

Exploring the benefit of arbuscular mycorrhizal *Glomus* spp. in improving available soil P and crop growth of sorghum cultivated on ex-coal mine soil

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Abstract. Prasetya B, Arfiyanti H, Arfarita N. 2024. Exploring the benefit of arbuscular mycorrhizal *Glomus* spp. in improving available soil P and crop growth of sorghum cultivated on ex-coal mine soil. *Biodiversitas* 25: 5112-5122. One main problem of ex-coal mining land is low soil phosphate (P) availability and easy binding of phosphate to Fe and Al (hydro)oxide. These conditions affect the abundance and activity of soil microorganisms. Unlike other microbes, mycorrhizae can help root uptake of P that is not available in the inorganic form and make it available for crops. The addition of slow-release phosphate sources - such as rock phosphate, bone meal, and fish meal - are good sources of P nutrients because they are not easily fixed and washed away. This study aimed to improve ex-coal mining land conditions by increasing the level of P availability in soil via the application of various P sources at different dosages to improve mycorrhizal spores and sorghum growth. The pot experiment used a factorial completely randomized block design (CRBD) with 3 different phosphate source type (P), consisting of 3 treatment levels, under the optimum concentration of - P1: rock phosphate; P2: bone meal; and P3: fish meal - and various dose of phosphate source (F) consisting of 5 treatment levels - F0: control; F1: 0.17 g/polybag; F2: 0.29 g/polybag; F3: 0.40 g/polybag; and F4: 0.57 g/polybag-with a total combination of 15 treatments with 4 replications. The combination of the P2F3 treatment (bone meal dose of 0.40 g/polybag) was the best dose. It was effective in increasing the number of Arbuscular Mycorrhizae (AM) spores by 273 and increasing soil available P by 28.24 mg/kg. The P1F4 (natural phosphate with 0.57 g polybag⁻¹) was the best treatment and produced the greater fresh weight and dry weight biomass of sorghum at about 31.86 g and 10.47 g, respectively. The plant height was increased by 5 to 35%, but there was no effect on the number of sorghum leaves.

Keywords: Bone meal, fish meal, mycorrhizae, P availability, rock phosphate

Abbreviations: AM: Arbuscular Mycorrhizae; FCRD: Factorial Completely Randomized Design; P: Phosphate

INTRODUCTION

Indonesia is a major coal mining country using open pit mining, resulting in damaging soil and environment (Nugraha et al. 2024). Coal mining activities will leave many large holes filled with water puddles and with high acid content (Amy et al. 2023; Nugraha et al. 2024). Open coal reduce soil biodiversity and lead to a loss of watershed functions, landform modification, and the release of metal(loid)s, so that land reclamation are required to minimize negative impacts (Amy et al. 2023; Nugraha et al. 2024). Harmful elements when in excess (i.e., sulfur, mercury, manganese, sulfuric acid, and lead), have been detected in aquatic environments near open ex-coal mining activities (Zaini et al. 2019), and low soil nutrient content was detected (Nugraha et al. 2024).

In acidic soils, such as ex-coal mining soil, phosphorous (P) is partly bound by Al and Fe (oxy)hydroxides (Johan et al. 2020; Zhao et al. 2023). P are key players in the formation of nucleic acids and phospholipids and in providing energy for cell division and formation. Additionally, inorganic phosphorus dissolved in the soil can be directly utilized by microorganisms (Cheng et al. 2023), but it can

be a restricting factor for soil microbial activity when limited (Mori et al. 2018). Efforts to improve soil P availability using soil microbe such as the application of Arbuscular Mycorrhizal (AM), are needed. AM are fungi that form a symbiotic relationship with plant roots. They can grow within the root's cortex, on its surface, or around epidermal cells. These fungi produce hyphae that extend into the soil to search for minerals, especially phosphates and nitrates, that promote plant growth (Chauhan et al. 2023).

Chauhan et al. (2023) proposed of using AM for ex-coal mining remediation. However, sourcing of AM from nature has been limited due to the limitation of spores produced and susceptibility to contamination (Huey et al. 2020). AM spore sources in ex-coal mining areas could be developed as a potential source of inoculants as they adapted well to this contaminant condition (Sudová et al. 2007; Huey et al. 2020). But, exploration and evaluation of the benefits of AM spore production from ex-coal mining areas are rarely reported. If any, in such reports, exploration of AM has been collected and identified from various ecosystems (i.e., acid sulfate soil (Higo et al. 2010), acid soil (Alotaibi et al. 2021); metal contaminated soil (Riaz et

al. 2021); and agro-forest soil (Prayogo et al. 2021) in order to improve crop growth, including AM from ex-coal mining soil (Wulandari et al. 2024). AM propagation efforts to obtain a high number of spores for colonization are needed (Huey et al. 2020).

The addition of P from an organic fertilizer can increase the amount of mycorrhizal colonization to a certain extent. Application of 25-50 mg P kg⁻¹ soil gave good results on colonization and spores number, but at levels >50 mg P/kg, the level of colonization decreased, and spores died (Mukhongo et al. 2016). Other alternative materials that contain a large P concentration and are classified as insoluble components include bone meal and fish meal (Panday et al. 2024). Bone meal is left over from slaughterhouses that are difficult to process while fish meal is flour derived from all excess parts of fish, they can be an alternative soil conditioner (Hart et al. 2022). Sorghum was chosen because of its fibrous roots and ability to grow shoots even after fruiting or harvesting. It is easily colonized by mycorrhiza because it has a greater number of fine roots than tap-rooted plants (Prayoga and Prasetya 2021).

