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Effect of Mycorrhiza and Phosphate-solubilizing Bacteria on Soybean Growth in Saline Soil Enriched with Liquid Smoke

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Abstract
Saline soils have high levels of Na^+ and Cl^- that adsorb phosphate, resulting in its decreased availability. Mycorrhiza (M) and phosphate-solubilizing bacteria (PSB) are both known to increase phosphate availability, but the difference in their ability to increase plant growth in saline soils has not been studied. This study aims to describe the effect of mycorrhiza and PSB on soybean growth in saline soil with the addition of liquid smoke which contains acetic acid as a precursor of gibberellin. Soybeans were given mycorrhiza, phosphate-solubilizing bacteria (PSB), or combination of both at various concentration. Data were analyzed descriptively and quantitatively. The maximum vegetative growth was shown in 40 g mycorrhiza with no additional PSB, for the parameters of root length, biomass, number of root nodules, number of active root nodules, and percentage of mycorrhizal infection. The highest generative growth was shown in combination of 20 g mycorrhiza and 50 ml PSB, for parameters of pod number and pod weight.

Keywords: plant growth, saline soil, soil microbes, agriculture.

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INTRODUCTION

Marginal land is low-productivity land with chemical, physical, and biological properties which are not quite optimal for plant growth. One of the causes for marginal lands is high salt content. High salinity can occur naturally on coastal lands, as well as due to human activities such as chemical fertilization, excessive irrigation, and the effects of climate change. Given the extensive occurrence of saline soils in Indonesia, it can be utilized as productive agricultural land (Hidayahtulloh and Setiawati, 2022). High NaCl levels in the soil can increase Na^+ ions, however it might reduce the availability of Mg, Ca, and K ions, thereby decreasing the absorption of other nutrients by plants. P and N are essential macronutrients that are needed for plant growth. The binding of P by Ca^{2+} in high pH soils, such as saline soils, causes the availability of phosphorus in the soil to decrease. The excess of Cl^- ions will also affect the absorption of NO^{3-}, which causes nitrogen deficiency and inhibition of mineralization in the soil, so that plant growth and production decrease (Mutmainah *et al.*, 2022).

In high salinity conditions, phosphorus level is commonly lacking. However, it is one of the macronutrients essential for plant growth and development. Phosphorus plays an important role in photosynthesis and carbohydrate metabolism by regulating the distribution of photosynthetic products between the source and reproductive organs, as well as the formation of cell nuclei (Albari *et al.*, 2018). In addition, abiotic stress affects the survival of functional bacteria, thus consequently also affects soil productivity as well as plant growth and yield (Widawati and Suliasih, 2016). For soil with high salinity, the main issue for plant growth is nutrient availability, which can be improved by various methods, for example applying mycorrhiza and phosphate-solubilizing bacteria.

Mycorrhiza is a type of soil microbe that makes an important contribution to soil fertility by increasing the ability of plants to absorb nutrients such as phosphate, water, and other nutrients, including nitrogen. Mycorrhiza infection in the root system of host plants produces intensive hyphae which can increase the capacity of host plants to absorb water and nutrients. The small size of the hyphae allowing it to infiltrate the soil micropores, allowing them to absorb water at very low water content conditions (Basri, 2018). Mycorrhizae are also found to produce the phosphatase enzyme. This enzyme can fix P bound by elements such as Ca-P or other forms via other uptake routes, converting organic phosphate (P) into primary orthophosphate that can be absorbed by plants (Rajmi *et al.*, 2018).

On the other hand, phosphate-solubilizing bacteria (PSB) are known to dissolve unavailable phosphate in the soil so that plants can absorb phosphate, one of which is *Pseudomonas fluorescens* (Rohmah *et al.,* 2013). PSB is capable of producing organic acids such as fumarate, lactate, and citrate, which can break down phosphorus (P) ions into compounds that are easily absorbed by plants (Alfiah *et al.,* 2016; Firdausi *et al.,* 2016).

