



Effects of Arbuscular Mycorrhizal Fungi combined with P Fertilizer on the System of Rice Intensification Cultivation

Elita, N., Yanti, R.*, Susila, E., Karmaita, Y.,
Andam, D.S. and Kurnia, A.I.

Food Crops Production Technology Study Program and Master Program in Applied Food Security,
Politeknik Pertanian Negeri Payakumbuh, Tanjung Pati, Km 7, Kecamatan Harau,
Kabupaten 50 Kota West Sumatera, Indonesia, 26271

*Correspondence: rinda_yanti@yahoo.co.id

ABSTRACT

This paper aimed to obtain appropriate indigenous arbuscular mycorrhizal fungi types and determine the right dosage of phosphate fertilizer to increase rice yields under the System of Rice Intensification Cultivation. The research design used a factorial randomised block design with three replications. Factor I was a type of arbuscular mycorrhizal fungi (*Glomus* sp. 2, *Glomus* sp. 3, and *Sclerocystis* sp) on Agam river sand media with the number of spores being 120 spores per 100 grams of sand media. The dose of arbuscular mycorrhizal fungi was 1.6 tons/ha or 15 g/plant. Factor II was the dose of phosphate fertilizer ($P = 0\%$, $P1 = 25\%$, $P2 = 50\%$, and $P3 = 75\%$) of the recommended dose which was applied for each treatment at these rates: $P = 0$ g/plot, $P1 = 20$ g/plot, $P2 = 40$ g/plot, and $P3 = 60$ g/plot. The data was analysed for variance using the SAS program. The results showed that *Glomus* sp. 3 with 50% phosphate fertilizer efficiency had statistically significant different results ($p < 0.05$) for plant height, number of tillers, number of panicles, number of filled grains, weight of 1000 seeds, and yield per hectare compared to the other treatments. The nutrient content of paddy fields pH, CN-ratio, total N, P available and CEC showed a statistically significant difference between the arbuscular mycorrhizal fungi types and the P fertilizer efficiency treatment. No statistically significant difference was found in the CN ratio between *Glomus* sp. 3 and *Sclerocystis* sp., but there was a statistically significant difference from *Glomus* sp. 2.

Keywords: *Glomus* sp3, indigenous rice, SRI, *Sclerocystis* sp

INTRODUCTION

Rice cultivation under the System of Rice Intensification (SRI) method uses an environmental sustainability approach. This system seeks to minimise the use of chemical fertilizers and pesticides. The SRI method integrates conventional approaches with more environment friendly methods such as the application of microorganisms (Farrar *et al.* 2014). Research results of several studies (Elita *et al.* 2018; Elita *et al.* 2020; Elita *et al.* 2021) show that microorganism application in rice cultivation using the SRI method increases rice yield and soil nutrient content in paddy fields.

As SRI is based on a sustainable farming system, it is said to produce higher-quality rice, and increases yields by reducing water requirements. SRI offers environmental benefits and gains of > 60%, as it reduces groundwater use by 60%. SRI based rice cultivation reduces costs per hectare (ha) of cultivation significantly (Gathorne-Hardy *et al.* 2016). The SRI method aids crop productivity by enhancing plant root growth and xylem exudation rate, leaf area index, light

interception by the plant canopy, and photosynthetic rate at the grain filling stage which result in higher seed yields, thereby increasing production by about 56% (Thakur *et al.* 2018).

The SRI method in the vegetative phase of groundwater under aerobic conditions, which allows decomposing microorganisms to live actively and develop properly and make them available in abundance to the plant. Among the microorganisms are arbuscular mycorrhizal fungi (AMF). Rhizobia in the rhizosphere of rice plants increase protein content and yield per hectare through the production of auxins and other growth-stimulating substances. Root exudate is a key factor in the various processes of the SRI method, which results in a larger root system and more rice tillers (Uphoff *et al.* 2015) .

AMF offers a symbiotic mutualism between fungi and the roots of higher plants. Plant roots are associated with AMF to obtain phosphorus (P) from the soil, including as a phosphate solvent. Improvement of soil health can take place quickly by utilising AMF as a bioremediator because AMF proliferates in unfavorable (marginal) environmental conditions. The presence of AMF in the roots of rice plants can increase the variety and amount of nutrients that can be absorbed by roots, especially P biologically, while also increasing the availability of soil P (Sarabia *et al.* 2018).

Indigenous AMF is more adaptive and effective in promoting plant development, as its ability to absorb nutrients is higher, thereby enhancing the speed of plant growth. According to Basu *et al.* (2018), the success of the association of AMF with plant roots is strongly influenced by the suitability of AMF species for the type of host plant. This association helps increase the supply of nutrients like nitrogen, phosphorus, and water to the plants, and in turn, the fungus gets 20% of the fixed carbon from the plants. Xu *et al.* (2018) added that there were differences in the behaviour of each AMF genus in obtaining nutrients from different plants. Therefore, their decomposition results in different bacterial and fungal communities. This effect is modulated by soil P availability which affects plant growth and production.

