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Effects of phosphate-solubilizing fungi on phosphorus availability, retention, and soil chemical properties in shallot cultivated on andisols of North Sumatra

Abstract. The availability of phosphorus (P) is one of the main limiting factors for plant productivity in Andisol soils due to the strong fixation of P by allophane clay minerals, aluminum and iron oxides. Giving the phosphate solubilizing fungi (PSF) is an alternative to increase available P which plants can use to increase the yield. This study aimed to evaluate the effectiveness of phosphate-solubilizing fungi (PSF) in improving P availability on Andisol. The experiment used a block randomized design (BRD) with three replications. The first factor is Andisol soil origin (Soil 1 and Soil 2) and the second is isolate of phosphate solubilizing fungi (*Aspergillus niger* P13, *A. niger* P21, *A. pseudodeflectus* BJ21, and *A. niger* BJ23). The research results showed that Soil 1 generally produces higher growth and yield of shallots than soil 2. *A. pseudodeflectus* BJ21 can increase available P by 48.46% and *A. niger* P13 by 45.71% compared to without phosphate solubilizing fungi. *A. niger* P13 can reduce P retention by 18.48% compared to without phosphate solubilizing fungi. *A. niger* BJ23 can increase plant P uptake by 35.35% compared to without phosphate solubilizing fungi. Inoculation of isolates *A. niger* P13 and *A. pseudodeflectus* BJ21 was able to adapt and grow well on Andisol soil which significantly increased the population compared to the control. Indigenous phosphate-solubilizing fungi (*A. niger* and *A. pseudodeflectus*) improved P availability and uptake in Andisol, reduced P retention, adapted well to the soil environment, and showed strong potential as environmentally friendly biofertilizers based on local resources.

Keywords: *Allium cepa* L · *Aspergillus niger* · *Aspergillus pseudodeflectus* · Phosphat · Yield.

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Introduction

Andisol (Andosol) is a volcanic ash–derived soil characterized by the dominance of short-range-order (amorphous) minerals such as allophane, imogolite, and ferrihydrite as well as Al–humus complexes, which confer unique physical and chemical properties including low bulk density and high porosity, high water-holding capacity, and a dark, friable surface horizon rich in organic matter with relatively high cation exchange capacity. These colloidal constituents also influence organic carbon stabilization through organo-mineral complexation, which is generally fertile but phosphorus-challenged (Takahashi & Dahlgren 2016).

Phosphorus (P), an essential nutrient, is responsible for photosynthesis, root formation, flowering, and bulb formation in shallots. Crop production is often affected by phosphate deficiency in many agricultural systems, particularly in Andisol soils, which are formed from volcanic ash and rich in allophane minerals and Al/Fe oxides, leading to high phosphate retention and reduced phosphate availability to plants (Marpaung et al., 2024a).

Most of the P nutrients found in soil are insoluble. Soil contains only around 0.1% of all plant P (Zhu et al., 2018). (Mehta et al., 2013), states that the biogeochemical cycle's fixation reaction mechanism results in cation deposition in the soil, immobilization, adsorption, and interconversion to organic forms. Large soil P reserves exist, but they are not readily available (Kishore et al., 2015). In order to get the best possible crop output, this leads farmers to apply a lot of inorganic phosphate fertilizer (Sharma et al., 2015). Low phosphate (P) availability is a concern in almost all tropical soils, including Andisol. High phosphate retention is caused by Andisol 97.8% concentration of allophane, the most reactive clay mineral (Sukarman & Dariah, 2014). Phosphate adsorption thus reduces the amount of accessible phosphate (P) in the soil, making phosphate unavailable to plants (Sukarman & Dariah, 2014; Marpaung et al., 2021).

High doses of inorganic phosphate fertilizers are traditionally used to manage phosphorus in Andisols. However, this method is not always effective because most of the applied phosphorus is bound and becomes unavailable. Alternative strategies that increase the utilization of phosphorus stored in the soil and reduce the need for inorganic fertilizers are needed because, in addition to efficiency issues, reliance on inorganic phosphorus fertilizers also poses economic challenges for farmers and has long-term negative environmental impacts (Silva et al., 2023).