Soil with high salinity levels allows only certain plants with high tolerance to survive, including those from the Leguminosae family (Rohmah *et al.,* 2013). Soybean is a leguminous plant that is sensitive to salinity, with a tolerance threshold of 2–4 dS/m (Mutmainah *et al.,* 2022). However, the need for soybeans in Indonesia increases every year, driven by population growth and rising per capita income. Therefore, to fulfil national soybean demand, additional soybean supplies must be imported overseas (Rohmah *et al.,* 2013). National soybean consumption in 2024 was expected to reach 3.03 million tons (Kementrian Pertanian, 2020). The increase local soybean productivity and fulfil national demand, soybeans can be grown in saline soil with improved nutrient availability and structure, for example through the application of mycorrhiza, PSB, or a combination of both (Zulaikah and Yuliani, 2018).

In addition to proper planting medium, it is necessary to add plant growth precursors, for spurring optimal plant growth and development, for example with liquid smoke supplementation. Liquid smoke is the result of condensation of vapor from combustion or pyrolysis directly or indirectly from high-carbon materials, as well as compounds such as cellulose, hemicellulose, and lignin (Istiqomah and Kusumawati, 2019). The largest components found in liquid smoke made from coconut shell are acetic acid at 46.56% and phenol at 12.93% (Jayanudin and Suhendi, 2012). Acetic acid is a primary metabolite compound found for plants in liquid smoke. Through the mevalonic acid pathway, acetate produces terpenoids. These terpenoid compounds serve gibberellin precursors, which then is synthesized in plant into active gibberellins (Taiz and Zeiger, 2002). Gibberellin is a growth hormone affecting flowering paternization, cell elongation and so on (Permatasari *et al.,* 2016).

Previous studies revealed that mycorrhiza and PSB was able to increase plant growth in saline soils (Rahayu, 2014; Zulaikhah and Yuliani, 2018), but comparation of the effect between both or the effect of their combination in saline soils has not been studied, considering both have a role in increasing phosphorus availability. This study aims to assess the effects of mycorrhiza, PSB, and their combination on the growth of soybean plants in saline soil enriched with liquid smoke. This approach considers the beneficial role of liquid smoke in stimulating the production of acetic acid, a recognized precursor of the hormone gibberellin, which plays a crucial role in regulating plant growth and development.

MATERIALS AND METHODS

This research was an experimental study carried out in January-May 2023. The research was conducted in Biology Laboratory, Faculty of Mathematics and Natural Sciences, Unesa. This study used a Randomized Group Design with 9 groups as the follows; M0P0 (0 g mycorrhiza + 0 ml PSB), M0P1 (0 g mycorrhiza + 50 ml PSB), M0P2 (0 g mycorrhiza + 75 ml PSB), M1P0 (20 g mycorrhiza + 0 ml PSB), M1P1 (20 g mycorrhiza + 50 ml PSB), M1P2 (20 g mycorrhiza + 75 ml PSB), M2P0 (40 g mycorrhiza + 0 ml PSB), M2P1 (40 g mycorrhiza + 50 ml PSB), and M2P2 (40 g mycorrhiza + 75 ml PSB). Each treatment was replicated 3 times, for a total of 27 units.

Planting medium was prepared by mixing regosol soil and sand in a ratio of 1:1, then portioned in 5 kg medium per polybag. Planting medium was watered using 1000mL 0.004 M seawater, once at the beginning, then 250 mL before treatment. The treatment was carried out for 1 week. After that, it was left to dry for 5 days. The soil was sterilized by adding 200 mL 2% formaldehyde per polybag, then left dry again for 5 days (Rahayu, 2014). For basic fertilizer, 40 mg of urea and 40 mg of KCI were given. Mycorrhiza was applied into 3-cm deep planting holes in the medium, as well as 40 g inoculum per polybag (Rahayu and Yuliani, 2019).