Furthermore, according to Sosa Hernández *et al.* (2017), in different soil types, the sources of AMF inoculants lead to different AMF communities. Their study indicated that AMF subsoil has a potential role as a reservoir of biodiversity. Double inoculation of AMF with rhizobium bacteria increased root biomass and nodule growth (Budiastuti *et al.* 2021).

Research on AMF has been carried out on various commodities, but the use of indigenous AMF species isolated from the rice rhizosphere and applied to rice using the SRI method lacks information. Therefore, a study was conducted on the effect of the type of AMF and the right dose of P fertilizer in increasing the yield of rice using the SRI method. The purpose of this study was to obtain the appropriate type of indigenous AMF and the right dose of P fertilizer to increase the yield of rice using the SRI method.

MATERIALS AND METHODS

Time and Place

The research was carried out on farmers' rice fields in Taram, Harau District, Limapuluh Kota Regency, Indonesia. The research was conducted from September 2022–January 2023.

Research Design

The field research used a factorial randomized block design with three replications. Factor I: Indigenous AMF of type *Glomus* sp.3, *Sclerocystis* sp, and *Glomus* sp.2 on Batang Agam river sand media with the number of spores being 120 spores per 100 grams of media. AMF was given at a dose of 1.6 tons/ha or 15 g/plant.

Factor II: For the treatment, based on the recommended dose of P fertilizer where P = 0%, P1 = 25%, P2 = 50%, and P3 = 75%) P fertilizer was applied to the plots as follows: P = 0 g/plot, P1 = 20 g/plot, P2 = 40 g/plot, and P3 = 60 g/plot.

To cultivate rice based on the SRI method, the soil had to be prepared. For the initial soil analysis, soil samples were collected and thoroughly mixed to prepare a composite sample of approximately 250 g as the working sample. The paddy fields were sanded with a tractor. The experimental plots measured 2.1 x 2.1 m and totalled 36 plots. The distance between plots in blocks was 0.5 m while the distance between replicates was 1 m. Each experimental plot was treated and eight weeks after planting, soil nutrient analysis was carried out by taking 250 g of soil samples from each experimental plot. Inoculant-type indigenous AMF was given according to the treatment at planting at a dose of 15 g/clump or 1.6 tons/ha (120 spores/100 grams).

Nurseries are mainly made up of manure. The seeds are soaked for 12 h, ripened for 12 h, and then sown in the nursery. Rice seedlings, aged 12 days, are ready for planting in the plot. Seedlings are planted one stem per planting point, at a distance of 25 x 25 cm. One plot will have 49 clumps of plants. Fertilization is given at half the recommended dose with urea (150 kg/ha or 63 g/plot) and KCl (50 kg/ha or 21 g/plot) at the time of planting, and urea is given twice (at the beginning and at 30 days old). SP-36 fertilizer was given based on the treatment. Water conditions were kept moist during the vegetative phase. In the flowering phase, the water level was maintained up to 5 cm above the soil surface. Ten days before harvesting, the soil was left to dry. Weeding was done by removing weeds and immersing them using a weeding tool to ensure a better soil air system. Harvesting was done at 110 days. After harvest, the grain was dried in the sun to attain a moisture content of 14%.

The following were observed: (i). plant height (cm), (ii) number of tillers, (iii) number of panicles per clump (panicle), (iv) number of pithy grains per panicle, (v) weight of 1000 seeds (g), (vi) production of dry grains per hectare (ton), (vii) analysis of soil nutrients before and after treatment, and (viii) development of AMF in the rhizosphere of rice plants.

Laboratory observations included root colonisation and spore density. To calculate spore density at the end of the observation, 10 g of AMF formula were inserted into 500 ml cup glass with 200

ml of sterile distilled water, stirred and left for 30 sec. Spore suspensions were filtered at sizes of 500 µm, 250 µm, 106 µm and 50 µm. The remaining were collected on a 50-µm sieve and transferred to a centrifuge tube to which was added 60% glucose and centrifuged for 10 min at 1000 rpm. The spores in the centrifuged tubes were poured into a 50-µm sieve and carefully washed with water to remove the glucose. The sponges retained on the sieve were transferred into a Petri dish and observed under a microscope.