The addition of natural phosphate, bone meal, and fish meal to mycorrhizal propagation media can be the right choice to produce the optimal number of mycorrhizal spores and increase soil P-availability (Adiningsih and Sitorus 2017) so that it can be utilized as an agent in remediating former coal mine land. Moreover, the acidity of technosol pH will induce Al phytotoxicity and improve phosphate binding to Al. This study aims to increase the P availability through the addition of different phosphorus sources and doses under arbuscular mycorrhiza inoculation on sorghum. The benefit of these applications will be indicated by the increase in plant growth and the number of arbuscular mycorrhizal fungal spores.

MATERIALS AND METHODS

Place, time, and materials

The research was conducted from April to November 2022. Soil chemical and biological analyses were conducted at the Department of Soils, Faculty of Agriculture, Universitas Brawijaya, Malang, Indonesia. Soil sampling of planting media and AM isolation were collected from ex-coal mining soil at Samboja Sub-district, Kutai Kartanegara District, East Kalimantan, which was previously recognized as a bare marginal land. Various types of grasses covered it with topographic conditions as moderately dissected and located at 500 m above sea level. Historically, the mining activities have been conducted for 15 years, which removed all topsoil and excavated underneath coal deposits. The isolation of mycorrhizal spores was conducted from composite soil sampling within the area of 65.50 ha; the mycorrhizae have been characterized as a single strain that has been adapted to the coal mining site. Based on the Schmidt and Ferguson climate type (Yasa et al. 2022), the research location has climate type A with a value of Q = 10.8%, rainfall ranges from 2000-2500 mm/year, and air temperature ranges from 20-30°C. The experiment was conducted under the controlled greenhouse

system including mycorrhiza propagation activities and sorghum cultivation.

Materials for mycorrhizal propagation were planting media, natural rock phosphate, bone meal, fish meal, *Glomus* spp. spores collected from the above location (Prayoga and Prasetya 2021), and local sorghum seeds selected from the local variety (Numbu variety). The natural phosphate used was produced by CV. Javamas Agrophos (a local producer), while bone meal and fish meal were obtained from UD. Bintang Terang (local distributor).

Soil preparation and mycorrhizal fungal spore isolations

Soil samples were collected from the rooting area of bull grass vegetation (*Paspalum fasciculatum* Willd. ex Flügge) at a depth of 0-20 cm. A total of 200 kg of ex-coal mine soil, which has been operated for 15 years and degraded, was taken, and then composited samples were collected and put into sacks. The soil was air-dried, sieved at a 2 mm sieve, and then sterilized by the steam method in a steamer at a temperature of 90°C for 4 hours on 2 consecutive days with a day break (Kozioł et al. 2017). The spore extraction technique used was wet sieving, and it was not conducted on sterilized soil. Each polybag was inoculated with 20 spores of *Glomus* spp.

Soil analysis

Soil pH was detected using a pH meter (Mettler Toledo F-20 series) calibrated using pH buffer solutions. The determination of soil C-organics used the Walkey and Black method. Total P was determined using the HNO₃ extraction method and detected using a spectrophotometer. The available P was conducted following the Bray II technique. Soil water content was determined using gravimetric methods.

Host plant preparation and AM propagation

Mycorrhiza propagation was done using the open pot method. This method was carried out for field tests and to ensure that the seedlings were truly colonized by mycorrhiza. The sorghum seeds used were sown for 7 days and then inoculated with mycorrhizal spores. These polybags were placed inside the greenhouse with regular irrigation using sterilized water, which was maintained at field capacity conditions.

Plant growth

Sorghum growth was routinely monitored every two weeks at 14, 28, 42, and 56 Days After Transplanting (DAT) by measuring plant height and number of leaves. At 56 DAT, the plants were cut to the base of the sorghum stem to determine the fresh weight (after taking a certain number of roots to observe mycorrhizal colonization) and the dry weight of sorghum biomass. Finally, 100 g of fresh sorghum biomass was collected and placed in the oven at 70°C for 24 hours to correct for determining moisture content.

Design, research parameters, and data analysis

The study used a Completely Randomized Factorial Design (CRFD) with the type of P source (P) as the first factor consisting of 3 treatment levels (natural phosphate,

bone meal, and fish meal) and a second factor of P source dose (F) consisting of 5 treatment levels (control; 0.17 g/polybag; 0.29 g/polybag; 0.40 g/polybag; and 0.57 g/polybag). The soil volume of each polybag was 2 kg, each inoculated with 20 mycorrhizal spores. Based on the design, there were 15 treatment combinations and 4 replications. Observation parameters included the number of spores, AM colonization of roots on roots, soil pH, soil P-total, soil P-available, plant height, number of leaves, fresh weight, and dry weight of sorghum. Data tabulation used Genstat and SPSS software, and further testing utilized Duncan's Multiple Range Test (DMRT) 5%.

Root colonization protocol

Root samples were collected at 56 DAT post-inoculation with AM fungi, washed with sterile water, and analyzed for AM colonization following a method described by Berruti et al. (2013) before their small fragments (1 cm) and placed into microscope slides using 36 root fragments. AMF structures were examined from cotton blue-stained roots under 40 x magnification of a DP70 Olympus camera.

RESULTS AND DISCUSSION

Soil properties and phosphate source analysis

Basic soil analysis aims to determine the initial soil condition before treatment (Table 1). Phosphate source analysis was also conducted to examine the chemical characteristics of the materials used as treatments (Table 2). This former coal mine soil has soil physico-chemical properties that can be a limiting factor for plant growth, including pH 3.67 (very acidic), C-organic 1.3% (low), and soil P-total 91.26 and soil available P 3.59 mg kg⁻¹ (very low) and sandy clay loam soil texture. For comparison, the ex-coal mining technosol in Tapin-South Kalimantan has a low pH value of 4.79 (acidic) and C-organic content of 1.46% (very low), total soil P of 342.1 mg kg⁻¹ (low), total soil K of 8.85 mg kg⁻¹ (very low), CEC of 82.7 cmol+kg⁻¹ (low), total base saturation of 57.11%, and Al levels of 82.7 cmolkg⁻¹, which mean that the CEC is saturated with Al, limit sorghum growth and increase metal toxicity (Sukarman and Gani 2020).

