41.22% on each host plant. Millet plants provide the optimum effect on increasing the number of Glomus spores on post-coal mine land as many as 4,340 spores pot-1 and the average percentage of colonization on the roots reached 52.48% compared to corn and sorghum plants. The combination of vermicompost dose of 2 g pot-1 and millet as a host plant resulted in the number of Glomus spores on post-coal mine land as many as 4,880 spores pot-1 with the percentage of colonization on the roots reaching 63.30%

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