Root samples were taken in the rhizosphere of rice plants after screening to observe root colonisation, including:

1. Percentage of mycorrhizal colonisation on plant roots, calculated using

$$\% \text{ colonisation} = \frac{\Sigma \text{ field of view (+)}}{\Sigma \text{ field of view}} \times 100 \%$$

2. The intensity of infection and classification of root infection rate are based on the classification of the Institute of Mycorrhizal Research and Development, USDA:

Class 1: Infection is 0-5 % (very low , +)

Class 2: Infection is 6-25 % (low, ++)

Class 3: Infection is 26-50% (medium, +++)

Class 4: Infection is 51-75% (high, ++++)

Class 5: Infection is >75 % (very high, +++++)

The higher the category of infection intensity, the higher the intensity of AMF infection in plant roots, an indicator of the effectiveness of AMF on plants. That is, the higher the rate of colonisation between AMF and plants, the higher the level of symbiosis between the two.

Statistical Analysis

To test the effect of treatment on the observed variable responses, analysis of variance was carried out using the Statistical Analysis System (SAS) program. The Duncan New Multiple Range Test (DNMRT) was conducted to see the difference in treatment at the 5% level.

RESULTS AND DISCUSSION

Vegetative Growth of Rice Plants

The results of the field experiment after statistical analysis showed no interaction between the type of AMF and the dose of P. The results of testing the type of AMF and the efficiency of P fertilizer on the vegetative growth of rice plants using the SRI method are presented in Figure 1.

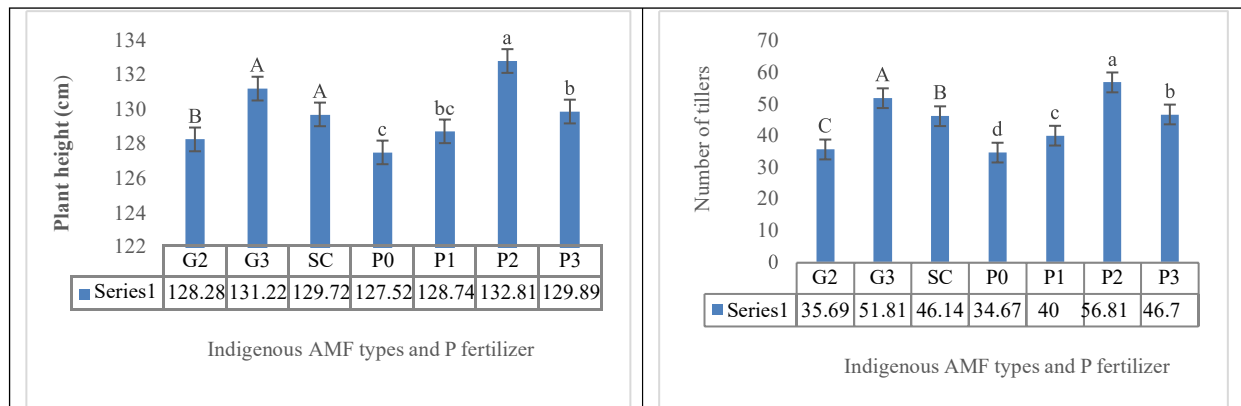


Figure 1. Vegetative observations of indigenous AMF inoculation and efficiency of P fertilizer in rice cultivation using the SRI method

Figure 1 shows that the highest plant height and number of tillers were found in *Glomus* sp3, with the difference not being statistically significant ($P < 0.05$) with *Sclerocystis* sp, but compared to *Glomus* sp.2, the difference was statistically significant. Among the three types of AMF isolates, compatibility differences were reflected in plant height and number of tillers. *Glomus* sp. 3 and *Sclerocystis* sp. species provided better nutrients than *Glomus* sp. 2.

Previous studies have reported the degree of compatibility of AMF isolates with plants to determine the effectiveness of AMF on plant growth decompose litter, thus accelerating the provision of nutrients from litter and organic matter Xu *et al.* (2018) . AMF can enhance plant nutrition with mineral nutrients, and vice versa while plant growth and development are triggered by organic carbon supplied from host plants (Moradi *et al.* 2017). Our study showed that the AMF type *Glomus* sp.3 on Batang Agam river sand carrier material was more suitable in that it could affect plant height and number of tillers in rice plants cultivated under the SRI method. A study by Omomowo *et al.* (2018) found that *Glomus* species were able to increase the growth and yield of cowpea.

With regard to the study on the efficiency of P fertilizer, the highest plant height and number of tillers were obtained at 50% fertilization efficiency (P2). The results of this study indicate that a maximum amount of P fertilizer is absorbed by rice plants in the presence of AMF. Rice plants under the SRI method go into a vegetative phase under dry conditions. In the presence of AMF symbiosis, rice plants have improved tolerance to drought stress and can be grown efficiently with the absorption of fertilizers, especially P (Quiroga *et al.* 2018).

AMF can suppress the loss of nutrients from the soil by enlarging the nutrient interception zone and preventing the loss of nutrients due to the leaching process by percolation of water which efficiently absorbs nutrients especially P (Cavagnaro *et al.* 2015). The results of this study indicate that *Glomus* sp3 and *Sclerocystis* sp are mycorrhizal isolates that are suitable for vegetative growth of rice plants under the SRI method.