Due to the low P level, Al phytotoxicity occurred. Phosphorus is a nutrient that is needed by sorghum plants and is the main limiting factor for sorghum plant production after nitrogen (Ajeigbe et al. 2018; Getinet and Atinafu 2022). The element P is needed for, among other things, the processes of photosynthesis, respiration, and generating energy in plant life activities (Flatian et al. 2018), and for the life of microorganisms, it is very important for growth and the formation of alkaline-phosphatase enzymes (Xie et al. 2019). Providing phosphate sources in the form of natural phosphate, bone meal, and fish meal can increase the P element in the soil. Here, total P concentrations in natural phosphate, bone meal, and fish meal were respectively 14.44% (quality C), 0.49% (quality I), and 0.79% (quality I) (Table 2).

P deficiency in crops could limit dry matter accumulation in leaves and branches (Feng et al. 2021). It

was also shown that low P levels inhibit plant growth. The absorption of other nutrients and photosynthetic performance decrease while the production of reactive oxygen compounds increases, so plant productivity is affected (Silva et al. 2023). When Al and Fe fix available P in the form of Al-associated P and Fe-associated P (Hosokawa et al. 2022) in the soil, it becomes unavailable to plants. Various sources of P in fertilizer have been proven to improve sorghum yield and growth (Flatian et al. 2018; Getinet and Atinafu 2022); however, the P source utilization from the fish and bone meal were rarely reported.

AM spore density

The treatment combination P3F3 (fishmeal with a dose of 0.40 g polybag⁻¹) produced the highest spore number at 299.2 spores 100 g soil⁻¹ (Figure 1). Our results were supported by Viandari et al. (2019), showing that the application of POC (liquid organic fertilizer) from fish waste resulted in soil P-availability of 46.30 mg kg⁻¹ with an increase of 2.76 times from the control. The application of fish meal in as much as 0.40 g polybag⁻¹ soil led to the highest spore number, and then this one decreased at a dose of 0.57 g polybag⁻¹. AM spore density was significantly affected by the increase in phosphate dosage and source. Fish meal application resulted in higher AM spore density compared to bone meal and natural rock phosphate. This is due to the rapid decomposition of this material. However, the highest AM colonization was detected under natural phosphate application. The highest AM spore density was found under fish meal treatment (P3), which for the greater AM colonization was obtained from rock phosphate treatment (P1). The different rate of substrate decomposition may influence AM spore density and their colonization in the sorghum root system.

A previous study identified that the addition of AM inoculum has affected AM spore density in corn cultivation (Ishaq et al. 2021). Spore density of AM per 100 g of soil in the rhizosphere of corn without inoculation (M0) was lower (30-40 spores 100 g soil⁻¹) than when inoculated with single inoculum (M1) (between 70 to 130 spores 100 g soil⁻¹) or mixed inoculant (M2) (80-90 spores 100 g soil⁻¹) at four levels of inorganic P fertilizer (P1 to P4) (Ishaq et al. 2021), which coincided with the range of AM spore density in this study. The P use in a slow-release form, such as natural phosphate, is more prudent and can give good results in the multiplication of mycorrhizal spores because it dissolves gradually (El-Sherbeny et al. 2022).

Table 1. Soil properties

Parameters	Coal mined land	Criteria *
pH (H ₂ O)	3.67	VA
C-Organic (%)	1.3	L
BO (%)	2.26	L
P-total HCl 25% (mg/kg)	91.26	VL
P-available (mg/kg)	3.59	VL

Notes: Source: (*) Soil Research Center 2009. VA: Very acidic; A: Acidic; SAC: Somewhat acidic; N: Neutral; SAL: Somewhat Alkaline; A: Alkaline; VL: Very low; L: Low

Table 2. Phosphate source properties

Parameter	Natural phosphate	Criteria *	Bone meal	Criteria **	Fish meal	Criteria **
P-total HNO ₃ + HClO ₄ (%)	14.4	Quality C	0.49	Quality I	0.79	Quality I
Water content oven 105°C (%)	9.59	Quality A	0.85	Quality I	6.54	Quality I

Notes: Source: (*) SNI 02-3776-2005; (**) SNI 01-2715-1996

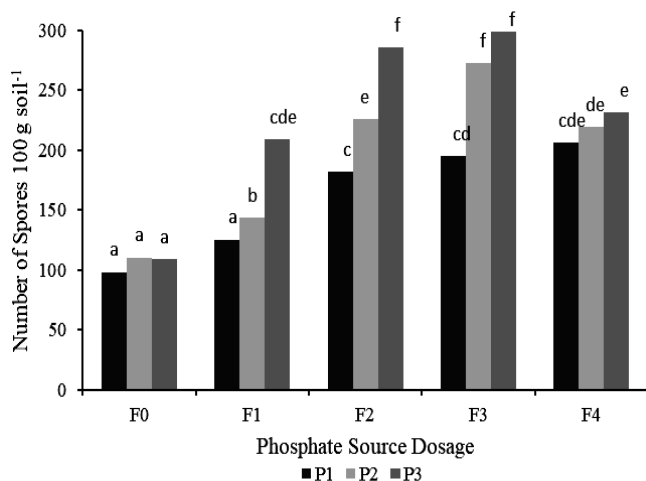


Figure 1. Average number of mycorrhizal spores at 56 DAT/100 g soil. Note: Numbers followed by the same letter indicate no significant difference (DMRT 5%)

The P content in bone meal and fish meal is not released easily since the components contain a long chain of protein and calcium phosphate (Cheluvraj et al. 2020). This characteristic makes the fertilizer based on such materials available slowly and gradually compared to an organic form. P availability affects the growth of pameo (*Citrus maxima*). (Meng et al. 2021), rice (*Oryza sativa*) (Mori et al. 2016), corn (*Zea mays*) (Jia et al. 2018), common bean (Namugwanya et al. 2014), cowpea (Jemo et al. 2017), coffee (Prayogo et al. 2021) and soybean (He et al. 2019).

