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Impact of Bioameliorants and *Trichoderma sp.* on Nutrient Availability, Plant Immunity, and Red Chili Productivity.

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ABSTRACT

The low productivity of chili plants needs to be increased through soil fertility and pest and disease control. The application of bioameliorants (50% biochar, 25% sugarcane blotong compost, 10% dolomite, 10% guano, 2.5% humic acid, and 2.5% biofertilizer) aims to improve the physical, chemical, and biological properties of the soil and enhance nutrient availability. Trichoderma sp. is needed to stimulate growth and induce resistance. The research aims to determine the best interaction and dosage of bioameliorants and Trichoderma sp. on the parameters of phosphate-solubilizing bacteria, available P, plant height, number of leaves, fruit weight, number of fruits per plant, and induced resistance. The research was conducted at the Bale Tatanen Experimental Garden of the Faculty of Agriculture, Universitas Padjadjaran. The research used a Randomized Block Design (RBD) factorial with nine treatments and three repetitions. The treatments included bioameliorants at 0 t ha^{-1}, 3 t ha^{-1}, 6 t ha^{-1} and Trichoderma sp. at 0 kg ha^{-1}, 8 kg ha^{-1}, and 16 kg ha^{-1}. The results of the application of bioameliorants and the biological agent Trichoderma sp., as well as the control treatment, showed no interaction between treatments. The application of bioameliorants and biological agents showed the best dose in the independent effect of bioameliorant at 6 t ha^{-1}, which increased available P by 86.6% compared to the control. The application of Trichoderma sp. showed the best dose at 16 kg ha^{-1}, which reduced the intensity of leaf curl virus disease by 66.6% compared to the control and reduced the intensity of fruit fly pest attacks at 16 kg ha^{-1} by 20.5% compared to the control.

Keyword: *attack intensity, growth, microbes, organic matter, Plant Immunity*

ABSTRAK

Produktivitas tanaman cabai yang rendah perlu ditingkatkan dari sisi kesuburan tanah dan pengendalian hama penyakit. Aplikasi bioamelioran (biochar 50%, kompos blotong tebu 25%, dolomit 10%, guano 10%, humic acid 2,5% dan pupuk bio 2,5%) guna memperbaiki sifat fisik, kimia, biologi tanah dan meningkatkan unsur hara. *Trichoderma* sp. diperlukan untuk memacu pertumbuhan dan ketahanan terimbas. Penelitian bertujuan mengetahui interaksi serta dosis terbaik pada pemberian bioamelioran dan *Trichoderma* sp. terhadap parameter bakteri pelarut fosfat, P-tersedia, tinggi tanaman, jumlah daun, bobot buah, jumlah buah per tanaman, dan ketahanan terimbas. Penelitian dilakukan di Kebun Percobaan Bale Tatanen Fakultas Pertanian Universitas Padjadjaran. Penelitian menggunakan Rancangan Acak Kelompok (RAK) Faktorial sembilan perlakuan dan diulang tiga kali. Perlakuan menggunakan bioamelioran 0 t ha^{-1}, 3 t ha-1, 6 t ha^{-1} dan *Trichoderma* sp. 0 kg ha^{-1}, 8 kg ha^{-1}, dan 16 kg ha^{-1}. Hasil pemberian bioamelioran dan Agen hayati *Trichoderma* sp. maupun perlakuan kontrol tidak terdapat interaksi antar perlakuan. Pemberian bioamelioran dan agen hayati *Trichoderma* sp. menunjukkan dosis terbaik pada pengaruh mandiri bioamelioran 6 t ha^{-1} terhadap P-tersedia meningkat sebesar 86,6% dari kontrol. Aplikasi *Trichoderma* sp. menunjukkan dosis terbaik pada pemberian *Trichoderma* sp. 16 kg ha^{-1} menurunkan intensitas penyakit virus keriting daun sebesar 66,6% dari kontrol dan menurunkan intensitas serangan hama lalat pada *Trichoderma* sp. 16 kg ha^{-1} sebesar 20,5% dari kontrol.

Keyword: bahan organik, intensitas serangan, mikrob, pertumbuhan, imunitas

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1. Introduction

Chili is one of the horticultural crops widely cultivated by the Indonesian people. Chili is a primary need for households, the food industry, large-scale industries, and other purposes. The distinctive characteristic of chili is its spicy taste from the capsaicinoid compounds, which are a group of alkaloids that make chili taste hot (Lestari, 2021). The spicy taste of chili is highly favored by the Indonesian population. The increasing population each year leads to a higher demand for chili production. In 2022, chili production in Indonesia reached 1,544,441 million tons, an increase of 11.4% with a harvested area of 187,849 ha and a productivity of 8.22 t ha^{-1} compared to 2021 (Badan Pusat Statistik, 2022). Despite the higher production figures, the productivity is still considered low. As explained by the Center for Agricultural Data and Information Systems (2023), chili productivity is considered low if it is below 10 t ha^{-1}. This indicates that chili productivity needs to be increased for higher yields.