Arbuscular mycorrhizal fungi (*Phylum glomeromycota*) are important components of the soil microbial community. AMF forms mutualistic associations with the roots of terrestrial plants, including many agricultural crops. In many agricultural crops, these mutualistic associations have

demonstrated the potential to increase crop productivity, thereby playing a key role in the functioning and sustainability of agroecosystems (Cosme 2023; Birhane *et al.* 2023).

The Generative Growth of Rice Plants

Field test results after statistical analysis, showed no interaction between AMF species and P dose. The results of inoculation of AMF types and efficiency of P fertilizer on the generative growth of rice plants using the SRI method are presented in Figure 2.

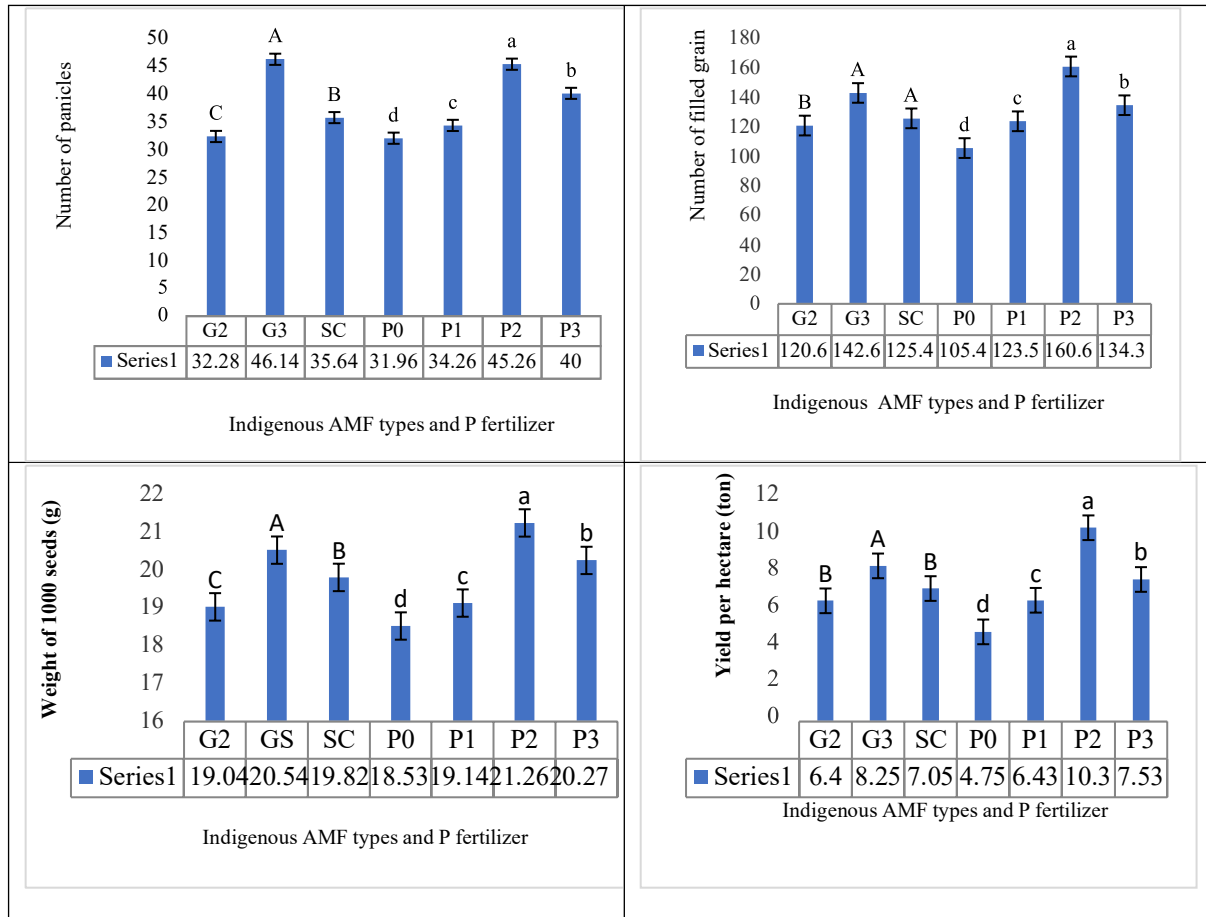


Figure 2. Generative observations of inoculation of indigenous AMF and efficiency of P fertilizer under SRI method of rice cultivation

Inoculation of *Glomus* sp.3 in rice plants under the SRI method showed a statistically significant increase ($P < 0.05$) in the number of panicles compared to *Sclerocystis* sp and *Glomus* sp.2. *Glomus* sp. 3 had more spores with an average size of > 250 m and a higher colonisation rate (Elita *et al.* 2018). *Glomus* sp.3 was found to provide better nutrients, sufficient for panicle formation.