Soybean seeds were sowed for 3 weeks, then transplanted to planting medium. PSB was applied first at 100 ml/polybag and then 150 ml/polybag. The next application was given at 50 ml/polybag and/or 75ml/polybag, applied at 9, 16, 23, 20, 37, and 44 days after planting (DAP). Liquid smoke was given at 1% of watering volume, 10 ml per polybag, applied on 7, 14, 21, 28, 35, and 42 DAP.

Plants were evaluated based vegetative growth parameters, including plant height, number of leaves, root length, plant biomass, number of root nodules, number of active root nodules, mycorrhizal infection, and P nutrient levels in plant leaves as well as plant generative parameters including age of flower emergence, number of pods and pod weight. Measurement of the number of leaves and plant height was performed at 15, 30, and 45 DAP. The data obtained were analysed descriptively and quantitatively.

RESULTS

Before conducting the research, soil condition data were measured to determine the levels of N, P, K, and Na elements. Soil conditions are also assessed through measurements of pH and soil moisture, as shown in Table 1. Figure 1 presents the harvested soybean plants at 45 DAP.

Table 1. Measurement of environmental parameters and nutrient levels of saline soil before treatments*

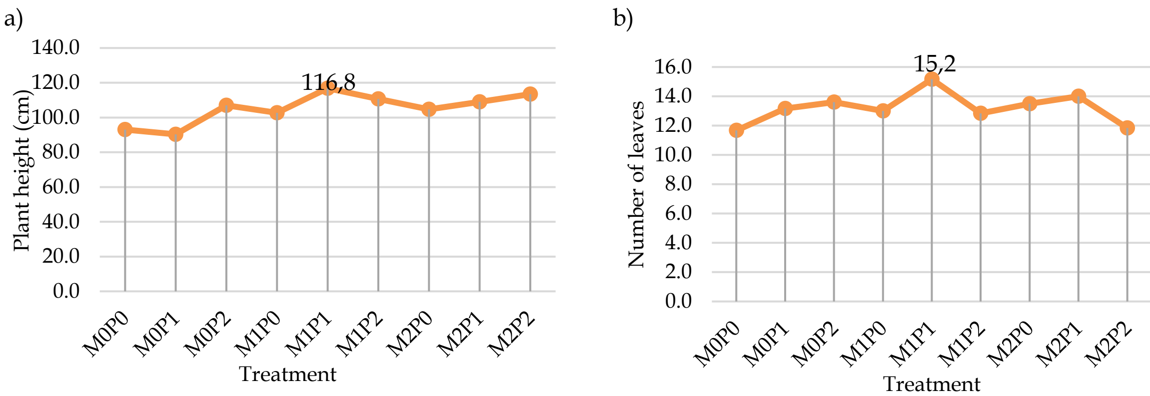
pH	Moisture level	Na (%)	N (%)	P (ppm)	K (%)
7,5	65%	1,20	0,30	36	0,37
Neutral	Moist	Very High	Medium	High	Low

*) criteria from Balittan soil test results by Sulaeman *et al*. (2005)

Figure 1. Evaluation of soybean plants after treatment. a = M0P0 (0 g mycorrhiza + 0 ml PSB), b = M0P1 (0 g mycorrhiza + 50 ml PSB), c = M0P2 (0 g mycorrhiza + 75 ml PSB), d = M1P0 (20 g mycorrhiza + 0 ml PSB), e = M1P1 (20 g mycorrhiza + 50 ml PSB), f = M1P2 (20 g mycorrhiza + 75 ml PSB), g = M2P0 (40 g mycorrhiza + 0 ml PSB), h = M2P1 (40 g mycorrhiza + 50 ml PSB), and i = M2P2 (40 g mycorrhiza + 75 ml PSB).

After application of mycorrhiza and PSB, the highest plant height was obtained from M1P1 (20 g mycorrhiza + 50 ml PSB) at 116.00 cm (Figure 2). Similarly, M1P1 also resulted in the highest number of leaves, at 15.2 leaves. On the other hand, highest plant root length and plant biomass was M2P0 (40 g mycorrhiza + 0 ml PSB).