The low productivity of chili peppers is influenced by several factors. These factors include soil quality, land management, soil fertility levels, and attacks by plant pests (Polii *et al.*, 2019). Soil quality and fertility significantly affect productivity. Excessive use of inorganic inputs in crop cultivation is not recommended because it has adverse effects on the environment. This results in challenges such as soil fertility reduction, organic matter content decrease, and soil structure deterioration. Efforts to address these challenges include the application of bioameliorants. Bioameliorants are soil conditioners that contain biofertilizers. According to Simarmata *et al*. (2023) stated that bioameliorants originate from a mixture of materials such as organic substances, biofertilizers, and soil conditioners (ameliorants) that can enhance soil fertility and health. Microbial activities from biofertilizers include nitrogen-fixing bacteria, phosphate-solubilizing microbes, and Plant Growth Promoting Rhizobacteria (Nosheen *et al.*, 2021). However, there are challenges that affect plants beyond soil fertility, namely disease and pest disturbances.

Chili plant diseases significantly impact production outcomes. According to Kurniastuti & Puspitorini (2021), approximately 90% of agricultural losses result from disease-related disruptions, which are highly detrimental to farmers. Diseases affecting chili plants include Fusarium wilt, bacterial wilt (Ralstonia), leaf curl disease caused by thrips, and yellow virus disease (Arsi *et al*., 2020). Control measures for chili plants can be implemented preventively by using environmentally friendly biological agents such as the fungus *Trichoderma* sp. *Trichoderma* sp. applied to chili plants plays a role in induced resistance. According to Purwantisari *et al*. (2016), *Trichoderma* sp. as an antagonistic fungus is beneficial for plants because it can induce plant resistance against pathogen invasion. The mechanism of induced resistance, also known as Induced Systemic Resistance (ISR), is activated indirectly by *Trichoderma* sp., which strengthens plant defense by suppressing pathogens (Devendra *et al*., 2007). *Trichoderma* sp. also aids in the decomposition of organic matter to enhance soil fertility, acting as an organic matter decomposer (Dedik & Gusti, 2017). Its ability as a decomposer accelerates the decomposition process of organic materials in bioameliorants. The breakdown products from *Trichoderma* sp. enhance nutrient availability for plant growth. Adequate nutrient supply results in high-quality chili fruit formation.

The application of *Trichoderma* sp. with bioameliorants has a beneficial interactive relationship that accelerates decomposition, enhances soil fertility, promotes plant growth, and improves chili fruit yield. Bioameliorants enhance soil phosphorus availability due to the presence of biofertilizers, including phosphate-solubilizing bacteria. These bacteria produce phosphatase enzymes that hydrolyze organic phosphates into inorganic phosphates, making phosphorus available to plants (Sonia & Tri, 2022). Phosphatase enzymes play a crucial role in the hydrolysis process of organic phosphates into inorganic phosphates (Fitriatin *et al*., 2020). Phosphate-solubilizing bacteria thrive actively and facilitate phosphorus solubilization in the soil, especially when supplied with high-energy sources from organic materials (Sinaga *et al*., 2018). This research aims to investigate the influence and interaction of bioameliorants and *Trichoderma* sp. on the population of phosphate-solubilizing bacteria, available phosphorus (P), induced resistance, and red chili yield.

2. Method

This research was conducted from July to December 2023 at the Experimental Field D3 Bale Tatanen, Faculty of Agriculture, Universitas Padjadjaran. Soil biological analysis was performed at the Soil Microbiology Laboratory, while soil chemical analysis was conducted at the Soil Fertility and Plant Nutrition Laboratory, both at the Faculty of Agriculture, Universitas Padjadjaran. The research method employed a Factorial Randomized Block Design (RBD) with 9 treatments and 3 replications, totaling 27 experimental units. There were 2 factors with 3 levels each: 1. Bioameliorant Factor (B);b0 = 0 t ha-1 Bioameliorant (without bioameliorant); b1 = 3 t ha-1 Bioameliorant equivalent to 215 g; b2 = 6 t ha-1 Bioameliorant equivalent to 430 g. 2. *Trichoderma* sp. Factor (T); t0 = 0 kg ha-1 *Trichoderma* sp. (without *Trichoderma* sp.); t1 = 8 kg ha-1 *Trichoderma* sp. equivalent to 0.57 g; t2 = 16 kg ha-1 *Trichoderma* sp. equivalent to 1.14 g