In terms of the number of pithy grains, statistically there was no significant difference between *Glomus* sp3 and *Sclerocystis* sp, but between *Glomus* sp2 and *Sclerocystis* sp, a statistically significant difference was observed. This result is related to the levels of the C/N ratio, where the difference was not statistically significant (Figure 3). These results are a clear indication that the

soil organic matter had decomposed well and was widely available, thus leading to a high amount of pithy grain.

The weight gain of 1000 seeds from plants with *Glomus* sp.3 type was significant ($P < 0.05$). The weight of 1000 seeds is a description of the results of good photosynthesis resulting in a larger grain size. *Glomus* sp.3 is able to provide better nutrition at a pH that has reached a normal level (6.05) resulting in organic matter and organic C being available for uptake of nutrients by the rice plants (Figure 3).

The highest production/ha was obtained with *Glomus* sp. 3. The ability of *Glomus* sp. 3 to provide good plant nutrients (Figure 4), as can be seen by the CEC value which increased from low to moderate (Table 1). This increase in CEC value facilitated the smooth transport (translocation) of carbohydrates from leaves to other plant parts, allowing photosynthesis to accumulate in the grain. These results indicate that *Glomus* sp.3 is able to increase the yield component under the SRI rice method. P2 fertilizer (50% efficiency) showed the highest yield for the component parameters of rice yields.

The most important function of this symbiotic association involves the transfer of nutrients such as organic C in the form of sugars and lipids (Jiang *et al.* 2017) (Luginbuehl *et al.* 2017) for fungi by plants and the transfer of P and N to plants by fungi (Smith-Ramesh *et al.*, 2017) The interaction between AMF and plant roots is one of the symbiotic relationships that makes a major and consistent contribution to the production of agricultural crops. Plant growth will depend on increased nutrient flow through the AMF network (Hamel and Plenchette 2017). According to Cosme *et al.* (2018), AMF play an important role in increasing agricultural yields and productivity with low inputs. Bernaola *et al.* (2018) state that in addition to the benefits accorded to plants, AMF can improve soil structure, reduce drought and salinity stress, and affect the diversity of plant communities.

Research results by Hidayati *et al.*(2016) state that the high yield of rice plants under the SRI method is a result of the significantly higher rate of photosynthesis, chlorophyll content, and N and P uptake. Rice plants under the SRI method in the generative phase (especially in the seed filling phase) were found to have the highest photosynthesis and lowest transpiration rates. The rice yield under the SRI method is higher (about 24%) compared to the conventional method of cultivation.

Rice fields are open to various types of beneficial microorganisms such as the following bacterial species: *Lactobacillus spp.*, *Klebsiella aerogenes*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas fluorescens*, *Azospirillum brasilense*, *Bacillus subtilis*, *Staphylococcus aureus*, *Enterobacter cloacae*, and *Micrococcus sp.*, especially cultivated under the SRI method. Research results by Okonji *et al.* (2018) state that AMF inoculation in the root zone of rice plants will have beneficial interaction with one of the above stated bacteria on soil fertility, which increases P availability, neutralises soil pH, and encourages C activity. Yield components of rice significantly increase due to the behaviour of two interacting microorganisms that encourage P uptake.

Nutrient Content of Paddy Fields before and after Application of AMF and P Fertilizer

An analysis of soil nutrient content was carried out before the soil was treated. The results of the initial soil analysis before treatment application are presented in Table 1.

TABLE 1
Results of the initial soil analysis of the study

Parameter	Results	Criteria
pH 1:1	5.71	Currently
C-Organic (%)	1.10	Very low
C/N	7.33	Low
N-Total (%)	0.15	Low
P (ppm)	9.2	Very low
CEC (me 100 g ⁻¹ soil)	12.70	Low

Soil analysis results subsequent to the inoculation of AMF type and the efficiency of P fertilizer on pH, C-organic, and CN-soil ratio showed no interaction between the two treatments. With regard to the main effect of AMF type, the results were significantly different, and in terms of P fertilizer efficiency, the results showed an increase in pH and CN-ratio, that was significantly different (Figure 3).

