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Effectiveness of Biological Agents in Improving the Growth of Cayenne Pepper (*Capsicum frustescens*) in Alfisol Soil

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Abstract

Tuban Regency is a major center for cayenne pepper production, but it is currently experiencing a decline in production due to the shrinking productive land area. As a result, farmers are opening new land in forest areas dominated by alkaline alfisol soil that lacks phosphorus elements. This study investigates the impact of biological agents *Bacillus subtilis* and CMA on the growth of cayenne pepper plants in alfisol soil. The study utilized a randomized block design with 8 treatments and 3 replications, resulting in a total of 24 beds: A0 (Control); A1 (SP-36); A2 (*Bacillus subtilis*); A3 (CMA); A4 (SP-36 + *Bacillus subtilis*); A5 (SP-36 + CMA); A6 (*Bacillus subtilis* + CMA); A7 (SP-36 + *Bacillus subtilis* + CMA). Plant growth measurements included dry weight, net assimilation rate, and crop growth rate (CGR). The research findings indicate that the combination of biological agents *Bacillus subtilis* and CMA in the A7 treatment (SP-36 + *Bacillus subtilis* + CMA) had the most significant impact on the growth of cayenne pepper plants across all parameters. This was confirmed by the results of the BNT test, which showed improvements in dry weight (23.253 grams), net assimilation rate (2.7950), and crop growth rate (9.790).

Keywords: Alfisol Land, *Bacillus subtilis*, Biological Agents, Chili Growth, CMA

1. Introduction

East Java Province is a significant contributor to cayenne pepper production in Indonesia. In 2021, the province produced 5,788,830 kilograms of cayenne pepper; in 2022, the production increased to 6,124,089 kilograms. The total production of cayenne pepper is a combined effort from all regencies in East Java Province, including Tuban Regency, which is ranked 5th in its contribution to cayenne pepper production (Rastono & Firgiyanto, 2024).

Tuban Regency is known as the center of cayenne pepper production in East Java due to its potential cayenne pepper producing areas, particularly Grabagan District, which produced 390,880 quintals of chili and Bancar District with 282,639 quintals in 2020 (Muzadi & Rastono, 2023). However, in 2021, cayenne pepper production in Tuban Regency declined in both areas, with Grabangan District producing 144,800 quintals and Bancar District producing 15,751 quintals (BPS, 2021). Despite the decline, cayenne pepper farming in Tuban Regency remains economically viable, necessitating a strategic approach to its development by understanding the influencing factors (Bete & Taena, 2018).

The decrease in cayenne pepper production can be attributed to the shrinking productive land area (Lukmana et al., 2023). Consequently, many farmers are clearing new land in the Tuban forest area. The forests in Tuban district are predominantly composed of red soil known as alfisol. Analysis of Tuban limestone Alfisol indicates a pH of 8.4 (alkaline), organic C content of 1.51% (low), K content of 0.77 me/100 g (high), Ca content of 10.83 me/100g (high), and Fe content of 1.42 ppm (low) (Taufiq, 2001). The high alkaline pH of 8.4 renders the soil alkaline, leading to limited availability of phosphorus (P) as it is bound by calcium (Ca), hindering its utilization by chili plants for root growth, assimilation, flowering, and seed ripening (Rastono & Firgiyanto, 2024). Research by Benggu & Ishaq (2022) suggests that alfisol soil is more susceptible to environmental stressors such as drought, water loss, salinity, and low soil pH.

Efforts to enhance alfisol land for improved plant growth and production necessitate the use of organic materials to ensure the soil can supply essential nutrients (Fajeriana & Gafur, 2023). Organic materials such as bokashi fertilizer, phosphate-solubilizing bacteria, and

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CMA can be utilized (Sulistiawati & Hemon, 2023). In their study, Sulistiawati & Hemon (2023) found that applying 600 grams of bokashi fertilizer per polybag led to an increase in organic C (0.34%), total N (0.11%), available P (25.66 ppm), CEC (53.19 me/100g), and had a positive impact on plant height, stem diameter, and fruit weight.

Sitanggang et al. (2017) reported that combining phosphate-solubilizing bacteria and P fertilizer application resulted in a 284.44% increase in P absorption and a 44.83% growth enhancement in corn plants. Arbuscular Mycorrhizal Fungi (AMF) can enhance the uptake of N, P, K nutrients and water through symbiosis with plant roots, meeting the plants' nutrient requirements. Sari et al. (2015) demonstrated that applying 10 tons ha-1 of manure along with 10 g of Arbuscular Mycorrhizal Fungi (AMF) per plant reduced the need for inorganic fertilizer by 40% without significantly affecting plant growth compared to using 100% inorganic fertilizer.