Chili seedlings were raised using a soil-seedling medium with a ratio of 2:1:1 of soil, cow manure, and rice husk ash. Seedlings were nurtured for approximately 28 days before being transplanted into polybags according to their respective treatments. Each polybag required 10 kg of growing media, adjusted with additional soil as necessary. The bioameliorant consisted of biochar (50%), sugarcane bagasse compost (25%), dolomite (10%), guano (10%), humic acid (2.5%), and biofertilizer containing nitrogen-fixing bacteria (1.25%) and phosphate-solubilizing bacteria (1.25%). *Trichoderma* sp. was applied into planting holes as per the treatments. Nutrient solution (AB mix) was administered every 3 days during the initial growth stages, with water given on alternate days. Concentrations were adjusted to 800 ppm (200 ml plant^{-1}) during 1-2 weeks after transplanting (WAP), 1200 ppm (400 ml plant^{-1}) during 3-4 WAP, and 1600 ppm (600 ml plant^{-1}) once the plants entered the generative phase.

Observations were conducted weekly from 1-6 weeks after planting (WAP) for plant height. Plant destruction was performed at the end of the vegetative stage (6 WAP), collecting soil samples for available phosphorus analysis and root zone samples for phosphate-solubilizing bacteria population assessment. Harvesting was carried out at intervals of 3 days, 1-5 times per harvest, to measure fruit weight per plant. Disease incidence was monitored throughout the growth period. Induced resistance (disease intensity during growth) against diseases such as chili leaf curl virus was calculated using the formula by Natawigena (1989):

$$P = \frac{A}{N} \times 100\%$$

Where:
- P = Plant damage level (%)
- A = Number of affected plants
- N = Total number of plants observed

3. Result and Discussion

3.1 Population of Phosphate-Solubilizing Bacteria

Based on the significant level analysis at 5% on the application of bioamelioran and *Trichoderma* sp. (Table 1) bioagent towards the population of phosphate-solubilizing bacteria there was no observed interaction effect between treatments. Bioagent on Phosphate-Solubilizing Bacteria Population at 6 WAP (weeks after planting). This is likely due to the suboptimal components of the bioamelioran in enhancing the population of phosphate-solubilizing bacteria. The low dosage of *Trichoderma* sp. is suspected to be insufficient in decomposing the organic materials from the bioamelioran.

Table 1. Independent Effects of Bioamelioran and Trichoderma sp.

Treatment	Phosphate-Solubilizing Bacteria (10^7 CFU mL^{-1})
Bioameliorant	
Control	0,85 a
3 t ha^{-1}	0,86 a
6 t ha^{-1}	0,87 a
Trichoderma sp.	
Control	0,83 a
8 kg ha^{-1}	0,87 a
16 kg ha^{-1}	0,89 a

Note: Mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% significance level.

Based on Table 1, there was no significant independent effect observed from the bioamelioran factor. However, the application of 6 t ha^{-1} bioamelioran slightly increased the population of phosphate-solubilizing bacteria compared to the control. The bioamelioran used in this experiment contains 1.25% biofertilizer with phosphate-solubilizing bacteria, which is suspected to contribute to the increase in phosphate-solubilizing bacteria population. The organic material component of bioamelioran, such as composted bagasse, is believed to be utilized by phosphate-solubilizing bacteria as an energy source for growth, thereby enhancing their population (Arifin *et al.*, 2021). This is suspected due to the bioamelioran component lacking cattle manure, thus not optimal in enhancing phosphate-solubilizing bacteria populations. This is supported by Situmorang *et al.* (2019), indicating that ameliorants containing 80% cattle manure, 20% coconut shell biochar, 5% dolomite, and guano increased phosphate-solubilizing bacteria populations compared to non-amended soils. This highlights the necessity of cattle manure in bioameliorants to boost phosphate-solubilizing bacteria populations. Cattle manure serves as organic matter, improving soil physical and biological properties, including the development of soil microbes, including phosphate-solubilizing bacteria (Oematan *et al*., 2022).

Table 3 results show no independent effect from *Trichoderma* sp. However, *Trichoderma* sp. at 16 kg ha^{-1} increased phosphate-solubilizing bacteria populations compared to the control, though not significantly different. Favorable environmental conditions and available substrates from Trichoderma sp. application support phosphate-solubilizing bacteria population growth (Hutamy, 2018). The application of *Trichoderma* sp. relates to providing energy sources for phosphate-solubilizing bacteria. The low dosage of *Trichoderma* sp. in this study, only 1.14 g per plant, is suspected to be insufficient, necessitating higher doses. This low dosage may not effectively decompose organic material from bioameliorants for phosphate-solubilizing bacteria energy sources, hence limiting bacteria population increases. Alori *et al.* (2017) research indicates that organically rich soils support microbial growth.