The use of phosphate-solubilizing microorganisms (including phosphate-solubilizing bacteria and fungi) is one approach that has received attention. Phosphate-solubilizing fungi (PSF) have the ability to solubilize limited inorganic phosphate through enzyme secretion, organic acid production, and release. Several laboratory and field studies have shown that PSF inoculation can increase soil-available phosphorus, plant phosphorus uptake, growth parameters, and yield in various food crops. However, the performance of PSF is strongly influenced by soil type, fungal species, environmental conditions, and fertilization techniques used (Arias et al., 2023). Research results from Marpaung, et al. (2024b) had been produced on phosphate-solubilizing fungi which can increase plant growth and yield. The phosphate-dissolving fungi *A. niger* PSF-8 isolated from mangrove plants is capable of dissolving phosphate and has the potential to be used as a bio fertilizer (Bhattacharya et al., 2015). Elias, et al. (2016a) found that the application of the phosphate-solubilizing fungi *Aspergillus* sp and *Penicilliun* sp can be able to increase growth, soil P and N content, and yield of *Phaseolus vulgaris* L.

The aim of this study was to evaluate the effect of phosphate-solubilizing fungi inoculation on soil available P, plant P uptake, and shallot yield on Andisol. This study hypothesizes that applying phosphate-solubilizing fungi to several soil sources can increase soil available P, plant P uptake, and shallot yield on Andisol.

Materials and Methods

The experiment was carried out in plastic houses located in Karo Regency, Indonesia, from January to June 2024. A factorial randomized block design was employed in the study. The treatment consisted of two factors: the first factor was the soil origin (soil 1 and soil 2). The second factor is the type of phosphate solubilizing fungi (Control, *A. niger* P13, *A. niger* P21, *A. pseudodeflectus* BJ21, *A. niger* BJ23). Each treatment consisted of 10 plants and was replicated three times.

Two Andisol samples were used: soil 1 collected from Dolat Rayat, Karo Regency, and soil 2 collected from Siborong-borong, North Tapanuli Regency. Isolate of phosphate solubilizing fungi is collection of Agriculture faculty of USU and BRIN from shallot rhizosphere. Isolate multiplication activities were carried out at the USU Laboratory. Phosphate-solubilizing fungal inoculants are prepared by first growing them on PDA media and incubating them for 2-4 days at 28^0C. Each pure culture of phosphate-solubilizing fungi was made until the fungal population density reached around 10^8 $CFUml^{-1}$ for the treatment. Each treatment's planting media was a 2:1 mix of soil and chicken manure. After the media has cooled and been sterilized, it is placed in a 3 kg polybag and covered with sterile water until it reaches field capacity. In a screen/plastic house, shallot seeds of the Batu Ijo variety are planted in polybags and treated with chemical fertilizers N 175 kg ha^{-1}, P_2O_5 90 kg ha^{-1}, and K_2O 120 kg ha^{-1} both during and one month after planting. Phosphate-solubilizing microbes were applied one week after planting at a dose of two millilitres per plant and five weeks after planting at a dose of ten millilitres per plant under the tested treatment, which was administered by watering it into the planting hole in the morning (Sriwantoko et al., 2020). Vegetable pesticides are sprayed as part of maintenance to stop pest and disease infestations. Depending on the severity of pest or plant disease attacks, spraying was done every four days. When the plants are 70–80 days following planting, harvesting can start.

The observation variables were secondary parameter is soil chemical properties before treatment and primary parameter are P available, P retention, P absorption (composite soil was taken from each treatment at 7 weeks after planting), pH, C-organic, and phosphate solubilizing fungi population in soil. The mean for each observation variable was analyzed using the F test and continued with the Tukey HSD test at the 5% level.