After treatment, M2P0 (40 g mycorrhiza + 0 ml PSB) had the highest root nodules at 29 nodules as well the most active nodules at 23. The same treatment also resulted in the highest mycorrhizal infection at 87%. Three groups all given 40 g mycorrhiza (M2P0, M2P1, M2P2) resulted in most phosphorus content at 0.24% (Figure 3).



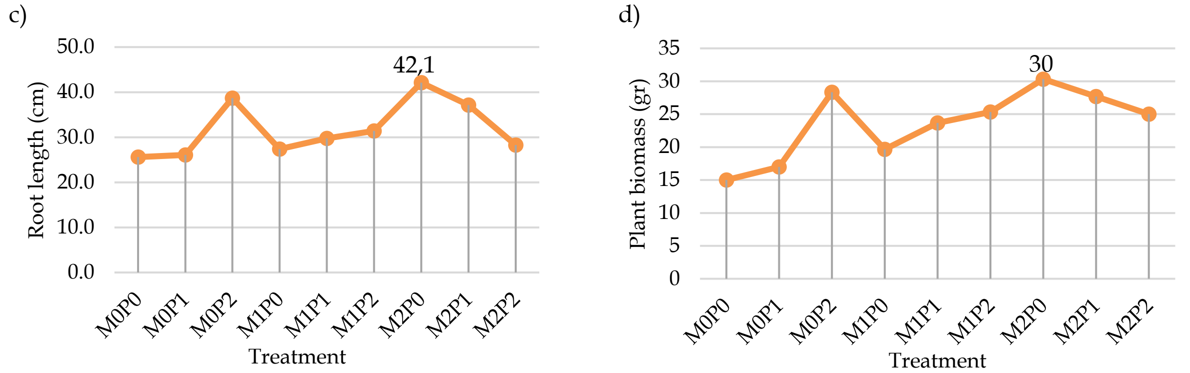


Figure 2. Effect of mycorrhiza, PSB, and their combination on a) plant height, b) number of leaves, c) root length, d) wet biomass of soybean plants. M0P0 = 0 g mycorrhiza + 0 ml PSB, M0P1 = 0 g mycorrhiza + 50 ml PSB, M0P2 = 0 g mycorrhiza + 75 ml PSB, M1P0 = 20 g mycorrhiza + 0 ml PSB, M1P1 = 20 g mycorrhiza + 50 ml PSB, M1P2 = 20 g mycorrhiza + 75 ml PSB, M2P0 = 40 g mycorrhiza + 0 ml PSB, M2P1 = 40 g mycorrhiza + 50 ml PSB, and M2P2 = 40 g mycorrhiza + 75 ml PSB.

