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Mycorrhizae and Biofertilizers Applications Stimulate Pineapple Growth in Acidic Soil

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Abstract

Pineapple is a leading commodity worldwide and can grow in a variety of mineral and organic soils. Excessive use of inorganic fertilizers has a detrimental impact on soil and crop health. Biofertilizers contain beneficial microorganisms that can enhance soil fertility, improve soil health, and promote sustainable crop production. This study aimed to investigate the crucial role of biofertilizers and mycorrhizae in promoting the vegetative growth of pineapple in acidic soils. The experiment was conducted using a completely randomized design with nine treatment levels and five replications. The results showed that the mycorrhizae applied at 5 g per plant, combined with full doses of chemical fertilizer (0.5 g ZA, 2 g DAP, and 2 g KCl per plant), produced the highest mycorrhizal colonization in treatment P4. Meanwhile, Provibio® biofertilizer at 5 mL per plant (diluted in 0.5 L water), mycorrhizae at 2.5 g per plant, and half doses of chemical fertilizer (0.25 g ZA, 1 g DAP, and 1 g KCl per plant (P8), improved vegetative growth and soil properties (pH, organic C, and total N) and supported the highest microbial population in the rhizosphere (26.05×10^4 CFU.g^{-1} soil). This study demonstrated that the combined application of biofertilizers and mycorrhizae in moderate doses is more effective than full single-dose applications, leading to healthier soils and stronger vegetative growth of pineapple in acidic soils.

Keywords: crop health, microbes, soil fertility, soil health, sustainable agriculture

Introduction

Pineapple (*Ananas comosus*) is a tropical fruit that grows in over 82 countries. In 2021, pineapple production was approximately 28.65 million metric tons. Indonesia is one of the leading countries in pineapple production worldwide in 2021, after the Philippines (FAO, 2021). According to the BPS-Statistics Indonesia (2021), the export volume of pineapple has reached 236,226 tons per year. Pineapple can grow in a wide range of soils (mineral and organic soil), preferably in sandy loam soil (FAO, 2021). In Indonesia, pineapple cultivation predominantly occurs in Ultisols, which are characterized by their acidic nature and low fertility rates. This necessitates a high reliance on inorganic fertilizers to enhance soil nutrient availability and support crop growth (Sutikarini et al., 2023). However, the continuous and excessive application of these fertilizers can lead to detrimental effects on soil health, including soil acidification and nutrient leaching, which ultimately compromise the yield of pineapple plants (Chen et al., 2023; Hanyabui et al., 2024). Research indicates that such practices not only degrade soil quality but also disrupt the microbial community structure, which is essential for maintaining soil fertility (Chen et al., 2023). Soil health is defined as the capacity of soil to function as a vital living system within ecosystem and land-use boundaries, to sustain plant and animal health and productivity, and maintain or improve water and air quality (Krasilnikov et al, 2022). Research indicates that the application of biofertilizers can increase the availability of essential nutrients like phosphorus and nitrogen in the soil, which are critical for plant growth (Marder et al., 2019; Saputro and Kurniawati, 2024). Furthermore, biofertilizers can partially ameliorate soil quality parameters, leading to a healthier soil ecosystem (Saputro and Kurniawati, 2024).

Mycorrhizal fungi form symbiotic relationships with plant roots, significantly enhancing nutrient uptake, especially phosphorus, and improving plant resilience against environmental stresses (Bellido et al., 2021; Saleh et al., 2021). The integration of mycorrhizae into agricultural practices not only boosts plant growth but also contributes to the overall health of the soil by promoting beneficial microbial communities (Bellido et al., 2021; Saleh et al., 2021). Studies have demonstrated that mycorrhizae can

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effectively replace chemical fertilizers, leading to more sustainable agricultural practices (Bellido et al., 2021). Moreover, the combined application of biofertilizers and mycorrhizae has enhanced soil microbial activity and diversity, which are essential for nutrient cycling and soil structure formation (Bhardwaj et al., 2014). Research indicates that mycorrhizal fungi can significantly improve the growth of various crops by increasing root biomass and enhancing nutrient uptake. For example, studies have shown that arbuscular mycorrhizae (AM) can lead to increased root surface area, which facilitates greater nutrient absorption (Subaedah et al., 2019).