Results and Discussion

Soil Chemical Properties Before Treatment. The results of the study of the chemical characteristics of the soil before treatment are shown in Table 1. The chemical characteristics of the soil before treatment showed that the soil in Soil 1had high levels of C-organic, N, and K_2O (4.91%, 52%, and 0.64 mg/100 g-1, respectively). The P_2O_5 (available P) content of the soil was relatively low, namely 2.36 ppm, while the total P (HCl 25%) and P retention was relatively high and very high, namely 54.45 mg/100 g^{-1} and 97.80%, respectively. In Soil 2, the C-organic content was high (4.37%), while the N and K_2O content were moderate (0.48% and 0.40 mg/100 g^{-1}, respectively). The P_2O_5 (available P) content of the soil was low, namely 2.21 ppm, while the total P (HCl 25%) and P retention was high and very high, namely 49.31 mg/100 g^{-1} and 93.90%, respectively. Soil pH in soil 1 and soil 2 is slightly acidic.

P Available, P Retention and P Absorption. The available P content of the soil is influenced by the interaction of the two treatments of soil origin and the type of PSF (Table 2). In general, the soil origin of Soil 2 produces significantly higher available P than Soil 1 Regency, namely 239.78 ppm compared to 192.72 ppm, respectively. The application of PSF, produces higher available P than the treatment without PSF, except for the PSF type of *A. niger* BJ23. In general, *A. niger* P13 and *A. niger* P21 produce significantly higher available P than the other treatments, namely 250.19 ppm and 235.26 ppm, respectively.

Table 1. Soil chemical properties before treatment

Criteria	Soil 1		Soil 2	
	Value	Note*	Value	Note*
C-organic (%)	4.91	High	4.37	High
P_2O_5 – Bray 1 (ppm)	2.36	Low	2.21	Low
P_2O_5 HCl 25% (mg/100 g)	54.45	High	49.31	High
P-Retention (%)	97.80	Very high	93.90	Very high
pH H_2O	5.9	Slightly acidic	5.88	Slightly acidic

Table 2. Interaction of soil origin and type of phosphate solubilizing fungi on P available

Treatments	P Available (ppm) Andisol soil origin		Average
	Soil 1	Soil 2	
Without PSF	143.35 d	242.54 ab	**192.95 abc**
A. niger P13	264.06 a	236.32 ab	**250.19 a**
A. niger P21	225.50 abc	245.03 ab	**235.26 a**
A. pseudodeflectus BJ21	165.07 cd	281.53 a	**223.30 ab**
A. niger BJ23	165.73 cd	193.50 bcd	**179.61 c**
Average	**192.74 b**	**239.78 a**	
CV (%)	**11.39**		

Note: Means followed by the same letter on the same rows and columns is not significant different by Tukey HSD test at 5% level

Table 3. Interaction of soil origin and type of phosphate solubilizing fungi on P retention

Treatments	P Retention (%) Andisol soil origin		Average
	Soil 1	Soil 2	
Without PSF	98.58 a	87.48 de	**93.03 a**
A. niger P13	80.36 f	88.62 cde	**84.49 c**
A. niger P21	92.24 bc	87.09 e	**89.67 b**
A. pseudodeflectus BJ21	97.48 a	90.11 cde	**93.79 a**
A. niger BJ23	96.16 ab	91.08 cd	**93.62 a**
Average	**92.96 a**	**88.88 b**	
CV (%)	**1.51**		

Note: Means followed by the same letter on the same rows and columns is not significant different by Tukey HSD test at 5% level

The interaction of the two treatments was able to increase the available P content of the soil, where the application of *A. pseudodeflectus* BJ21 in the soil origin of Soil 2 resulted in the highest available P, which was 281.53 ppm and was not significantly different from the treatment of *A. niger* P13 in the soil origin of Soil 1 Regency, which was 264.06 ppm. Meanwhile, the lowest available P was found in control treatment in the soil origin of Soil 1 Regency, which was 143.35 ppm. This indicates that the application of PSF was able to dissolve bound P so that the available P increased compared to the soil before treatment, which was 2.21-2.36 ppm.

The interaction between the two treatments of soil origin and PSF type significantly affected the P content of soil retention (Table 3). The soil origin of Soil 1generally produced significantly higher P retention than that of Soil 2, except for the PSF type *A. niger* P13 in the soil origin of Soil 1which produced the lowest P retention.

The interaction of the two treatments significantly affected P retention, with the lowest P retention occurring in soil from soil 1 with *Aspergillus niger* P13 as PSF, at 80.36%. The highest P retention was found in soil from soil 1 without PSF application, at 98.58%. This suggests that PSFF plays an important role in increasing phosphorus (P) solubility for plants by reducing P retention in the soil. The main mechanisms used by PSF in dissolving P include the production of organic acids, phosphatase enzymes, and siderophores.