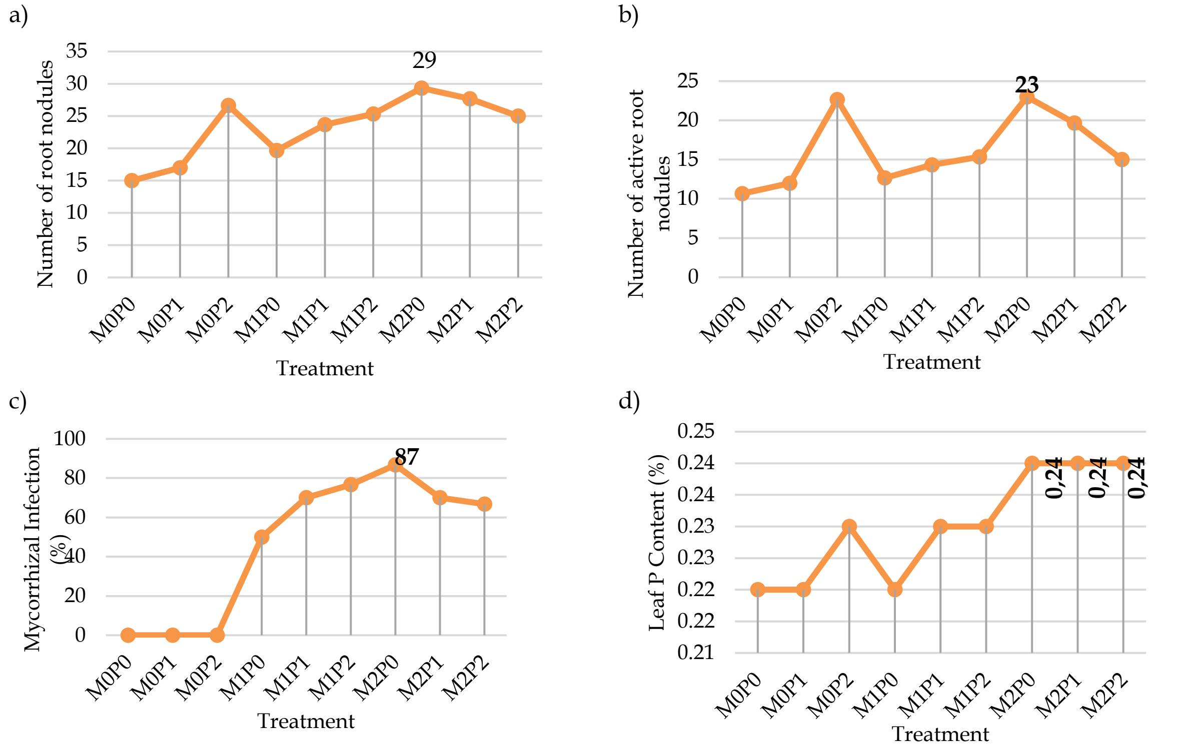


Figure 3. Effect of mycorrhiza, PSB, and their combination on a) number of root nodules, b) number of active root nodules, c) mycorrhizal infection, d) leaf P content of soybean plants. M0P0 = 0 g mycorrhiza + 0 ml PSB, M0P1 = 0 g mycorrhiza + 50 ml PSB, M0P2 = 0 g mycorrhiza + 75 ml PSB, M1P0 = 20 g mycorrhiza + 0 ml PSB, M1P1 = 20 g mycorrhiza + 50 ml PSB, M1P2 = 20 g mycorrhiza + 75 ml PSB, M2P0 = 40 g mycorrhiza + 0 ml PSB, M2P1 = 40 g mycorrhiza + 50 ml PSB, and M2P2 = 40 g mycorrhiza + 75 ml PSB.

The results of the application of mycorrhiza and PSB (Figure 3) obtained that the average age of flower emergence was the fastest in the treatment of mycorrhiza 0 grams + PSB 75 ml, mycorrhiza 40 grams + PSB 50 ml, and mycorrhiza 40 grams + PSB 75 ml, namely on day 13 DAP. The average number of plant pods and weight of plant pods are the highest in the mycorrhiza treatment of 20 grams + PSB 50 ml with value of 12 pods and 9 grams, respectively.

Overall, the data show that at the vegetative growth stage, the treatment that yields the highest growth is mycorrhiza 40 grams + PSB 0 ml, across the parameters of root length, biomass, number of root nodules, number of active root nodules, and percentage of mycorrhizal infection. Meanwhile, the generative growth of plants that produced the highest results was in the mycorrhiza treatment of 20 grams + PSB 50 ml, for the parameters of the number of pods and pod weight.