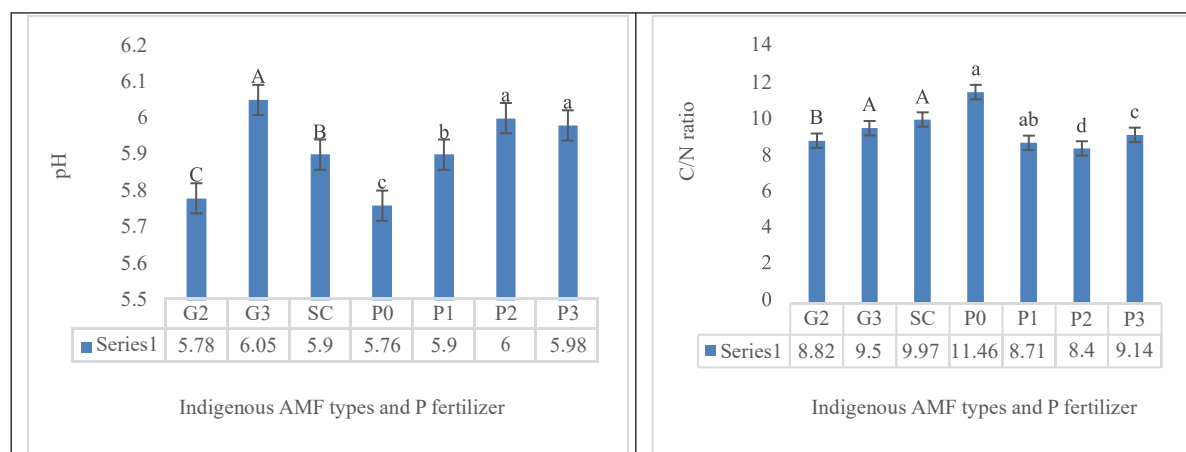


Figure 3. Analysis of soil nutrients after inoculation of AMF and the efficiency of P fertilizer on pH and CN-ratio

In Figure 3, it can be seen that the main effect of AMF was on soil pH which was significantly different; the highest pH was obtained in the treatment of *Glomus* sp. 3. In terms of the efficiency of P fertilizer, the observations of the highest soil pH at P2 were not significantly different from P3, but significantly different from P1 and P0. When compared with the initial soil pH (5.71), there was an increase in pH for all treatments; the highest increase in pH was obtained in *Glomus* sp. 3 treatment.

This shows that the application of *Glomus* sp3 is the most suitable for increasing soil pH for lowland rice under the SRI method, with further effects on root absorption, root development, and higher metabolic activity. The role of AMF is to increase the nutrients N and P in the soil, coupled with P fertilization, so that it results in an increase in soil pH (Wu *et al.* 2021). Acidic soil pH has a detrimental effect on rice plant growth, reducing root length by up to 31%. Decrease in chlorophyll content which affects the process of photosynthesis (Awasthi *et al.* 2022).

The C/N value of the application effect of *Glomus* sp. 3 and *Sclerocystis* sp. was not significantly different, but significantly different from that of the *Glomus* sp. 2 fungus, indicating that these two types of fungi have strong activity in decomposing soil organic matter. The main effect of the efficiency of P fertilizer is shown by the highest C/N value at P0, which indicates lower AMF activity without P fertilizer mineralising organic matter. According to Sun *et al.* (2021), rhizosphere microorganisms interact with plant roots to accelerate the mineralisation of soil organic matter to obtain nutrients. Root-mediated changes in soil organic matter mineralisation are highly dependent on root-derived carbon inputs and soil nutrient status. Root morphology, rather than root biomass, is positively related to C/N mineralisation. There was interaction between the application of AMF and the efficiency of P fertilizer on organic C, total N, CEC, and P-available paddy soil (Figure 4).