The application of the PSF type treatment significantly affected plant P uptake, while the soil origin treatment and the interaction between the two treatments did not significantly affect it. The soil from soil 1 resulted in higher P uptake in shallots than the soil from soil 2, although the statistical difference was not significant (Table 4).

P uptake by shallot plants with PSF application on shallot plants resulted in significantly higher P uptake than without PSF application, except for the Aspergillus niger P21 treatment. Application of PSF type *A. niger* BJ23 resulted in the highest P uptake by plants, which was 1.98% and the lowest was found in the control treatment, which was 1.28%. The type of *A. niger* BJ23 produced P uptake that was not significantly different from the treatments of *A. pseudodeflectus* BJ21 and *A. niger* P13. This shows that providing the right type of phosphate-solubilizing microbes will help the availability of P that can be directly absorbed by plants.

Table 4. Effect of soil origin and type of phosphate solubilizing fungi on P absorption

Treatments	P Absorption (mg plant^{-1})
Soil origin	
Soil 1	1.63
Soil 2	1.45
Phosphate solubilizing fungi	
Without PSF	1.28 b
A. niger P13	1.51 ab
A. niger P21	1.28 b
A. pseudodeflectus BJ21	1.65 ab
A. niger BJ23	1.98 a
CV (%)	**19.85**

Note: Means followed by the same letter on the same columns is not significant different by Tukey HSD test at 5% level

Table 5. Effect of soil origin and type of phosphate solubilizing fungi on pH and C-organic

Treatments	pH	C-Organic
Soil origin		
Soil 1	6.10 a	7.59 a
Soil 2	5.82 b	6.07 b
Phosphate solubilizing fungi		
Without PSF	6.10	6.70
A. niger P13	5.95	6.88
A. niger P21	5.95	6.91
A. pseudodeflectus BJ21	5.92	6.92
A. niger BJ23	5.88	6.73
CV (%)	**3.47**	**5.31**

Note: Means followed by the same letter on the same columns is not significant different by Tukey HSD test at 5% level

pH and Organic Carbon Soil. Soil chemical properties showed that soil origin treatment had a significant effect on soil pH and C-organic, while phosphate-solubilizing fungus type treatment and the interaction of the two treatments had no significant effect (Table 5). Soil origin treatment gave a significant difference between treatments on soil pH and C-organic, where soil origin from Soil 1produced significantly higher pH and C-organic than soil origin from Soil 2, namely 6.10 and 7.59%, respectively.

This is consistent with the higher pH and organic carbon content before treatment. After treatment, the pH and organic carbon of each soil source increased. This is related to the addition of phosphate-solubilizing microbes, resulting in an increase in pH and organic carbon. In general, the application of PSF resulted in a lower pH compared to the treatment without phosphate-solubilizing fungi. Meanwhile, organic carbon content increased after the PSF treatment, although not statistically significantly.

Phosphate Solubilizing Fungi Population. The results showed that the type of phosphate solubilizing fungi (PSF) and soil origin (Soil 1 and Soil 2) influenced the population of phosphate solubilizing fungi in Andisol soil. In general, Soil 2 produced a larger PSF population than Soil 1, with an average value of 7.53 × 10^8 CFU g^{-1}. The treatment without PSF (control) showed the lowest PSF population in both soil types, namely 1.87 × 10^8 CFU g^{-1} in Soil 1 and 3.57 × 10^8 CFU g^{-1} in Soil 2. Compared with the control, inoculation of phosphate solubilizing fungi significantly increased the PSF population. The population values of *A. niger* P13 and *A. pseudodeflectus* BJ21 were the highest (8.75 × 10^8 and 7.42 × 10^8 CFU g^{-1}), but *A. niger* BJ23 was the lowest (5.01 × 10^8 CFU g^{-1}). The interaction between soil origin and PSF type showed that some isolates, especially *A. niger* P13, were able to grow better on Soil 2 than Soil 1 (Table 6).