A previous study stated that biofertilizers contain microorganisms such as *Azotobacter*, *Azospirillum*, and *Rhizobium*, which can improve soil fertility through nitrogen fixation, enhance soil health, and increase crop productivity. Biofertilizer application can increase nutrient absorption in pineapples, especially by accelerating the decomposition of organic matter (Hazra et al., 2019). A combination of Arbuscular Mycorrhizal Fungi (AMF) biostimulants and fertilizers improves maize production (Akpode et al., 2024). Additionally, mycorrhizal associations have been linked to improved plant resistance to diseases and environmental stresses, further contributing to enhanced growth and productivity (Olaleye and Fagbola, 2020). Arbuscular Mycorrhizal Fungi (AMFs) contribute to improving soil fertility on oil carbon sequestration, nutrient contents, microbial activities, and soil structure (Fall et al., 2022). This issue addresses determining the important role of biofertilizers and mycorrhizal fungi on the vegetative growth of pineapple in acid soil.

Materials and Methods

Experimental Site

This study was conducted from June 2022 to November 2022 in the Terbanggi Besar pineapple field in Lampung, Sumatra, Indonesia. The average temperature ranges from 20-35°C, and the humidity is typically 60-80%. The soil used in this study had highly acidic pH conditions, low organic C, N, and C/N ratios, moderate amounts of available P, and very low amounts of available K. Previous crops included cassava (K1 and K2) and pineapple (B1, B2, and B3). This condition is consistent with the chemical analysis of the Ultisol soil in the study by Janket et al. (2021) and Anwar et al. (2023), except condition P, which was classified as moderate. Additionally, the reference to the chemical analysis of Ultisol soil in the study by Anwar et al. (2023) can be corroborated by the findings in the literature that discuss the nutrient dynamics and soil characteristics of Ultisol, particularly in relation to cassava cultivation. Table 1 shows the results of soil chemical analyses.

Data Collections

Data was collected for soil properties and microbial analysis. Soil property parameters measured included biological properties (total microbes, nitrogen-fixing bacteria (*Azotobacter* sp.), phosphate-solubilizing bacteria (MPF), and potassium-solubilizing bacteria (MPK)) and chemical properties (soil pH (H_2O), C-Organic (determined using Walkley and Black), total nitrogen (Kjeldahl), C/N ratio, total phosphorus (Bray extract), and total potassium (HCL 25% extract). The parameters of vegetative plant measurements included plant height, number of leaves, root weight, and plant root health. The observation period was monthly.

Data collection for the mycorrhizal analysis. Mycorrhizal parameters observed included analysis of mycorrhizal colonization, number of mycorrhizal spores, and types of mycorrhizal spores. The soil media was determined based on the diversity in the number of spore types for planting pineapple. The pouring–filter method (Pacioni, 1992) was used in isolation, followed by centrifugation (Brundrett et al., 1996). Percentage of root colonization by viewing the effectiveness of mycorrhizae in infecting roots by observing vesicles, arbuscules, and hyphae on roots,

Table 1. Initial soil chemical properties used for the study.

Treatment Code	pH	Organic C (%)	N (%)	C/N	P_2O_5 (mg.100 g^{-1})	K_2O (mg.100 g^{-1})
K1	3.60 (ha)	1.03 (l)	0.13 (l)	7.77 (l)	27.62 (m)	2.22 (vl)
K2	3.79-3.83 (ha)	1.07 (l)	0.17 (l)	6.17 (l)	25.29 (m)	2.25 (vl)
B1	3.81-3.91 (ha)	0.88 (vl)	0.15 (l)	5.87 (l)	24.06 (m)	2.07 (vl)
B2	3.75 (ha)	1.07 (l)	0.19 (l)	5.63 (l)	24.3 (m)	2.28 (vl)
B3	3.80-3.83 (ha)	1.03 (l)	0.15 (l)	6.87 (l)	23.57 (m)	2.25 (vl)

Notes: K1 and K2= previously used to grow cassava; B1, B2, and B3= previously used to grow pineapples; ha= highly acidic; vl= very low; l= low; m= moderate (BPSI, 2023).

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and calculated using the calculation developed by Rajapakse and Miller (1992) as follows:

$$\text{Root colonization (\%)} = \frac{\text{Infected root (\%)}}{\text{Area of observing plate}} \text{ x } 100\%$$

The claim of mycorrhizal biofertilizer spores includes the types *Glomus etunicatum*, *Glomus maniholtis*, *Acaulospora* sp., and *Gigaspora* sp., containing 626 spores per gram of fertilizer. Mycorrhizal colonization in plants was analyzed by calculating the rate of root infection and spore counts stored in the roots and soil samples, categorized as mycorrhizal infection according to the Institute of Mycorrhizal Research and Development, USDA Forest Service.