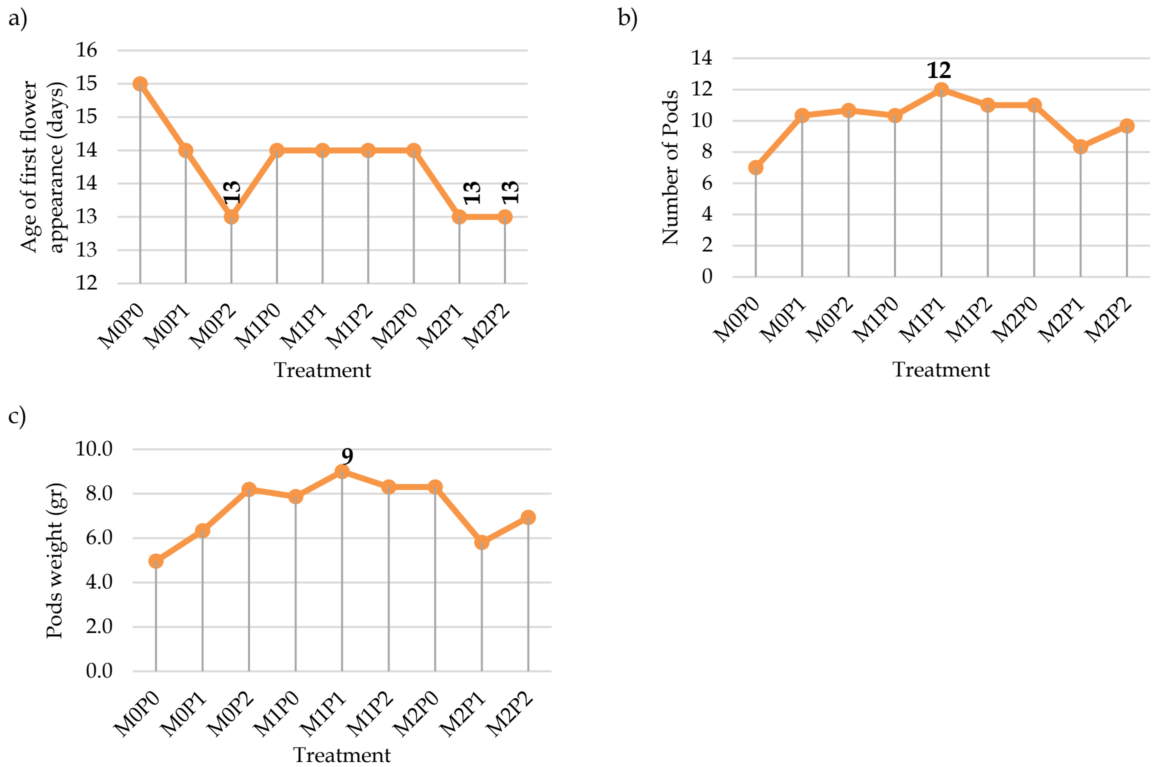


Figure 3. Effect of mycorrhiza, PSB, and their combination on a) age of first flower appearance, b) number of pods, c) pod weight. M0P0 = 0 g mycorrhiza + 0 ml PSB, M0P1 = 0 g mycorrhiza + 50 ml PSB, M0P2 = 0 g mycorrhiza + 75 ml PSB, M1P0 = 20 g mycorrhiza + 0 ml PSB, M1P1 = 20 g mycorrhiza + 50 ml PSB, M1P2 = 20 g mycorrhiza + 75 ml PSB, M2P0 = 40 g mycorrhiza + 0 ml PSB, M2P1 = 40 g mycorrhiza + 50 ml PSB, and M2P2 = 40 g mycorrhiza + 75 ml PSB.

DISCUSSION

Plant growth and development is affected by many factors, one of which is the growing medium. The growing medium provides nutrients that support plant growth and reproduction (Febriani *et al*., 2021). The pH of medium concocted was 7.5 and the moisture content was 65% (Table 1). Soybeans grow optimally at a pH of 6-6.8. A higher pH indicates higher levels of base ions in the soil. Meanwhile moisture level is ideal for soybean growth, with optimal levels around 60-70% (Kementrian Pertanian, 2020). The soil chemistry test results showed that the N content moderate at 0.30%, the K content of 0.37 cmol+/kg was in the low category, the P content of 36 ppm of the high category, and the Na content of 1.20% was in the very high category (Sulaeman *et al*., 2005).