Table 6. Effect of soil origin and type of phosphate solubilizing fungi on phosphate solubilizing fungi population

Treatments	Phosphate Solubilizing Fungi Population (10^8 CFU g^{-1}) Andisol soil origin		Average
	Soil 1	**Soil 2**	
Without PSF	1.87 c	3.57 bc	**2.72 b**
A. niger P13	6.84 ab	10.66 a	**8.75 a**
A. niger P21	6.25 abc	6.84 ab	**6.55 a**
A. pseudodeflectus BJ21	4.28 bc	10.56 a	**7.42 a**
A. niger BJ23	3.98 bc	6.04 abc	**5.01 b**
Average	**4.64 b**	**7.53 a**	
CV (%)	**1.36**		

Note: Means followed by the same letter on the same rows and columns is not significant different by Tukey HSD test at 5% level

The soil chemical composition before treatment showed several characteristics of Andisol, namely high organic carbon content, acidic-slightly acidic pH, low P_2O_5 (available P), and high P retention. This is in accordance with Anindita et al. (2023), which states that Andisols, particularly those under forest or agricultural land use, can contain significant amounts of organic carbon due to interactions with amorphous minerals like allophane that help protect carbon. These characteristics make Andisol suitable for agricultural use. This property is also consistent with the characteristics of Andisol, which includes low available phosphorus due to significant phosphorus fixation by allophane (Zhu et al., 2018). The soil pH value ranged from 5.88 to 5.90, classified as slightly acidic. According to (Sukarman & Dariah, 2015), most Andisol in Indonesia are acidic, with a pH range of 3.4 to 6.7. Soil 1 has higher organic C, N, and K_2O content, thus providing a better source of energy and nutrients for plants. However, Soil 1 also has very high P retention, so that initial P availability remains low even though total P is high. In contrast, Soil 2 has still high organic C but lower N and K_2O, so that soil fertility is relatively more limited, but the slightly lower P retention value compared to Soil 1 allows phosphate-solubilizing fungi to develop better and be more effective in dissolving bound P.

In Andisol soil, the relative surface charge of the cell will bind with the charge of the soil clay minerals, thereby reducing the adsorption capacity of the clay minerals to phosphate (Herzberg & Elimelech, 2008). Additionally, by obstructing or competing for soil adsorption sites on Al and Fe oxides, organic acid anions generated by phosphate-solubilizing microorganisms can decrease phosphate fixation and increase soil phosphorus availability(Alori et al., 2017). Furthermore, P absorption activity through the release of organic acids such as fumarate, lactate, and citrate can break down P ions bound to soil cations such as Al, Ca, Fe, and Mg and then convert them into forms available for plant absorption (Tian et al., 2021; Timofeeva et al., 2022). The principle of the phosphate mineral dissolution mechanism is the production of organic acids and the acid phosphatase enzyme, which plays a role in the mineralization of organic phosphate in the soil (Setiawati & Pranoto, 2015). According to (Istina & Nurhayati, 2019), native phosphate-solubilizing microbes can increase the soil P nutrient content.

Phosphate-solubilizing microbes produce organic acids such as citric, oxalic, and malic acids that can dissolve inorganic phosphate bound to soil minerals, thereby increasing the availability of P for plants. Producing phosphatase enzymes, PSF secrete phosphatase enzymes that hydrolyze organic phosphate compounds into inorganic forms that can be absorbed by plants. Producing siderophores, some PSF produce siderophores that chelate metal ions such as Fe^{3+} and Al^{3+}, which normally bind phosphate in the soil. By binding these metal ions, phosphate becomes more available to plants and P soil retention will decrease. Insoluble phosphate can be dissolved and mineralized using different phosphate-solubilizing microbes that produce phosphatase enzymes, low-molecular-weight organic acids (Zhu et al., 2018). According to Campos, et al. (2018), the activity of the PSF group that is able to secrete organic acids can extract phosphate from an insoluble form to become available so that plants can absorb P elements to meet their needs.