The impact of P sources, in addition to sorghum, has been published and becoming a major interest in many studies (Sun et al. 2017; Wang et al. 2019; Kumar et al. 2024; Matheus et al. 2024); however, the comparison of a different material of P sources, particularly fish and bone. The impact of combining the application of AMF and different types of P sources to AM spore and sorghum growth and productivity was determined by the source of phosphate and dosage. As expected, mycorrhizae have many benefits to crops. In this study, the increase of AM colonization in the sorghum root system influenced sorghum growth and biomass under the different sources and dosages of phosphate. The AM colonization has a strong relationship with the AF number of spores. However, some studies also reported that there was no impact on sorghum performance (Sun et al. 2017). Previous findings by Wang et al. (2019) showed that the different levels of salinity affected AM spore and colonization. AM proved to extend the crop root system by producing hyphae, which then the

hyphae improved nutrient uptake by scavenging nutrients, particularly P, which was far from the root system.

According to Campos et al. (2018), mycorrhizal plants have more massive roots so that they can reach wider soil (Huey et al. 2020). The main advantage of mycorrhizal symbiosis for plants is the effective provision of P through mycorrhizal pathways in large volumes directly to cortical cells (Bagyaraj et al. 2020; Wilkes 2021). Mycorrhizal external hyphae will reach areas outside the depletion zone that roots cannot reach, reducing the impact of P depletion and improving plant nutrition (Jiang et al. 2020). In this study, soil P was greater under the natural phosphate application compared to bone and fish meal, which triggered higher AM colonization. The findings prove that a higher concentration of soil P will raise AM colonization.

AM colonization of roots

The P1F3 treatment (natural phosphate at a dose of 0.40 g polybag⁻¹) produced the highest percentage of AM colonization at 93% (Figure 2) compared to bone and fish meal application. Under the highest natural phosphate application, the AM colonization declined. The AM colony in the root system is a complex process; the colonization was also affected by the rising soil pH and soil P availability. According to Hindumathi and Reddy (2011), sorghum plants of several cultivars produced colonization values ranging from 56.5 to 83.9%, which was comparable to AM colonization in this study. Colonization levels of AM at 24 days after application ranged from 3 to 5% and raised to about 27 and 44% at 45 days after application, which was lower than AM colonization in this study. Colonization of AM was similar under differences in sorghum cultivar or P addition (Lendzemo et al. 2007).

The highest AM colonization was in P1F3, but the highest number of spores was in P3F3. This was because the value of P-available in the natural phosphate treatment at the beginning of planting was high. Mycorrhiza was not optimal in producing spores, and the pH was also low. The highest concentration of P under natural phosphate application may reduce AM spore production but, in the longer term, improve AM colonization, but in higher dosages of P sources, that colonization declined. A similar trend of AM root colonization was also detected under different treatments of Na (Wang et al. 2019).

The addition of P fertilizer in high doses can reduce mycorrhizal colonization of roots, especially by inhibiting the formation and development of structures such as arbuscules and spores (Liu et al. 2016). Spore production is influenced by many factors (Pangaribuan 2014), including the type of host and the exudates it produces. Based on the nature of phosphate availability and its environment, strategies, methods, and technologies are

needed to improve the efficiency of using and applying fertilizers to plants. In terms of phosphate absorption efficiency, the activity of microorganisms that can increase phosphate solubility is needed, one of which is the beneficial application of arbuscular mycorrhiza and their interaction to increase host plant growth (Bagyaraj et al. 2015; Prayogo et al. 2021; Silva et al. 2023).

Soil pH

The soil pH values tend to increase with increasing doses of phosphate source (Table 3). The phosphate source P3 produced the highest pH value of 5.31 (acidic). The pH value (5.42) at the highest phosphate source dose was classified as acidic in the F4 treatment. Additionally, the use of bone and fish meal increases pH up to two units compared to the original soil (3.67). The ability of bone (P2) and fish meal (P3) to raise pH is higher compared to natural phosphate rock (P1).

Soil pH is an important factor in the process of dissolving P from the bone fraction, as the solubility of P in the soil is influenced by soil pH (Penn and Camberato 2019). Dissolution in the bone requires H^+ ions, so P in the bone fraction will dissolve faster under acidic conditions. However, the more soluble Al^{2+} has more opportunity to bind available P quickly. The use of phosphate rock and microbes (such as phosphate-solubilizing bacteria) can affect nutrient availability, increase plant length, biomass, and uptake of N, P, and K. It can also lower Na^+ and Cl^- availability and pH and increase soil enzymes and microbial diversity (Nacoon et al. 2021).

The increase in soil pH due to fishmeal reported by Adiningsih and Sitorus (2017) from 4.5 to 6.9 was due to the increasing dose of fish bones and the increasing Ca content. The high Ca content causes the pH value to change because Ca has alkaline properties and Ca could reduce toxicity and the availability of Al^{3+} . Fishmeal, with its potential to increase soil pH, offers a hopeful prospect for agricultural improvement. Fishmeal increases soil pH because Ca^{2+} in fertilizer will replace H^+ and Al^{3+} so that the concentration of OH^- increases (Panday et al. 2024). On the other hand, P is bound by calcium (Ca) elements but still becomes an essential factor for supporting the growth of both vegetative and generative phases of plants (Xie et al. 2019). Bonding Ca-P in calcareous soil can be reduced by the application of organic sources of P and biofertilizers to improve corn yield (Prayogo et al. 2019).