Data collection for root health index scores. Root health index assessments were carried out at the fourth month after planting (4th MAP). Pineapple plants were collected from polybags, washed with clean water, and dried. The plant fresh weight, root fresh weight, main root length, fibrous roots, non-fibrous roots, and fine fibrous roots were measured. The following formula was used to calculate root health:

$$\text{Total of roots} = \text{AS} + \text{ANS} + \text{ASH} \quad (1)$$

$$\%\text{AS} = \frac{\text{Number of fibrous root}}{\text{total of roots}} \quad (2)$$

$$\%\text{ANS} = \frac{\text{Number of non-fibrous root}}{\text{total of roots}} \quad (3)$$

$$\%\text{ASH} = \frac{\text{Numbers of fine fibrous root}}{\text{total of roots}} \quad (4)$$

Note: AS= number of fibrous roots, ANS= number of non-fibrous roots, and ASH= number of fine fibrous roots.

RHI was calculated as an index (RHI):

$$\text{RHI} = 30\%\ \text{PAU} + 20\%\ \text{AS} + 10\%\ \text{ANS} + 30\%\ \text{BBA} + 10\%\ \text{ASH} \quad (5)$$

Note: PAU= main root length; BBA= fresh plant weight.

Research Design

The experiment followed a completely randomized design (CRD) with nine treatment levels, each replicated five times. Treatments included control, the use of chemical fertilizer (NPK), biofertilizer, mycorrhizae, and different combinations of these inputs. The standard or "100% fertilization rate" was defined as 0.5 g ZA per plant (equivalent to 50 $kg.ha^{-1}$ or 10.5 kg $N.ha^{-1}$), 2 g DAP per plant (200 $kg.ha^{-1}$ or 92 kg $P_2O_5.ha^{-1}$), 2 g KCl per plant (200 $kg.ha^{-1}$ or 100 kg $K_2O.ha^{-1}$), 5 g mycorrhizae per plant, and 10 ml Provibio® biofertilizer diluted in 1 L water. Provibio® contains nine beneficial microorganisms, namely *Azospirillum lipoferum* ICBB 6088, *Bradyrhizobium japonicum* ICBB 9251, and *Lactobacillus* sp. ICBB 6099, *Saccharomyces cerevisiae* ICBB 8808, *Microbacterium lactium* ICBB 7125, *Phanerochaete* sp. ICBB 9182, *Paenibacillus macerans* ICBB 8810, and *Bacillus thuringiensis* ICBB 6095. A summary of the treatments is provided in Table 1.

Pineapple Cultivation

Experiments using pineapple crown seedlings. Pineapple seedlings were planted in 15 kg soil-filled polybags for a period of four months after planting. Inorganic fertilizer (nitrogen-phosphate-potassium fertilizer, abbreviated as NPK) was applied at planting, whereas mycorrhiza and biofertilizer were applied one month after planting. The polybag was then shaded 75% with a nylon net.

Treatment codes	Description	ZA (g)	DAP (g)	KCl (g)	Mycorrhizae (g)	Provibio® ($ml.L^{-1}$)
P0	Control (no input)	0	0	0	0.00	0.0
P1	100% NPK	0.5	2	2	0.00	0.0
P2	100% Provibio® + 100% NPK	0.5	2	2	0.00	10.0
P3	100% Provibio® + 50% NPK	0.25	1	1	0.00	10.0
P4	100% Mycorrhizae + 100% NPK	0.5	2	2	5.00	0.0
P5	100% Mycorrhizae + 50% NPK	0.25	1	1	5.00	0.0
P6	100% Provibio® + 100% Mycorrhizae + 50% NPK	0.25	1	1	5.00	10.0
P7	75% Provibio® + 75% Mycorrhizae + 50% NPK	0.25	1	1	3.75	7.5
P8	50% Provibio® + 50% Mycorrhizae + 50% NPK	0.25	1	1	2.50	5.0

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Data Analysis

Vegetative growth data were analyzed using ANOVA, followed by the 5% DMRT (Duncan Multiple Range Test) to determine the significance of the variables studied. Pearson's correlation analysis of soil property data was used in this study.

Results and Discussion

Soil Chemical Properties

After four months, the soil's pH and organic C content increased compared to the initial chemical conditions of the soil (Table 2). An increase in organic C content indicates an increase in the number and activity of microorganisms in the soil (Patoine et al., 2022). The rise in pH due to mycorrhizae and biofertilizers can be attributed to the enhancement of organic compounds in the soil, which play a crucial role in binding cations within the adsorption complex. This phenomenon leads to a higher concentration of base saturation in the soil. Mycorrhizal fungi are known to improve soil structure and increase the availability of nutrients, including phosphorus, which can indirectly influence soil pH by enhancing organic matter content and cation exchange capacity (CEC) (Herawati et al., 2021; Fikrinda et al., 2022) microorganisms play an important role in the decomposition of organic matter. This affects the increase in pH because the decomposition process releases organic compounds, whether organic acids or base cations, which can increase the soil pH (Latief et al., 2017).