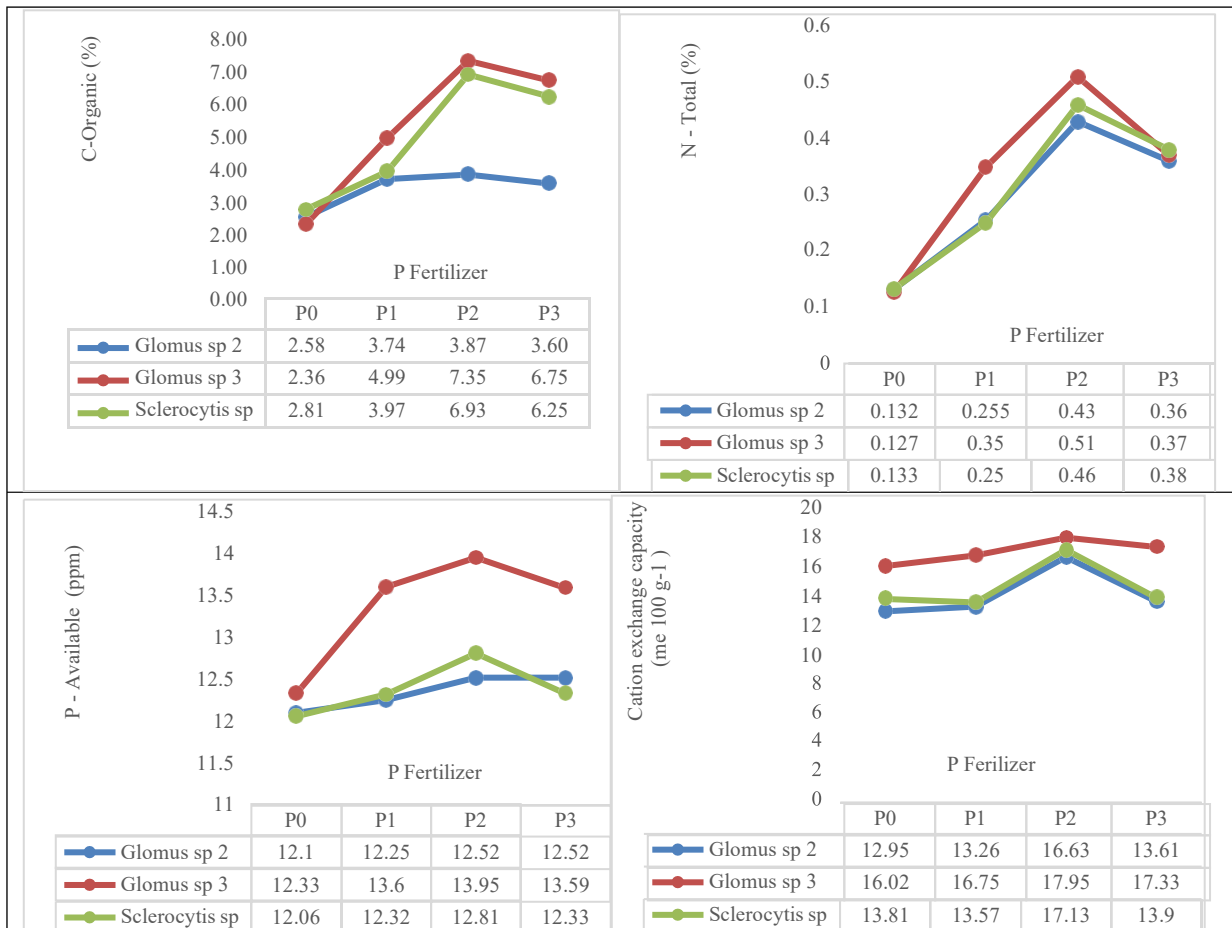


Figure 4. Interaction of AMF species with efficiency of P fertilizer on organic C, total N, P available and CEC

Fungus *Glomus* sp 2, *Glomus* sp 3 and *Sclerocystis* sp interacted with organic C, Total N, available P and Cation Exchange Capacity (CEC) at doses of P2. The highest interaction was given by *Glomus* sp 3 at doses of P2. Competition for the fungus *Glomus* sp 3 is more beneficial in increasing soil nutrients compared to *Glomus* sp2 and *Sclerocystis* sp. According to Wu et al (2021) interspecific competition of fungi is more beneficial than intraspecific competition for increasing soil nutrients.

The average N-total soil applied with *Glomus* sp 3 was the highest (0.34%), whereas in combination with P2 it was the highest and significantly different from the others (0.42%). The combination of *Glomus* sp3 and P2 has the highest total soil N-value (0.51%), this indicates that *Glomus* sp 3 and P2 can provide N nutrients so that they are suitable for plant needs, especially lowland rice with the SRI method which requires high N. The impact of this condition has a further effect on increasing root absorption, root development and higher metabolic activity. Tan et al (2021) stated that the mycelium of the AMF fungus can increase litter decomposition and accelerate the release of N nutrients through the decomposition of nitrogen compounds in litter. Yang *et al* (2019) stated that competition for specific fungal species greatly intensified plant nutrition.

The highest interaction in *Glomus* sp. 3 with a dose of P2 fertilizer with a value of 17.95 increased the CEC status of the soil at the medium criteria level that was higher than the initial CEC (Table 1) of 12.70 (medium). The increase in CEC value is a result of AMF, especially the *Glomus* sp. 3 species at P2 dose, resulting in a faster rate of ion exchange for potassium calcium and magnesium which have an impact on increasing soil pH (Figure 3). An increase in soil pH has an impact on root absorption of soil nutrients leading to an increase in the number of pithy rice grains (Figure 2). This encourages an improvement in soil quality and better absorption of plant roots.

AMF variety of *Glomus* sp. 2, *Glomus* sp. 3 and *Sclerocystis* sp. interact with the application of P fertilizer so as to increase available P. The increase in available P from very low levels (see Table 1) had a positive impact on the availability of P. AMF can help the hyphae absorb phosphorus far from the reach of the roots and increase metabolic energy to form a higher number of seeds, resulting in an increase in the number of pithy seeds and production.