The growing medium shows high levels of Na (Table 1), causing salinity stress that inhibits plant growth due to a decrease in soil osmotic potential (Sulaeman *et al*., 2005). High salinity causes the accumulation of Na and other ions such as boron and molybdenum, which interfere with the availability of calcium (Ca), magnesium (Mg), phosphorus (P), iron (Fe), manganese (Mn), and zinc (Zn) due to increased soil pH, preventing roots from absorbing these nutrients (Karolinoerita and Yusuf, 2020). Phosphorus (P) level in the medium was considered high, at 36 ppm (Table 1). P in soil with high salinity is bound by Na, preventing it from being absorbed by plants. Low availability of N, P, and K can inhibit the preparation of the generative phase (Oktaviani *et al*., 2014)

During the vegetative phase, 40 g mycorrhiza without PSB (M2P0) yielded the highest results in root length, plant biomass, number of root nodules, number of active root nodules, and mycorrhizal infection rate. Mycorrhiza can increase phosphorus uptake from the soil through its hyphae. Mycorrhiza also secreted phosphatase and carbonate compounds to produce primary orthophosphate compounds that convert organic phosphorus in saline soils into absorbable compounds (Rajmi *et al*., 2018). These compounds can increase nutrient uptake by increasing the concentration of acidic compounds in the root zone, thereby increasing the solubility of nutrients in the rhizosphere and making previously bound nutrients more easily absorbed by the plants (Alias *et al*., 2021).

Vesicular-arbuscular mycorrhizae infect host plant roots through hyphal networks that spread inside and outside the roots, helping with water and macronutrient absorption, especially phosphorus (P), by penetrating small pores in the soil. External hyphae absorb and convert phosphorus into polyphosphate, which is transferred to internal and arbuscular hyphae. In the arbuscular structure, polyphosphate compounds are broken down into organic phosphate and then channeled to the host plant cells. Compared with plants not infected with mycorrhiza, the presence of external hyphae significantly increases nutrient absorption, especially of phosphorus (Nasution *et al*., 2013). In line with the results of this study the research by Oktaviani *et al* (2014) reveals that mycorrhizal treatment has a significant effect on plant height parameters because it increases the availability of phosphorus (P), which plays a role in cell division and plant tissue formation.

The absorption of element P by plants is directly proportional to the formation of ATP and NADPH; this is proven by CO_2 being reduced and producing large amounts of carbohydrates (Ginting *et al*., 2018). Phosphorus plays a role in plant growth because it contributes to the formation of phospholipids, carbohydrates, and paradoxical enzymes, thereby enhancing vegetative growth. Mycorrhiza also helps overcome water imbalances caused by salinity stress by providing water through hyphae that penetrate soil pores, thereby meeting the plant's water needs (Maulana and Harahap, 2023).

Nitrogen level in the medium was at 0.30% to be used by mycorrhiza as energy source. In addition, the decomposition products form ammonium and nitrate that can be used by roots for plant metabolic growth (Hapiza *et al*., 2014). Nitrogen plays a role in the formation of proteins, DNA, and RNA, and together with carbon, hydrogen, and magnesium, it forms chlorophyll (Taiz and Zeiger, 2002). The highest number of root nodules and active nodules was also produced by M2P0 (40 g mycorrhiza with no PSB), in line with the high percentage of mycorrhizal infection and P level. However, addition of 50 ml PSB (M2P1) and 75 ml PSB (M2P2) along with 40 g mycorrhizae showed the same results for leaf P level (Figure 2).

Mycorrhizae can increase the absorption of nitrogen (N) and phosphorus (P), to supports the formation of root nodules due to symbiosis between plant roots and *Rhizobium* bacteria, which begins when plants release photosynthetic exudates to attract bacteria as a nutrient source (Simanungkalit *et al*., 2006). *Rhizobium* utilizes carbohydrates, proteins, and oxygen from plants to live, with oxygen requirements controlled by leghemoglobin. Deficiencies in P and N inhibits root nodule formation (Taufik and Sundari, 2013), so mycorrhiza ability to increase the absorption of both P and N can multiply and activate root nodules.