The available nutrients also changed, with N and K increasing, but P decreasing. Increased availability of N and reduced availability of P in soil occurs due to the mutual association between mycorrhizae and N-fixing microbes, which has been reported by Hestrin et al. (2019), where N-fixing microbes require high amounts of P to produce nitrogenase enzymes so that mycorrhizae help provide P for plants and also for microbes around them (Püschel et al., 2017). The increased availability of K is one of the advantages of mycorrhizal biofertilizers (Begum et al., 2019). The condition where available P is lower than before treatment indicates that plants can absorb nutrients well. According to Natalia et al. (2018), on pineapple cultivation land with high productivity, more available nutrients are absorbed by plants, leading to a lower amount of nutrients available after harvest on the land compared to plants. Table 2 shows the analysis results of the soil chemical properties, four months after planting.

Soil Biological Properties

Table 3 shows the results of the analysis of soil biological properties, including the total population of *Azotobacter* sp., total microbes, MPF, and MPK. The population of *Azotobacter* sp. was the most abundant in P6 (6.15 × 10^4 CFU.g^{-1}) and the lowest in P2 (0.45 × 10^4 CFU.g^{-1}). Nitrogen levels can also be affected by the dose of the fertilizer applied. The application of biofertilizers increased the value of N-total in the chemical properties of the soil. The highest yield of the total microbial population was found in sample P8, which was 26.05 × 10^4 CFU.g^{-1}, and the lowest population was found in sample P6, with a value of 5.20 × 10^4 CFU.g^{-1}. This is consistent with a study

Table 2. Soil chemical properties after four months of mycorrhizae and biofertilizers application

Treatment codes	pH	C-org (%)	N (%)	C/N	P2O5 (mg.100g^{-1})	K2O (mg.100g^{-1})
P0	5.16-5.75 (a)	1.64 (l)	0.31 (m)	5.29 (l)	5.60 (vl)	5.35 (vl)
P1	4.83-5.82 (a)	2.43 (m)	0.34 (m)	7.15 (l)	4.88 (vl)	4.04 (vl)
P2	5.28-5.82(a)	2.26 (m)	0.29 (m)	7.79 (l)	4.95 (vl)	6.15 (vl)
P3	4.68-5.39 (a)	2.07 (m)	0.32 (m)	6.47 (l)	4.75 (vl)	2.45 (vl)
P4	5.43-5.57 (a)	2.33 (m)	0.31 (m)	7.52 (l)	4.86 (vl)	3.19 (vl)
P5	5.34-5.61 (a)	2.58 (m)	0.29 (m)	8.90 (l)	5.06 (vl)	1.84 (vl)
P6	5.22-5.59 (a)	2.84 (m)	0.31 (m)	9.16 (l)	4.93 (vl)	6.41 (vl)
P7	5,17-5,84 (a)	3.10 (h)	0.31 (m)	10.00 (l)	5.78 (vl)	7.19 (vl)
P8	5.16-5.75 (a)	1.64 (l)	0.31 (m)	5.29 (l)	5.60 (vl)	5.35 (vl)

Notes: a= acid; vl= very low; l= low; m= moderate; h= high; vh= very high (BPSI, 2023). P0 (Control), P1 (NPK 100%), P2 (100% Provibio® biofertilizer + 100% NPK), P3 (Provibio® biofertilizer 100%+ NPK 50%), P4 (Mycorrhizae 100% + NPK 100%), P5 (Mycorrhizae 100% + NPK 50%), P6 (Provibio® biofertilizer 100% + Mycorrhizae 100% + NPK 50%), P7 (Provibio® biofertilizer 75% + Mycorrhizae 75% + NPK 50%), and P8 (Provibio® biofertilizer 50% + Mycorrhizae 50% + NPK 50%).

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by Susilawati et al. (2013), who found that the total microbial count in the soil is influenced by various growth conditions such as temperature, humidity, aeration, energy sources, and cultivation practices.

The results of the MPF population showed that the highest population was found in the P5 sample, with a value of 3.00 × 104 CFU.g^{-1}, and the lowest was in the P0 sample with a value of 0.20 x 104 CFU.g^{-1}. Phosphate-solubilizing bacteria (MPF) are known to colonize the rhizosphere and root tissues, where they play a crucial role in making phosphorus available to plants by solubilizing it from insoluble forms (Adhyaningtyas et al., 2023; Agboola et al., 2023; Syahri et al., 2023). The results of the MPK population analysis were much lower than those of the other bacterial analyses. The lowest yield was 0.15 × 104 CFU.g^{-1} found in sample P8, and the highest yield was 1.30 × 104 in sample P6. This small total population may have been affected by the pH value. Furthermore, the study by Adebajo et al. (2021) emphasized that potassium solubilization by different bacterial genera, such as *Pseudomonas* and *Bacillus*, varies with soil conditions, including pH, and that these bacteria can effectively solubilize potassium in soils with a pH around 5-8. Applications of biofertilizers have the advantage of increasing the diversity and activity of soil microbial populations, repairing soil structures, and increasing crop yields (Adhyaningtyas et al., 2023; Agboola et al., 2023). Furthermore, other biological characteristics, such as populations of *Azotobacter* sp., MPF, and MPK, exhibited a low number of populations due to soil sampling, which exposes them to herbicides that can negatively impact bacterial populations.