The Development of AMF in the Rice Rhizosphere

AMF application increases soil microbial activity, especially microbes around the rhizosphere of rice plants. This can be seen from the observations shown in Figure 5.

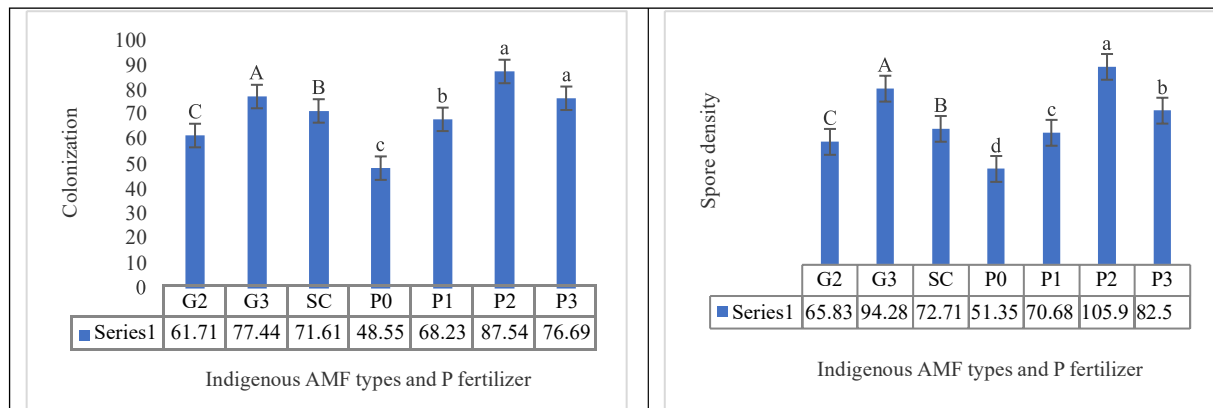


Figure 5. Comparison of AMF colonisation and AMF spore density with P. fertilizer efficiency

All plant treatments showed mycorrhizal colonisation and spore density. The colonisation effect and the highest spore density were found in *Glomus* sp. 3 and P2 treatment ($p < 0.05$), which significantly increased rice yield. Colonisation and density of mycorrhizal spores increased soil nutrients, including organic C, N-total, CEC, and P-available soil (Figure 5). In addition to the nutritional effects, AMF fungi also have an effect on biotic resistance in host plants (Campo *et al.* 2020) and provide basic resistance to plants which enables better plant growth (Wang *et al.* 2021).

AMF are important microorganisms in rice fields and several other wetlands (Wang *et al.* 2015). The development of AMF fungus is not depressed in flooded conditions but is very responsive to the growth of rice plants in non-flooded rice fields (Vallino *et al.* 2014).

Varied growth response of rice plants to the inoculation of AMF was obtained in several studies ranging from positive to negative. Most studies reported that AMF increased rice plant biomass, grain yield, and P uptake under flooded conditions (Gewaily 2019). According to Wang *et al.* (2021), AMF colonisation triggers a strong defense response in rice plants in shaded conditions and has a good influence on rice plant metabolism. In contrast, several other studies found that AMF inoculation resulted in a reduction in the amount of dry matter and rice production under flooded conditions (Bao *et al.* 2019). In line with the results of this study, in the SRI method of rice cultivation, the vegetative phase of groundwater conditions was not flooded. This unstressed growth conditions led to a good development of AMF, which could have had the effect of increasing growth, rice plant production, and soil nutrients.

CONCLUSION

Glomus sp. 3 and 50% P fertilizer efficiency increased the highest rate of vegetative and generative growth in rice cultivation under the SRI method and the pH of paddy soil. The *Glomus* sp. 3 type increased rice yields up to 8.25 tons/ha, with 50% P fertilizer efficiency, reaching 10.27 tons/ha. It also increased the nutrient content of paddy soil planted with rice using the SRI method. The best CN ratio was obtained from *Glomus* sp. 3 and *Sclerocystis* sp. The fungi *Glomus* sp.2, *Glomus* sp.3, and *Sclerocystis* sp. interacted on organic C, total N, available P, and CEC at doses of P2. The highest rate of interaction was given by *Glomus* sp. 3 at a dose of P2. Competition for the fungus *Glomus* sp. 3 was found to be more beneficial in increasing soil nutrients compared to

Glomus sp. 2 and *Sclerocystis* sp. AMF colonisation and AMF spore density by *Glomus* sp. 3 were 77.44% and 94.28 spores, respectively, while the percentages on the effect of 50% P fertilizer efficiency were 87.54% and 105.90 spores.

ACKNOWLEDGEMENTS

I would like to thank the Directorate General of Vocational Education. I am also grateful to P3M and the Director of Politeknik Pertanian Negeri Payakumbuh for assistance throughout this research.

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