Total P soil

Phosphate source type P1 had the highest P-Total value (125.29 $mg\ kg^{-1}$), classified as low. Phosphate source dose F4 displayed the highest P-Total of 122.18 $mg\ kg^{-1}$, classified as low but for timer higher than control (Table 4). The greater the dose given the greater the total soil P (Table 4). The phosphate source of P1 and phosphate source dose at F4 showed the highest total soil P value due to high concentrations of P_2O_5 in that material (natural rock phosphate) - as much as 82.31 $mg\ kg^{-1}$. This finding highlights the significant impact of phosphorus application on soil P levels; the more phosphorus applied, the more

phosphorus is bound by the soil so it will increase P-total soil. When viewed as a whole, the soil P-Total value at 56 DAT decreased compared to the beginning of planting. This indicates the role of mycorrhiza in the process of improving P uptake through extending crop root systems.

Available soil P

The P1F4 treatment (natural phosphate with 0.57 $g\ polybag^{-1}$) produced the highest P-available value of 34.89 $mg\ kg$. Higher doses of phosphate also increased the P-available value (Figure 3). The increase in P-available value was caused by the addition of P fertilizer or under the application of soil conditioner where the P contained therein was released slowly in the soil (Flatian et al. 2018; He et al. 2019; Getinet and Atifanu, 2022). The use of natural rock phosphate, a naturally occurring mineral that is high in phosphorus, with AM was able to increase plant growth and production beyond the use of each separately has been shown to improve legume (*Centrosema pubescens*) productivity (Indriani et al. 2016).

Table 3. Effect of treatments on soil pH

Treatment	pH
Types of phosphate sources	
P1	4.98 a
P2	5.16 ab
P3	5.31 b
Phosphate source dosage	
F0	4.68 a
F1	5.10 b
F2	5.22 bc
F3	5.35 bc
F4	5.42 c

Note: Numbers followed by the same letter indicate no significant difference (DMRT 5%)

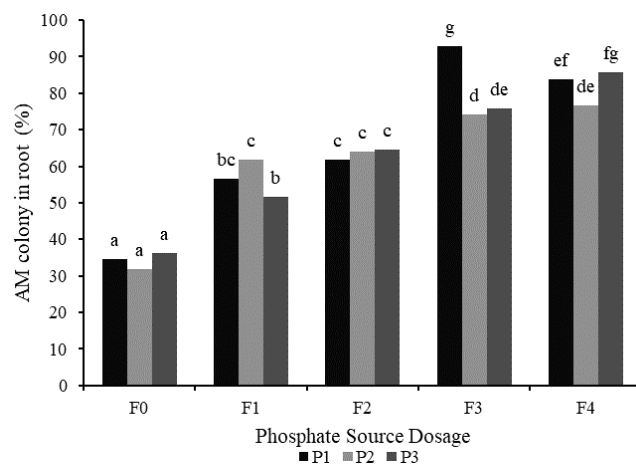
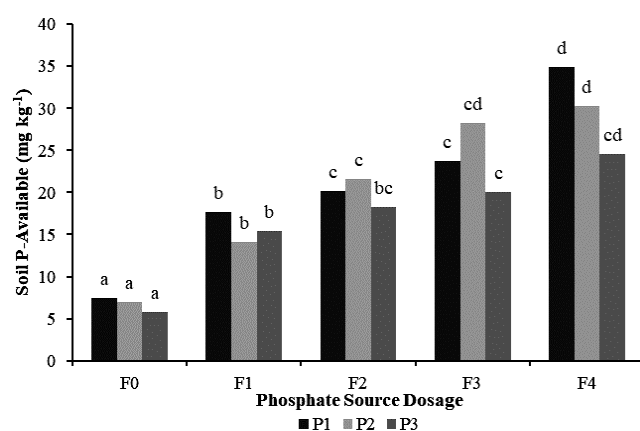


Figure 2. Average AM colonization at 56 DAT. Notes: Numbers followed by the same letter indicate no significant difference (DMRT 5%)

Table 4. Effect of treatment on total soil P

Treatment	Total soil P (mg/kg)
Types of phosphate sources	
P1	125.29 c
P2	93.97 b
P3	77.40 a
Phosphate source dosage	
F0	40.06* a
F1	73.07 b
F2	98.99 c
F3	122.18 d
F4	160.14 e

Notes: Numbers followed by the same letter indicate no significant difference (DMRT 5%). *: Soil P content without phosphate addition

**Figure 3.** Mean values of available soil P at 56 DAT. Note: Numbers followed by the same letter indicate no significant difference (DMRT 5%)

In the following year, only AM played a significant role in plant growth and production of the Barley system (Campos et al. 2018). In this study, the addition of natural rock phosphate and the greater dosage of that application significantly improved soil P availability by 20% higher than that addition of particularly at the highest dosage of application (F4). This may be due to the excess of nutrients such as Ca or Mg, which maintains the level of soil pH close to a neutral condition and binds the remaining Al^{3+} .

The previous findings showed that the application of AM in the NK treatment has a similar effect on plant growth, grain yield, and P uptake to the NPK treatment and increased available soil P concentrations 40 days after application. This evidence indicated that inoculated roots mobilize more P, and plants have a greater opportunity to absorb more P from soil (Cozzolino et al. 2013).

This study also revealed the higher dosage of P source exaggerated soil P availability. Similarly, the increasing dosage of P fertilizer showed improved wheat grain yield. Moreover P efficiency was detected under low P application than the higher P application, whereas the *Glomus* spp. application was more effective than the indigenous

mycorrhizae under wheat cropping systems (Ortas and Bykova 2018).