Growth Responses

Table 4 shows observations of plant height and number of leaves at 1 Month After Planting (MAP) and 2 MAP; the plants had high uniformity. The plant height data at 3 and 4 MAP showed significant differences for each new treatment. This occurred because pineapple roots generally appear when pineapple plantlets have reached a height of approximately 30 cm (Reinhardt et al., 2017), and seedlings without roots and pineapple plantlets cannot take advantage of external inputs provided through the soil. The DMRT follow-up test analysis showed that the plant height data at 3 and 4 MAP yielded significant differences between the treatments. At four MAP treatments, P1 yielded the best results, and treatments P2, P4, P5, P6, and P8 were not significantly different from treatment P1. This showed that treatment with P2, P4, P5, P6, and P8 matched the growth of P1 (NPK 100%). The presence of bacteria in biofertilizers is crucial for enhancing plant growth, primarily due to their ability to produce phytohormones such as auxins, cytokinin, and gibberellins (Setiawati et al., 2023).

Table 5 shows that the fresh and dry weights of the plants were the highest with the combination of mycorrhizal and biofertilizer treatments (P4, P5, P6, and P8), and the highest weight was 100% inorganic fertilizer (P1). Observation of fresh and dry weights is one way to determine biomass changes during treatment (Huang et al., 2019). According to Luo et al. (2022), plants under drought conditions and lacking nutrients tend to grow roots. Moreover, Luo et al. (2022) stated that the presence of mycorrhizae in plant roots causes the plants to be more resistant to drought and nutrient deficiency; therefore, biomass growth is focused on the parts of the plant above the ground.

Table 3. Soil biological properties due to the application of mycorrhizae and biofertilizers.

Treatment	Population of microorganisms ($\times 10^4$ CFU.g^{-1})			
	Azotobacter	MPK	MPF	Microbe total
Control	1.25	0.20	0.35	9.25
NPK 100%	0.55	1.10	0.85	11.00
100% Provibio® biofertilizer + 100% NPK	0.45	1.10	0.85	21.72
Provibio® biofertilizer 100%+ NPK 50%	1.80	1.65	0.30	15.40
Mycorrhizae 100% + NPK 100%	1.25	1.40	0.60	7.20
Mycorrhizae 100% + NPK 50%	3.00	3.00	0.95	11.04
Provibio® biofertilizer 100% + Mycorrhizae 100% + NPK 50%	6.15	0.40	1.30	5.20
Provibio® biofertilizer 75% + Mycorrhizae 75% + NPK 50%	3.40	0.35	0.80	9.60
Provibio® biofertilizer 50% + Mycorrhizae 50% + NPK 50%	1.50	2.15	0.15	26.05

Notes: Phosphate-solubilizing bacteria (MPF) and potassium-solubilizing bacteria (MPK).

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Table 4. Plant height and number of pineapple leaves due to the application of mycorrhizae and biofertilizers on an acid Ultisol

Treatments	Plant height (cm)			
	1 MAP	2 MAP	3 MAP	4 MAP
Control	16.53a	26.30a	36.50a	46.87a
NPK 100%	20.91b	33.75bc	49.88e	59.27f
100% Provibio® biofertilizer + 100% NPK	20.18b	34.04c	49.40e	57.61cde
Provibio® biofertilizer 100%+ NPK 50%	18.67ab	31.47bc	46.89cd	56.97bcd
Mycorrhizae 100% + NPK 100%	18.76ab	32.68bc	49.47e	56.63bcd
Mycorrhizae 100% + NPK 50%	19.04ab	31.90bc	48.43e	58.03de
Provibio® biofertilizer 100% + Mycorrhizae 100% + NPK 50%	19.39ab	32.49bc	48.32d	58.39ef
Provibio® biofertilizer 75% + Mycorrhizae 75% + NPK 50%	18.46ab	31.11b	45.68bc	56.62b
Provibio® biofertilizer 50% + Mycorrhizae 50% + NPK 50%	19.80ab	32.09bc	45.25b	59.14f
	Leaf number			
Control	46.00ab	56.33bc	63.00bc	66.67bc
NPK 100%	46.40b	55.47bc	62.73bc	66.33bc
100% Provibio® biofertilizer + 100% NPK	43.67a	52.40a	59.47a	67.95c
Provibio® biofertilizer 100%+ NPK 50%	46.87b	57.13c	64.07c	68.33c
Mycorrhizae 100% + NPK 100%	46.40b	56.02bc	62.80bc	63.93a
Mycorrhizae 100% + NPK 50%	45.47ab	54.27b	61.47ab	65.53ab
Provibio® biofertilizer 100% + Mycorrhizae 100% + NPK 50%	45.73ab	55.47bc	62.40bc	67.07bc
Provibio® biofertilizer 75% + Mycorrhizae 75% + NPK 50%	46.33b	56.40bc	62.73bc	67.27bc
Provibio® biofertilizer 50% + Mycorrhizae 50% + NPK 50%	46.07ab	55.47bc	62.20bc	67.33bc