Phosphate-solubilizing microbes have the ability to dissolve bound P into plant-available P through the production of organic acids, phosphatase enzymes, and siderophores. *A. niger* is a phosphate-solubilizing microbe that can dissolve insoluble P compounds (Rojas et al., 2019). These microorganisms can increase phosphorus uptake by releasing phosphorus compounds bound to the soil and making them available to plants (Kumar et al., 2018). (Baumann et al., 2018) stated that phosphate-solubilizing microorganisms can dissolve fixed phosphorus by releasing organic acids, namely by lowering pH, through chelation activity, competing with phosphorus for adsorption sites, and releasing phosphorus through the formation of soluble complexes with metal ions that will bind phosphorus strongly. The excretion of organic acids by phosphate-solubilizing microbes will chelate Fe and Al ions so that P is released. In addition, PSM produces phosphatase enzymes that play a role in the mineralization of organic P so that orthophosphate release occurs from soil Po sources (Bi et al., 2020). Siderophores are also produced by several types of phosphate-solubilizing microbes, which are a group of secondary metabolites with high affinity for Fe (Gu et al., 2020), so that Fe will be chelated and P will be released.

Soil acidity is a crucial factor in ensuring phosphorus immobilization in the soil (Zhu et al., 2018). Microbial growth and activity are significantly influenced by changes in the acidity of their environment. Bacteria generally have maximum solubility at pH 5.5-6, while fungal spores have a pH of 5-6. The pH activity of fungal isolates decreased significantly at pH 7-8. Research by (Wang et al., 2021) indicates that a lower pH does not necessarily translate to a higher P solubilization capacity. However, according to Li, et al. (2019), there is a strong correlation between decreased pH and P solubilization.

The application of PSF increased the population of phosphate-solubilizing fungi in Andisol soil. This indicates that the applied fungi have the ability to thrive and adapt well to volcanic soils (Takahashi & Dahlgren, 2016); (Takahashi & Dahlgren, 2016; Kalayu, 2019). This soil has a high phosphate fixation capacity due to the presence of active Al and Fe, but the unique characteristics of Andisols, which are rich in organic matter and dominated by amorphous minerals such as allophane and imogolite, provide physical conditions that support the growth of microorganisms in the soil (Takahashi & Dahlgren, 2016). Initial soil characteristics, such as organic matter content, pH, and soil chemistry, influence the success of phosphate-solubilizing fungi colonization. Differences in fungal populations in Soil 1 and Soil 2 demonstrate this (Alori et al., 2017). Populations of *A. niger* P13 and *A. pseudodeflectus* BJ21 isolates were larger than those of other isolates; This indicates a better physiological and metabolic ability to utilize soil carbon sources and dissolve bound phosphate through the production of organic acids such as oxalic and citric acids (Kalayu 2019). These results indicate that the compatibility between microbial isolates and Andisol soil characteristics greatly influences the effectiveness of phosphate-solubilizing fungus-based biofertilizers (Alori et al., 2017; Takahashi & Dahlgren, 2016). According to the results of research by Elias et al., 2016b), the *Aspergillus* genus can dissolve phosphate qualitatively.

The results of the study on the use of indigenous phosphate-solubilizing fungal isolates *A. niger* and *A. pseudodeflectus* in Andisol soil were able to improve soil chemical properties by increasing organic carbon, available P, P uptake by shallot plants in particular and reducing P retention. In addition, this type of fungus can also adapt and grow well. This shows the potential for developing agricultural technology based on local resources. The use of phosphate-solubilizing fungi as biofertilizers supports a more efficient and environmentally friendly food production system by reducing dependence on inorganic phosphate fertilizers.

Conclusion

Soil 1 generally produces higher growth and yield of shallots than soil 2. *A. pseudodeflectus* BJ21 can increase available P by 48.46% and *A. niger* P13 by 45.71% compared to without phosphate solubilizing fungi. *A. niger* P13 can reduce P retention by 18.48% compared to without phosphate solubilizing fungi. *A. niger* BJ23 can increase plant P uptake by 35.35% compared to without phosphate solubilizing fungi. Application of phosphate solubilizing fungi can increase shallot yields by 63.19%-72.39% compared to without phosphate solubilizing fungi. Inoculation of isolates *A. niger* P13 and *A. pseudodeflectus* BJ21 was able to adapt and grow well on Andisol soil which significantly increased the population compared to the control.

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