Mycorrhizal infection also similarly increased in M2P0, resulting in high P levels in plants, although combinations with 50 ml (M2P1) and 75 ml PSB (M2P2) showed similar results. Root infection triggers the release of sugar exudates and organic acids as part of a mutualistic symbiosis for plants to obtain P, while mycorrhiza obtain carbon from plants (Kartika *et al*., 2016). PSB also requires root carbon for metabolism, while plants need carbon for protein synthesis along with nitrogen. Due to carbon competition, only high mycorrhizal doses were effective for symbiosis. Thus, the highest mycorrhiza concentration at 40 g was most optimal for enhancing plant growth during the vegetative phase.

During the generative phase, mycorrhiza was found to be insufficient to support plant growth, thus PSB role was needed to increase P availability by converting P_2O_5 into HPO_4^- and $H_2PO_4^-$. In saline soils with high pH, an increase in cations such as Na^+, Ca^{2+}, Mg^{2+}, and Al^{3+} causes P to become bound and unavailable to plants. PSB dissolved P through the production of organic acids to chelate compounds, releasing $H_2PO_4^-$ ions that can be absorbed by plants (Mutmainah *et al*., 2022). Organic acids also help reduce stress caused by soil salts. Soluble phosphate from organic or mineral sources are absorbed by plants through chemical and biological processes (Simanungkalit *et al*., 2006). Previous study shows that *Pseudomonas fluorescens* PSB increased soybean growth in saline soils (Zulaikah and Yuliani, 2018)

Mycorrhiza and PSB combination help release phosphorus (P) bound by abundant sodium (Na) in saline soils. PSB releases P through the secretion of organic acids, while mycorrhiza absorbs it through hyphae and frees bound P using phosphatase enzymes (Sabilu *et al*., 2021). The combined effects increase the availability and absorption of P via plant roots. In the generative phase, P is very important for the transport of photosynthetic products and the formation of pods and biomass (Zulputra *et al*., 2014). The emergence of the first flower at 13 DAP indicated the transition from the vegetative to the generative phase, which was generally observed at 35–39 HST (Pertanian, 2020).

The acceleration of the generative phase occurred due to the addition of liquid smoke in all treatments. Liquid smoke contains high levels of acetic acid, acting as a precursor for gibberellin hormones production via acetic acid-mevalonate pathway that produces terpenoids (Taiz and Zeiger, 2002). Mycorrhizae play an important role in pod and seed formation and improve yield quality through symbiosis with plant roots (Ardiansyah *et al*., 2014). The combination of mycorrhiza and PSB also plays a role in increasing P availability for pod and biomass formation. Noviani and Rahayu (2022) showed that the highest soybean productivity, characterized by pod number and biomass, was affected by the combination of *P. fluorescens*, *Azospirillum* sp., and local microorganisms.

Based on the results of the study, the best treatment during the vegetative phase of the plant was 40 g mycorrhiza without PSB, as mycorrhiza alone is sufficient to support plant growth during this phase. During the generative phase, the best treatment was 20 g mycorrhiza and 50 ml PSB combination, which was able to increase both pod number and pod weight, due to the combination was able to increase phosphorus availability for the plants.

CONCLUSION

Addition of mycorrhiza and PSB affected the vegetative and generative growth of soybean plants in saline soil enriched with liquid smoke. The best treatment during vegetative growth of soybean was observed in 40 g mycorrhiza with no PSB. Meanwhile, the best treatment for soybean generative phase was 20 g mycorrhiza combined with 50 ml PSB. As different types of development occur in plant during vegetative and generative phase, different treatments are required to support optimal growth.

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CONFLICT OF INTEREST

There is no conflict of interest.

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