The presence of mycorrhiza can help increase the mineralization of inorganic nutrients in plants through the formation of hyphae that reach deep into the soil so that P elements become available within modified crop root systems (Suharno et al. 2017; Sun et al. 2018; Suparno et al. 2023). According to Kumar et al. (2018), mycorrhiza can improve soil quality by improving soil aggregation, providing P nutrients, and being resistant to drought conditions. The development of AM in plants will help convert organic P into inorganic P ($H_2PO_4^-$ and HPO_4^{2-}), which plants can absorb (Sun et al. 2018; Jiang et al. 2020)

Plant height

Sorghum plant height increased every week from 14 DAT to 56 DAT (Table 5). At the ages of 14 and 28 DAT the treatment combination did not significantly affect sorghum plant height. This was due to the slow-release nature of the phosphate fertilizer given and the time it would take to dissolve the phosphorus in it. At the age of 42 DAT, the P1F4 treatment (natural phosphate with 0.57 g polybag⁻¹) showed the highest plant height (67.75 cm). The highest plant height at the age of 56 DAT was in the P1F3 treatment (100.88 cm). Overall, the application of phosphate fertilizer types and doses increased the height of sorghum plants compared to the control after 42 DAT.

In general, the application of AM with the addition of natural rock phosphate has a better impact on increasing plant height than bone and fish meal material; this was due to the long-term effect and slow release of P from the natural rock phosphate. Evidence showed that the increase in natural rock phosphate dosage raised plant height by 30%, from 71.12 cm to 100.88 cm at 56 DAT (Table 5) by 20%. Here, the plant height was lower than the previous results of Sowmen et al. (2019), in which sorghum was treated with natural phosphate 0.51 g polybag⁻¹ and reached 155.3 cm. In another study, sorghum height under the application of P ranging between 0, 15, 30, 45, and 65 kg ha⁻¹ was between 175.6 cm and 216.5 cm (Ajeigbe et al. 2018).

Natural phosphate, such as rock phosphate, applied to the planting media, supplies P into the soil. This phosphorus has a major role in the plant processes and metabolic pathways of biosynthesis, such as respiration, cell division photosynthesis, early flowering, seed maturity, and root development (Sharif et al. 2014; Mansyur et al. 2021). Mycorrhiza helps plants absorb nutrients, especially P, so that their growth increases, while plants provide carbohydrates in return (Nuridayati et al. 2019; Jiang et al. 2020). The application of AM with a combination of composts of farmyard manure, humic acid, and press mud showed the greatest plant height of 147 cm, which was higher than the sorghum height in this study (100.88 cm) (Table 5).

Bone meal and fish meal were more efficient due to their abundance and availability. These materials were recognized as residue from marine activity or the waste of fish factories and processing, which was rarely used for soil amendment material enriched with P sources. Other than that, it has a lower price compared to natural rock phosphate.

Number of leaves

The type of phosphate source did not significantly affect the number of leaves at all observation times, while the dose of phosphate source significantly influenced the number of leaves at 42 and 56 DAT (Table 6). Our present results agreed with those of a previous study by Togatorop et al. (2021), which stated that the addition of bone meal doses of 0.15-0.50 g polybag⁻¹ increased the number of leaves of sorghum plants to a range of 6.38 to 6.58. Phosphorus has a major influence on the process of photosynthesis and will trigger the growth of new leaves in response to the availability of P in the soil (Sun et al. 2018; Feng et al. 2021; El-Sherbeny et al. 2022). When metabolic processes run well, this triggers the formation of vegetative tissue (leaves) and increased cell size so that the plant size and the number of leaves increase (Feng et al. 2021).

A well-functioning photosynthesis process will then spur the formation of carbohydrates and proteins, thereby increasing plant growth and productivity (Mansyur et al. 2021). Muhammad et al. (2018) that measurements on the number of leaves of sorghum were more than two times higher compared to the result of this study, with the highest number of leaves attained in plants treated with phosphorus in the form of NPK fertilizer at the dose of 45 kg ha⁻¹ (15.89 cm), while the lowest was obtained without phosphorus source at 60 days after sowing (11.78 cm).

This difference may be due to different sorghum varieties, genetic factors, climatic factors, and management. Novri et al. (2015) mentioned that genetic factors are the major factors that influence sorghum growth and yield, whereas different genotypes have varying advantages in utilizing environmental factors such as water, light, and nutrients, which affect plant development and yield (Jaenudin et al. 2023).

Plant fresh weight

The P1F4 treatment (natural phosphate 0.57 g polybag⁻¹) produced the highest root + shoot fresh weight of 31.86 g plant⁻¹ at 56 DAT. The root + shoot fresh weight of sorghum plants significantly increased by 66.85% compared to the control (Figure 4).

Plant dry weight

The P1F4 treatment (natural phosphate with 0.57 g polybag⁻¹) produced the highest root + shoot dry weight of 10.47 g plant⁻¹ (Figure 5). The application of natural phosphate to sorghum by Salam et al. (2016) showed that the dry weight of plants could reach 23.81 g with an increase of 18.43% compared to the control. Previous findings by Silveira et al. (2018) showed that the application of natural phosphate rock under the combination of with limestone (43.34 g plant⁻¹) and without limestone (53.82 g plant⁻¹), which was 4 times higher than dry weight sorghum biomass of this study. As rock phosphate may contain other minerals, such as Ca and Mg, they can raise soil pH by reducing the excess of Al³⁺ and Al/Fe phosphate bindings. This material was slowly degraded, so that the release of P and other nutrition (Ca, Mg) from rock phosphate is gradual, which secures plant nutrient requirements during the period of sorghum growth.