3.2 Available P

Based on the 5% significance level analysis of variance, the effect of bioamelioran and the biotic agent Trichoderma sp. (Table 2) on available P shows no interaction between treatments. However, there is a significant independent effect indicating an increase in available P from the application of bioamelioran

Table 2. Independent Effects of Bioamelioran and *Trichoderma* sp. on Available P at 6 WAP (weeks after planting)

Treatment	Available P (ppm)
Bioamelioran	
Control	7,48 a
3 t ha^{-1}	10,69 ab
6 t ha^{-1}	13,97 b
Trichoderma sp.	
Control	9,01 a
8 kg ha^{-1}	9,63 a
16 kg ha^{-1}	13,51 a

Note: Mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% significance level.

Table 2 shows that the independent treatment effect is observed only with the application of bioamelioran. The variance analysis at the 5% significance level indicates that the application of bioamelioran at a dose of 6 t ha^{-1} shows significantly different results from the control treatment. However, the application of bioamelioran at a dose of 3 t ha^{-1} is not significantly different from 6 t ha^{-1}. The increase in available P can be attributed to the application of bioamelioran containing components like compost from sugarcane filter cake and biofertilizer (phosphate-solubilizing bacteria) which have the ability to enhance available P. According to Leovisi (2012), compost from sugarcane filter cake has the additional benefit of neutralizing the effects of Al-dd, thereby increasing the availability of P in the soil. The study by Hidayat et al. (2023) shows that the parameter of available P increases from low to medium-high categories due to the inoculation of phosphate-solubilizing bacteria. Fitriatin *et al*. (2021) found that the application of biofertilizer and organic ameliorant significantly increased available P by up to 79.8% compared to the control.

Table 2 shows no independent effect of the biological agent *Trichoderma* sp. on available P. However, the application of *Trichoderma* sp. at 16 kg ha^{-1} slightly increased available P compared to the control. The lack of independent effect is likely due to the low dosage of *Trichoderma* sp. According to Elita *et al*. (2021), available P can increase with the application of *Trichoderma* sp. at a dose of 20 g per plant. Biofertilizer enriched with *Trichoderma* sp. in the rhizosphere region can enhance the available P content (Cai *et al*., 2015). Available P can increase due to the activity of *Trichoderma* sp. in the soil, which can bind metals such as Fe, Al, and Mn, releasing P ions and increasing the availability of P in the soil (Elita *et al*., 2021). Furthermore, Elita *et al*. (2021) explain that the activity of *Trichoderma* sp. in the soil not only increases available P but also mineralizes more organic compounds. *Trichoderma* sp. produces the enzyme alkaline phosphatase, which can solubilize phosphate, making it available in the soil (Eslahi *et al*., 2019).

3.3 Disease Intensity

The observation results indicated that the disease affecting the chili plants was leaf curl virus (Appendix). Based on the 5% significant level variance analysis, the application of bioameliorant and the biological agent *Trichoderma* sp. showed no interaction effect on the intensity of leaf curl virus disease. However, there was an independent effect of the *Trichoderma* sp. treatment (Table 3). According to Anwar *et al*. (2018), *Trichoderma* sp. is used to control plant diseases or as a biocontrol agent.

Table 3. Independent Effects of Bioameliorant and Biological Agent *Trichoderma* sp. on Disease Intensity

Treatment	The intensity of leaf curl virus disease (%)
Bioamelioran	
Control	33,33 a
3 t ha^{-1}	33,33 a
6 t ha^{-1}	33,33 a
Trichoderma sp.	
Control	50,00 c
8 kg ha^{-1}	33,33 b
16 kg ha^{-1}	16,67 a

Note: Mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% significance level.

Table 3 shows that the independent effect of applying the biological agent *Trichoderma* sp. significantly impacts the intensity of leaf curl virus disease. The variance analysis at the 5% significance level indicates that applying *Trichoderma* sp. at 16 kg ha^{-1} significantly reduces the intensity of leaf curl disease compared to the control (Figure 16). The application of *Trichoderma* sp. at 8 kg ha^{-1} and 16 kg ha^{-1} was able to reduce the intensity of leaf curl virus disease by 33.3% and 66.6%, respectively, compared to the control. This demonstrates that the application of the biological agent *Trichoderma* sp. is effective in reducing the intensity of leaf curl virus disease. *Trichoderma* sp. triggers the plant's response to induce systemic resistance as a reaction to leaf curl virus attack.