Notes: Values in each row and column followed by the same letter are not significantly different based on the DMRT multiple range test at 5%.

Table 5. Fresh and dry weight of plants due to the application of mycorrhizae and biofertilizers on an acid Ultisol

Treatments	Fresh weight (g)	Dry weight (g)	% Dry / fresh weight
Control	547.25a	57.26a	10.46
NPK 100%	725.44d	99.51d	13.72
100% Provibio® biofertilizer + 100% NPK	631.00c	85.77c	13.60
Provibio® biofertilizer 100%+ NPK 50%	575.78b	71.03abc	12.34
Mycorrhizae 100% + NPK 100%	709.22d	85.11e	12.00
Mycorrhizae 100% + NPK 50%	707.89d	71.45abc	10.09
Provibio® biofertilizer 100% + Mycorrhizae 100% + NPK 50%	684.33d	74.29abc	10.86
Provibio® biofertilizer 75% + Mycorrhizae 75% + NPK 50%	564.58ab	62.02a	10.98
Provibio® biofertilizer 50% + Mycorrhizae 50% + NPK 50%	707.89d	73.97abc	10.45

Notes: Values in each row and column followed by the same letter are not significantly different based on the DMRT multiple range test at 5%.

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Table 6 presents the root health index (RHI), indicating that the combination of mycorrhizal and biofertilizer treatments yielded moderately healthy roots. Healthy roots protect plants from pathogen attacks and root diseases (Weng et al., 2022). Root health conditions also affect the ability of roots to absorb water and nutrients, protect plants from root diseases, repair soil structures, and shelter beneficial microorganisms, such as mycorrhizae, in symbiosis with plants through roots (Ryan et al., 2016).

Mycorrhiza Root Colonization

Table 7 shows that mycorrhizal roots (P4, P5, P6, P7, and P8) had a higher range of root infections than those without mycorrhizal treatments (P0, P1, P2, and P3). Mycorrhizal 100% had the highest root infection rate, ranging from 21% to 67%, which falls under the Class 4 category, or high mycorrhizal infection, according to the Institute of Mycorrhizal Research and Development and the USDA Forest Service.

The P5 treatment yielded the highest spores (13-26) per 50 g of sample. According to Triguna et al. (2024), there are significant increases in spores and arbuscular mycorrhizal fungi (AMF) colonization in mungbeans, indicating variability in spore counts depending on treatment and environmental conditions. Similar to Alayya and Prasetya (2022), the spore count in a mycorrhizal colony is determined by various factors, such as environmental conditions, the type of host plant, and the type of mycorrhizal association.

Table 6. Root health index (RHI) of pineapple plants due to the application of mycorrhizae and biofertilizers on an acid Ultisol

Treatments	Root health index*	Notes*
Control	3.30	Moderately healthy
NPK 100%	3.00 - 3.10	Moderately healthy
100% Provibio® biofertilizer + 100% NPK	3.60	Healthy
Provibio® biofertilizer 100%+ NPK 50%	3.00 - 3.60	Moderately healthy- Healthy
Mycorrhizae 100% + NPK 100%	3.00 - 3.10	Moderately healthy
Mycorrhizae 100% + NPK 50%	2.50 - 3.60	Moderately healthy
Provibio® biofertilizer 100% + Mycorrhizae 100% + NPK 50%	3.30	Moderately healthy
Provibio® biofertilizer 75% + Mycorrhizae 75% + NPK 50%	2.50 - 3.00	Moderately healthy
Provibio® biofertilizer 50% + Mycorrhizae 50% + NPK 50%	3.30 - 3.60	Moderately healthy- Healthy

Notes: *= The root health index based on PT. Great Giant Pineapple standards.