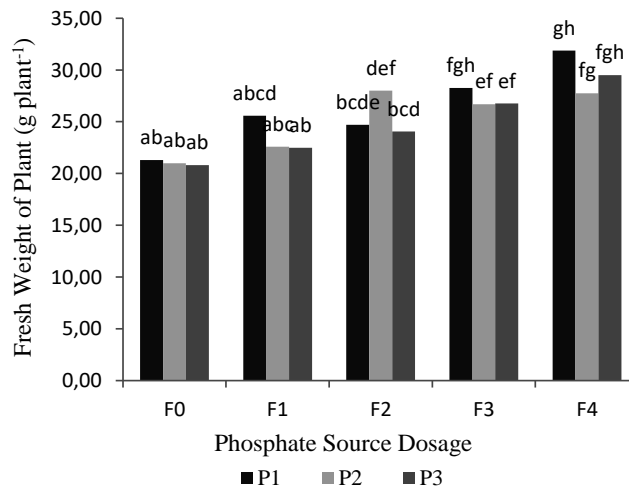


Figure 4. Plant fresh weight at 56 DAT. Note: Numbers followed by the same letter indicate no significant difference (DMRT 5%)

Table 5. Average value of sorghum plant height

Treatment	Plant height (cm)			
	14 DAT	28 DAT	42 DAT	56 DAT
P1F0	12.35	18.75	41.18 ab	71.12 a
P1F1	16.13	24.88	44.80 abc	93.00 cde
P1F2	14.70	23.63	55.08 de	76.75 ab
P1F3	14.95	22.75	60.73 ef	100.88 e
P1F4	15.05	23.13	67.75 f	97.62 de
P2F0	13.60	18.25	40.88 a	71.83 a
P2F1	16.83	23.55	51.20 cd	87.00 bcd
P2F2	16.20	22.88	48.08 abcd	81.88 abc
P2F3	16.55	24.25	50.50 cd	82.62 abc
P2F4	15.28	24.38	53.85 de	87.00 bcd
P3F0	10.13	16.53	42.33 ab	72.65 a
P3F1	14.18	19.00	48.50 abcd	74.17 a
P3F2	14.35	18.85	49.25 bcd	74.25 a
P3F3	16.28	19.58	50.75 cd	76.00 ab
P3F4	14.13	23.70	52.50 cd	79.31 ab

Notes: Numbers followed by the same letter indicate no significant difference (DMRT 5%), DAT: days after transplanting

Table 6. Average values of leaf number of sorghum plants

	Number of Leaves			
	14 DAT	28 DAT	42 DAT	56 DAT
Types of phosphate sources				
P1	3.95	3.75	6.05	5.95
P2	3.90	3.55	6.30	6.30
P3	3.75	3.85	6.20	6.20
Phosphate source dosage				
F0	3.75	3.58	5.67 a	5.50 a
F1	3.92	3.83	6.08 b	6.08 b
F2	3.83	3.58	6.58 c	6.25 bc
F3	3.83	3.58	6.17 bc	6.33 bc
F4	4.00	4.00	6.42 bc	6.58 c

Notes: Numbers followed by the same letter indicate no significant difference (DMRT 5%), DAT: days after transplanting

Relationship between AM root colonization and spore count

We observed that the higher the number of AM colonies produced, the higher the number of spores with a coefficient of determination ($R^2 = 0.47$) (Figure 6). Mycorrhizal development begins with colonization to form structures within the roots. The degree of colonization depends on the availability of elements, especially nitrogen and phosphorus. Mycorrhiza increases plant nutrient uptake by expanding the absorbing surface of the roots. The first stage of mycorrhizal infection in plants begins with plant roots releasing sugars and hormones to form associations with mycorrhizal spores so that the spores begin to germinate (Huey et al. 2020). The next stage will form an appressorium so that hyphae can penetrate the root cortex and form an arbuscular structure (Widyati 2017).

The application of P fertilizer efficiently supplies P nutrients in the planting media. Mycorrhiza utilization significantly increases the availability of this phosphorus in the soil by expanding crop root systems with the development of AM hyphae; its structures, such as external hyphae, can develop very extensively in the soil and expand the scope of nutrient absorption (Liu et al. 2016; Ishaq et al. 2017; Nuridayati et al. 2019). In the root zone of plants, a P depletion zone will be formed due to limited available phosphorus. P accumulated in the external hyphae will be converted into orthophosphate compounds with the help of phosphatase enzymes. This compound will then be forwarded to the arbuscule, which is then broken down into inorganic phosphate and distributed throughout the plant tissue (Nurhayati 2019; El-Sherbeny et al. 2022).

In addition, the mycorrhiza used in this study came from one strain of the *Glomus* sp. genus, which has a good level of adaptation to various environments. According to Hasyati et al. (2018), *Glomus* sp. has a wide distribution level with high adaptation because it is tolerant of extreme environments and develops well at pH 4-6.

P-available soil and number of spore relationship

The presence of mycorrhiza can help increase soil P-availability. The organic form of P will be converted into inorganic P that plants can absorb through the mineralization process. Mycorrhizal hyphae can access root pores where root hairs cannot penetrate due to their smaller diameter (20-50 μm). The maximum number of spores was obtained when soil P-availability reached 28.58 mg kg^{-1} , resulting in a spore count of 235 spores 100 g soil^{-1} (Figure 7). Based on the results of soil P-availability analysis at 56 DAT, P2F3 treatment was close to the maximum value. The P2F3 treatment gave good results on the number of spores (273), AM colonization of the roots of 74.25% (high), and soil P-availability of 28.24 mg kg^{-1} (very high). Symbiosis of plants with mycorrhiza will occur when the (available) soil P amount is low; if phosphorus in the soil is too high, mycorrhiza will not develop and colonize the roots. When high phosphorus in the soil, mycorrhiza will go dormant, becoming ineffective (Huey et al. 2020).