Trichoderma sp. effectively suppresses disease through the induction of systemic resistance or induced systemic resistance (ISR). Induced resistance in plants can be observed by the obstruction of pathogen penetration processes into plant tissues, thereby inhibiting pathogen infection in the plant (Harman *et al*., 2004). The impact of *Trichoderma* sp. on disease infection in plants results in a systemic resistance response. This response is attributed to the accumulation of phytoalexins and increased activity of several inducing enzymes, such as chitinase and β-1,3-glucanase, which can inhibit the growth and development of pathogens (Shahbaz *et al*., 2009).

3.4 Fruit Weight and Number of Fruits per Plant

The results of the analysis of variance at the 5% significance level indicate that the independent effects of bioamelioran and *Trichoderma* sp. on fruit weight and fruit number per plant (Table 4) show no interaction between treatments.

Table 4. The independent effect of bioamelioran and *Trichoderma* sp. bioagent on fruit weight and fruit number per plant

Treatment	Fruit Weight (g plant^{-1})	Number of Fruits per Plant (fruit)
Bioamelioran		
Control	118,83 a	9,11 a
3 t ha^{-1}	129,31 a	10,78 a
6 t ha^{-1}	129,41 a	12,22 a
Trichoderma sp.		
Control	120,09 a	10,89 a
8 kg ha^{-1}	120,68 a	10,78 a
16 kg ha^{-1}	136,77 a	10,44 a

Note: Mean values followed by the same letter are not significantly different according to Duncan's Multiple Range Test at the 5% significance level.

This is likely due to the low nutrient content in the bioamelioran components, which may not sufficiently meet the nutrient availability in the soil. The dose of *Trichoderma* sp. also appears insufficient to decompose the organic matter from the bioamelioran, which is needed to produce nutrients essential for the formation of chili fruit. Table 4 shows no independent effect of bioamelioran application on fruit weight and fruit number per plant. However, bioamelioran at 6 t ha-1 increased fruit weight compared to the control. The number of fruits per plant also increased with the application of bioamelioran at 6 t ha-1 compared to the control. The lack of significant differences due to bioamelioran application is likely because the components used in the bioamelioran do not contain optimal nutrients. Bioamelioran components are expected to be more effective if they include manure fertilizer, which can improve soil fertility and provide nutrients for soil microbes. This is supported by Situmorang *et al*. (2019), who found that bioamelioran components consisting of 72% manure fertilizer, 18% coconut shell biochar, 10% dolomite, and guano improved fruit weight and fruit number per chili plant compared to the control. Manure fertilizer contains N, P, K, and organic C, which are essential for fruit formation and can increase fruit weight (Novizan, 2007). According to Meylia & Koesriharti (2018), nutrient availability is one of the factors influencing the number of fruits per chili plant.

The table 4 shows that there is no independent effect of Trichoderma sp. application on fruit weight and fruit number per plant. Application of Trichoderma sp. at 16 kg ha-1 slightly increased fruit weight compared to the control. However, the treatment without Trichoderma sp. resulted in a higher number of fruits per plant compared to the application of Trichoderma sp. This indicates that Trichoderma sp. application does not directly affect fruit weight and fruit number per plant. The dosage of Trichoderma sp. application is suspected to need an increase. This aligns with the study by Fitria *et al*. (2018), which found that application of Trichoderma sp. at 20 g per plant showed better responses in terms of fruit weight and fruit number per chili plant. The generative phase requires phosphate nutrients for fruit and seed development (Qibtyah & Mariyatul, 2015). According to Poulton *et al*. (2011), phosphate nutrients can be obtained with the assistance of Trichoderma sp. fungi, which aid plants in nutrient absorption. This is also explained by Sianturi (2010), who stated that phosphorus plays a crucial role in fruit formation, stimulates flower and fruit formation, and accelerates fruit ripening. The application of Trichoderma sp. contributes to the decomposition of organic matter to release bound nutrients, especially nitrogen and phosphorus (Pandriyani & Supriyati, 2010).

4 Conclusion

The interaction between bioamelioran and *Trichoderma* sp. did not show significant differences in the population of phosphate-solubilizing bacteria, available P, disease resistance, and red chili yield. Trichoderma sp. application effectively reduced the intensity of leaf curl virus disease, with Trichoderma sp. at 8 kg ha^{-1} reducing it by 33.3% and Trichoderma sp. at 16 kg ha^{-1} by 66.6%.

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