Table 7. Root colonization, number, and type of spores in an acid Ultisol due to the application of mycorrhizae and biofertilizers

Treatment	Root infection	Infection rate	Spore count	Type of spore
P0	0%	Class 1	5	-[z]
P1	3-11%	Class 2	3-16	-
P2	0-11%	Class 2	0-5	-
P3	3-6%	Class 2	3-13	-
P4	21-67%	Class 4	3-14	*Acaulospora* sp.
P5	15-38%	Class 3	13-26	*Acaulospora* sp.
P6	14-24%	Class 3	7-13	*Acaulospora* sp.
P7	13-17%	Class 2	1-15	*Acaulospora* sp.
P8	3-18%	Class 2	5-8	*Acaulospora* sp.

Notes: [z]: no mycorrhizal spores; rate of infection based on the guidelines of The Instate of Mycorrhizal Research and Development, USDA Forest Service, Athena, Georgia, USA (Setiadi and Setiawan, 2011). P0 (Control), P1 (NPK 100%), P2 (100% Provibio® biofertilizer + 100% NPK), P3 (Provibio® biofertilizer 100%+ NPK 50%), P4 (Mycorrhizae 100% + NPK 100%), P5 (Mycorrhizae 100% + NPK 50%), P6 (Provibio® biofertilizer 100% + Mycorrhizae 100% + NPK 50%), P7 (Provibio® biofertilizer 75% + Mycorrhizae 75% + NPK 50%), and P8 (Provibio® biofertilizer 50% + Mycorrhizae 50% + NPK 50%).

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However, spore count does not directly correlate with root infection (Setiadi and Setiawan, 2011), which is key to successful mycorrhizal inoculation in plants. However, the decline in mycorrhizal spores was due to the soil pH, as there are differences between the optimum germination pH of each type of spore. *Glomus* sp. develops optimally at pH 5.6-7, *Acaulospora* sp. at pH 4-5, and *Gigaspora* sp. at pH 4-6. Based on Debashis et al. (2022), arbuscular mycorrhizal fungi (AMF) symbionts are highly beneficial in planting for abiotic stress tolerance and pH. Figure 1 shows the root infections caused by mycorrhizae and *Acoulospora* sp.

Correlation Analysis

Table 8 shows the correlation between chemical and biological soil properties, including total populations of microbes, *Azotobacter* sp., MPF, and MPK, and soil chemical properties, including pH, K-total K, P-total P, organic C, and N-total N. Total microbes had a positive value and a positive correlation with a pH value of 0.649, meaning that as the pH value increased, the number of microbes increased. The effect of pH on bacterial growth is related to enzyme activity. Bacteria require enzymes to catalyze reactions related to bacterial growth. If the pH of the medium or environment is not optimal, it will interfere with bacterial growth (Susilawati et al., 2013). The correlation between *Azotobacter* sp. and N-total N had a value of -0.615 (perfect negative correlation), indicating that the relationship between the two variables is always inverse. The N-total N value resulted from the addition of inorganic fertilizers, which directly provided sufficient nitrogen to the soil. The N-total N level was adequate; therefore, the treatment with *Azotobacter* sp. had no significant effect because the N-total N in the soil was already available.