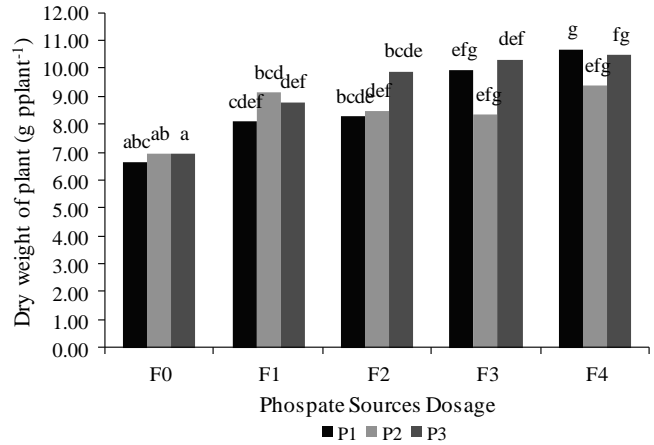


Figure 5. Plant dry weight at 56 DAT. Note: Numbers followed by the same letter indicate no significant difference (DMRT 5%)

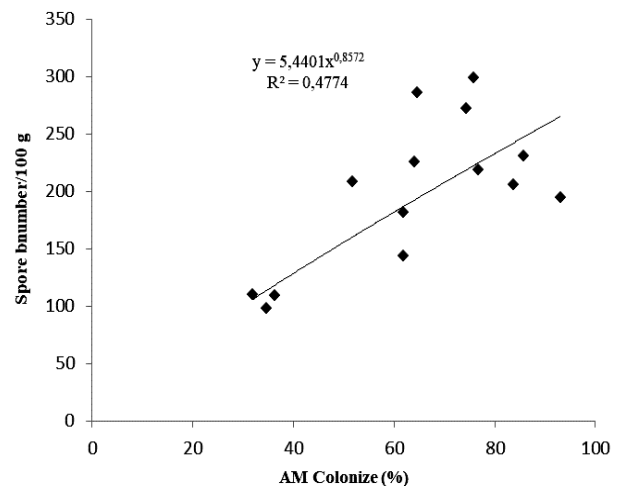


Figure 6. Relationship between AM colony and spore density

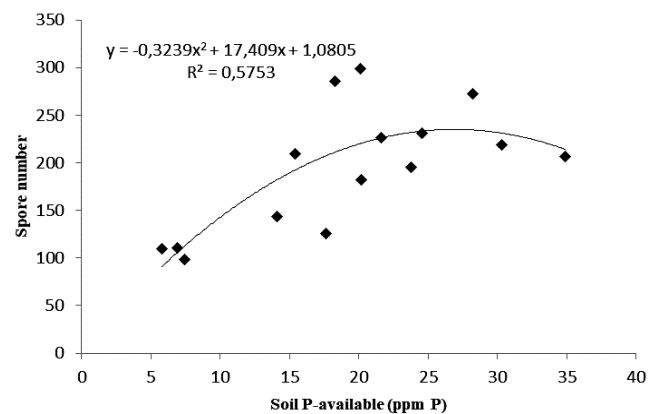


Figure 7. Relationship between P-soil availability and number of spores

High soil P concentrations will inhibit the development of AM colonization and reduce AM plant symbiosis. High P-available concentrations will result in slower infection, reducing the ability of AM to form spores, arbuscular, vesicles, and other mycorrhizal structures, except near plants that have a very high level of dependence on mycorrhiza (Vosnjak et al. 2021). Tenzin et al. (2022) suggest using a variety of host plants to be able to obtain spores more efficiently. The relationship between soil P available and AM spore number is presented in Figure 7. That relationship was followed by a polynomial curve, in which it was indicated that the increasing soil P-available will be followed by the raising of the AM number of spores until it reaches a maximum value before it declines in higher concentration ($R^2 = 0.57$) (Figure 7).

Acidic soil conditions are an environment favorable for mycorrhiza because they have acidophilic properties. Soil pH is important in relation to the nature of P in the soil, which easily reacts with elements such as Al and Fe in acidic soils (Jia et al. 2018; He et al. 2019). P is one element that sorghum needs abundantly besides N. Sufficient P in plants can help accelerate growth and form cells in roots and growing shoots, but when P deficiency occurs, it can inhibit the emergence of new shoots, the number of leaves is small, and the plants are short (Jiang et al. 2019; Crous and Ellsworth 2020; Mansyur et al. 2021). Mycorrhiza tends to increase symbiosis with plants and form more spores under stressful conditions (Prasetya and Ukhtansyah 2020). In this study, the acidity was due to the excess of Al^{3+} and lack of organic matter, P availability, and the existence of other nutrients such as Ca and Mg. As the soil pH raising as the impact of the addition of rock phosphate or bone/fish meal, the AM number of spores also increased before it declined in higher soil P concentrations. This may be due to the soil P availability having reached the saturated condition for AM to produce spore or the limitation of the size of the media.

In conclusion, the results of the present study revealed that the application of AM in combination with natural rock phosphate, bone meal, and fish meal constitutes a feasible and sustainable strategy to enhance the availability of P, but also a suitable method to reclaim former coal mines for agriculture production following sorghum cultivation. The natural rock phosphate with higher dosage proved to increase AM colonization, which then affected plant height, number of leaves, fresh and dry roots, and shoot biomass. The wider perspective was related to the opportunity strategies for options to better land management of ex-coal mining soil, which has low pH, lack of organic matter, and P sources. The soil amendment use is considered a massive waste from the fish industry (bone and fish meal) as potential sources of P need to be used and applied to degraded ex-coal mining soil.

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