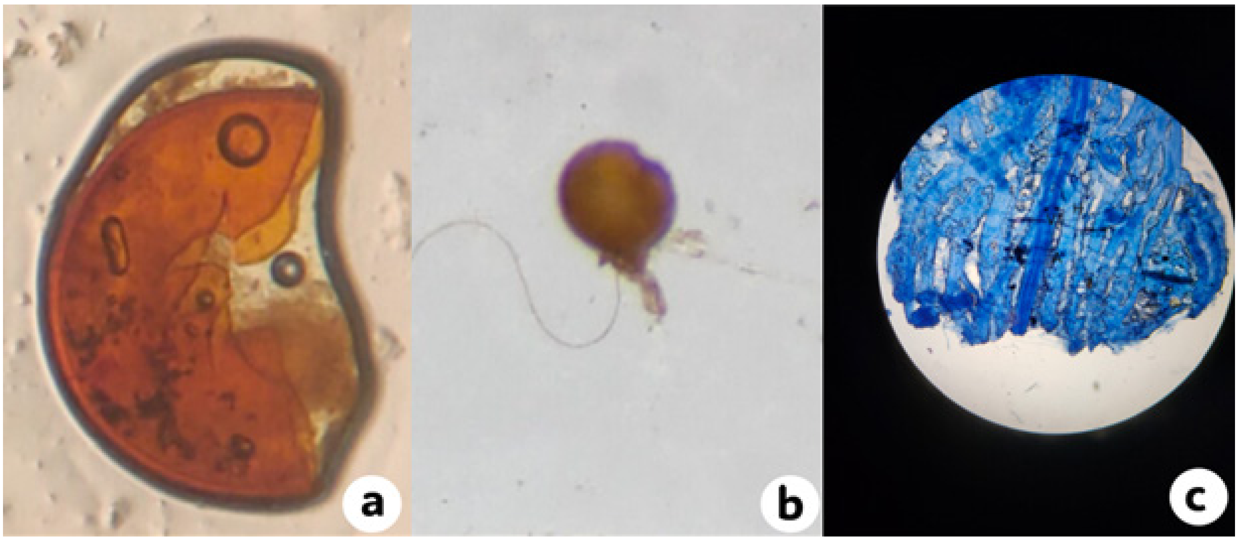

Figure 1. Mycorrhizal spores and infected roots. a) *Acaulospora* sp. at 400X magnification, b) *Acaulospora* sp. at 400X magnification, c) Root of pineapple infected by mycorrhizae at 400X magnification.

Table 8. Correlation of biological properties with soil chemical properties

Parameter	pH	Total microbes	*Azotobacter*	MPF	MPK	Total potassium	Total phosphate	Organic C	N
pH	1	0.972	0.126	0.506	0.639	0.941	0.393	0.775	0.021
Microbe total	0.649	1	0.177	0.244	0.144	0.366	0.222	0.344	0.311
Azotobacter	0.151	-0.496	1	0.633	0.088	0.255	0.577	0.407	0.077
MPF	0.282	0.400	-0.165	1	0.625	0.566	0.849	0.762	0.889
MPK	-0.216	-0.529	0.597	-0.197	1	0.024	0.374	0.439	0.976
K-total	0.012	0.340	-0.424	-0.211	-0.734	1	0.221	0.277	0.899
P-Total	-0.122	0.452	-0.217	0.086	-0.337	0.452	1	0.724	0.702
C-organic	0.588	0.355	0.315	-0.117	-0.295	0.406	0.137	1	0.75
N-total	0.000	0.377	-0.615	0.013	-0.011	-0.049	-0.148	-0.061	1

Notes: Below the diagonal is the correlation value, and above the diagonal is the P-value.

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The mycorrhizal colonization analysis revealed that 100% mycorrhizae + 100% nitrogen-phosphate-potassium fertilizer (P4) enhanced root infection. However, the application of mycorrhizae did not result in significant differences in the vegetative growth. This finding is consistent with the study by Sutrisno et al. (2018), which reported no significant difference in the number of leaves between the treatment without mycorrhizae and the treatment with mycorrhizae. This is because the pineapple plant has a long lifespan, and the seeds used are crowns, which require a longer time to grow (Utama et al., 2007).

The optimal yield results for vegetative growth, soil properties, and root health index (pH, C-Organic, N-total, the highest microbial population of 26.05 × 104 CFU.g^{-1}) were observed in the P8 treatment (50% Provibio® biofertilizer + 50% Mycorrhizae + 50% NPK), which is a combination of biofertilizers. Mycorrhizae increased soil health in acidic soil. Various studies related to the improvement of acidic soil for pineapple plant growth, such as liming (Sutrisno et al., 2018), FABA and lignite (Fajarindo et al., 2023), and organic fertilizers (Hazra et al., 2019) and biofertilizers (Krishan et al., 2017), which drive beneficial microorganisms, play an important role in soil health. This indicates that improving soil health is a key determinant of sustainable agriculture. Pineapple is one of the important fruit crops worldwide and belongs to the CAM group, which requires a lot of water (Akpode et al., 2024). This results in high nutrient leaching, leading to water pollution through nutrient uptake. However, this research was conducted during the pineapple's vegetative period. Data on the generative period and replication of experiments in other locations with different microclimates are needed to obtain a more comprehensive picture of the technology in the field, particularly in relation to the use of a combination of mycorrhizae and biofertilizers as part of building sustainable agriculture in the future.

Conclusions

Applying mycorrhizae at 5 g per plant together with the full dose of chemical fertilizer (0.5 g ZA, 2 g DAP, and 2 g KCl per plant) produced very high levels of root infection, as shown by the mycorrhizal colonization parameters. On the other hand, a balanced combination of Provibio® biofertilizer (5 ml per plant, diluted in 0.5 L water), mycorrhizae (2.5 g per plant), and half the chemical fertilizer dose (0.25 g ZA, 1 g DAP, and 1 g KCl per plant) led to better vegetative growth, improved soil properties (pH, organic C, total N), and a higher root health index. This treatment also yielded the largest microbial population in the rhizosphere (26.05 × 10^4 CFU.g^{-1} soil). Overall, these results suggest that using moderate and balanced doses of biofertilizer, mycorrhizae, and NPK is more effective than relying on full single-dose applications, thereby helping to create healthier soil and promoting stronger pineapple growth in acidic conditions.

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