Research combining BPF P. fluorescens with CMA has shown promising results in increasing the yield and quality of peanut seeds (Asih & Wartapa, 2022). This study utilized various BPF densities and CMA doses, with the best combination being P. fluorescens (109cfu ml-1) + CMA (10 gr/plant) in Latosol soil. The upcoming research will involve phosphate-solubilizing bacteria Bacillus subtilis (10-9cfu ml-1) and CMA (10 gr/plant) in alfisol soil. In Tuban district, there is a lack of research on using biological agents to enhance plant production, particularly in chili cultivation. This study aims to assess the impact of biological agents Bacillus subtilis and arbuscular mycorrhizal fungi (CMA) on the growth of cayenne pepper to improve its production.

2. Material and Methods

This research was conducted from June to October 2023 in Tuban Regency between Latitude -6.9649208S and Longitudinal 111.7894193.3E with an altitude of 46 MDPL. The materials used were 300m2 of alfisol land, 30ton/ha bokashi fertilizer, 300 Kg/ha urea, 300kg/ha SP36, and 250kg/ha KCL, Bacillus subtilis (10-9cfu ml-1), CMA, chili seeds, and pesticides. Tools: hoe, sickle, water pump, analytical scales, oven, stationery, scissors, digital camera, meter, ruler, vernier, and hand sprayer.

The implementation of the study used a group randomized design (RAK) with 8 treatments and 3 replications then obtained 24 beds in the study, namely A0 (Control); A1 (SP-36); A2 (Bacillus subtilis); A3 (CMA); A4 (SP-36 + Bacillus subtilis); A5 (SP-36 + CMA); A6 (Bacillus subtilis + CMA); A7 (SP-36 + Bacillus subtilis + CMA). The implementation of the research was first carried out by cultivating 300m2 of alfisol land by alternating to improve soil structure. Sprinkle 30ton/ha of fermented bokhasi fertilizer on the alfisol soil as a base fertilizer. Homogenize the soil and bokashi fertilizer and then make a 1.5 x 1.5 m2 bed. Let the land stand for 14 days so that bokashi fertilizer as organic matter can improve the soil's physical, chemical, and biological properties (Fitriany & Abidin, n.d.). The recommended planting distance for cayenne pepper is 70 cm x 50cm (Litbang, 2019). P fertilizer, Bacillus subtilis and CMA treatments were applied at the beginning of planting. Pests and diseases of cayenne pepper plants are treated when symptoms appear. Supplementary fertilization was carried out at 15, 28, 42 HST using urea and KCl fertilizers at the doses provided. The parameters observed in this study were:

a. Dry Weight

Dry weight was measured by taking samples of chili plants (roots, stems, leaves, and stems) after harvest at 12 MST, then in an oven at a temperature of 105OC until constant. The oven results were weighed using an analytical scale to determine the weight.

b. Net Assimilation Rate (Net Assimilation Rate)

Net Assimilation Rate (NAR) measures plant photosynthesis's efficiency in producing dry biomass per unit leaf area per unit time. This parameter describes the ability of plants to convert light energy into organic matter after accounting for carbon loss due to respiration:

$$NAR = \frac{W2 - W1}{A.(t_2 - t_1)}$$

c. Crop Growth Rate (CGR)

CGR is the rate or speed of plant growth over a certain period. Typically, CGR refers to the amount of biomass (such as dry weight) produced by a plant per unit of time, such as grams per square meter per day (g/m²/day) or kilograms per hectare per day (kg/ha/day) ((Afriani et al., 2021). Crop Growth Rate (CGR) is calculated using the following formula:

$$CGR = \frac{W2 - W1}{T2 - T1}$$

W2 – W1 = dry weight changes of leaves (g)
T2 – T1 = change of time (day)

Data from parameter observations were collected and then analyzed using analysis of variance (ANOVA), if there is a real effect, then the DMRT test is continued at the 5% level. Then the research flow is shown in Figure 1 flow diagram as follows:

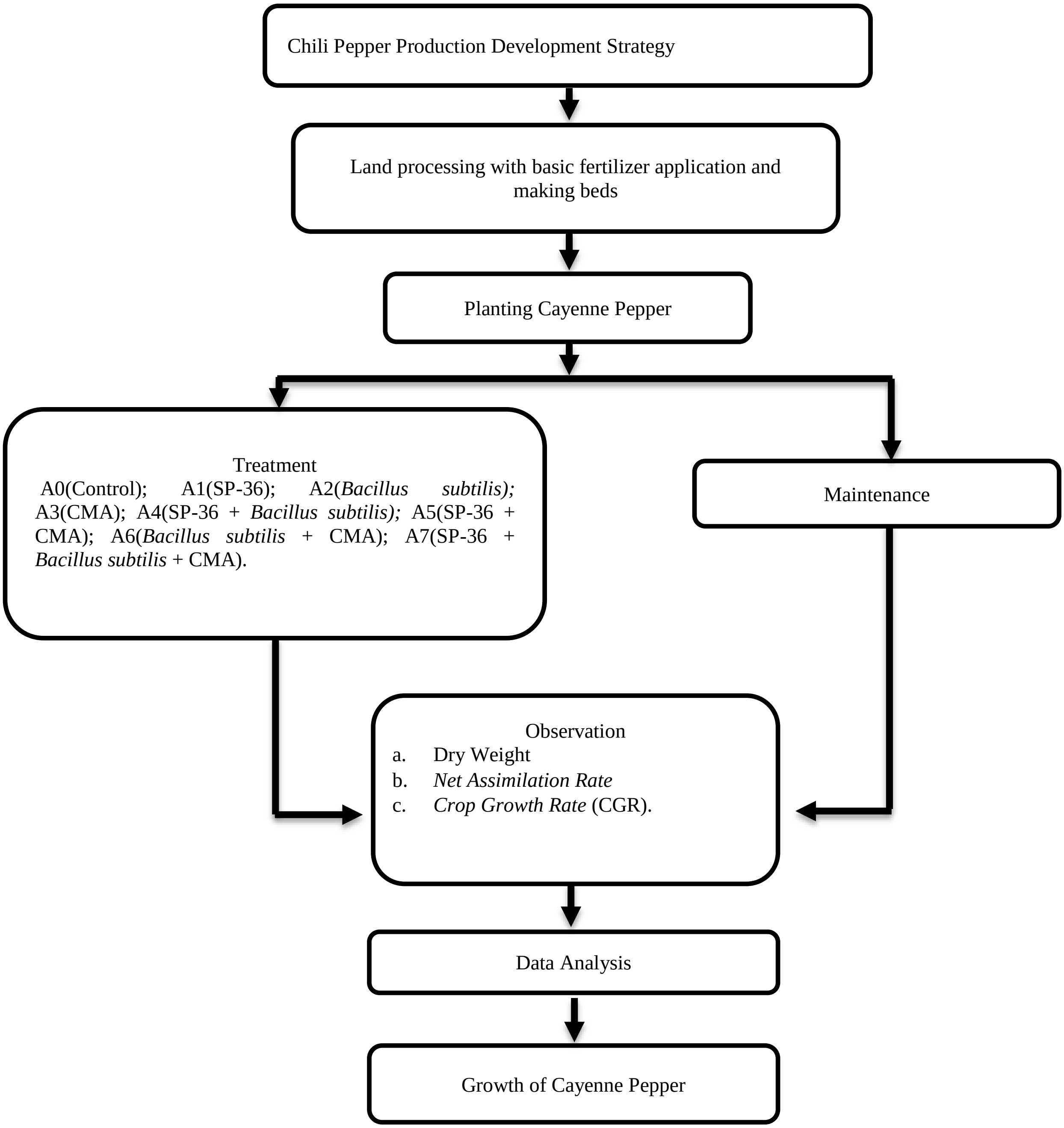

Figure 1. Research flow diagram

3. Results and Discussion

3.1. Dry Weight

Plant dry weight is a measure of the amount of biomass accumulated by plants. It is an important indicator of plant growth and development as it represents the accumulation of organic compounds synthesized by plants. Plant dry weight is reflective of the plant's nutritional status and serves as an indicator of overall plant health and growth. Therefore, it is closely linked to nutrient availability (Husna et al., 2021). In the study, the dry weight of the stover was recorded, as shown in Table 1.

The results of the analysis of variance in Table 1 show that the dry weight of cayenne pepper plants under 12 MST treatments (A0, A1, A2, A3, A4, and A5) were not significantly different at the 5% level. However, treatment A0 had the lowest dry weight value. Treatments A1, A2, A3, A4, A5, and A6 were also not significantly different at the 5% level. Treatments A6 and A7 in dry weight showed no significant difference at the 5% level, but both significantly differed from the dry weight of treatment A0.

The weakness of alfisol land in Tuban Regency is the low phosphorus (P) element content, which is evident in the soil's quick water absorption and tendency to dry out easily (Rastono & Firgiyanto, 2024). As a result, chili plants in the control treatment, which received basic fertilizer (manure) without adding biological agents, did not undergo optimal lateral meristem division. This is supported by the findings of Lovitna et al. (2021), who stated that the P element plays a crucial role in triggering embryonic cell division from the apical meristem for height growth and stabilizing metabolism to increase stem diameter through the development of lateral meristem cells. Supit et al. (2022) further emphasized that lacking P elements can disrupt cell division and meristem tissue development, leading to decreased plant height growth and a lower number of dry-weight leaves.

Table 1. Dry weight of cayenne pepper plant stalks

Treatment	After Harvest 12 (MST)
A0=Control	6.830±6.51[c]
A1=SP-36	11.800±0.75[bc]
A2=*Bacillus subtilis;*	10.883±8.86[bc]
A3=CMA;	16.090±2.99[abc]
A4=SP-36 + *Bacillus subtilis;*	11.513±9.97[bc]
A5=SP-36 + CMA	13.977±5.85[abc]
A6=*Bacillus subtilis* + CMA	19.427±3.21[ab]
A7=SP-36 + *Bacillus subtilis* + CMA.	23.253±2.85[a]

Notes: Numbers in the same column followed by the same letter are not significantly different (P>0.05) in the 5% BNT test.

Based on the results of the variance test Table 1, it can be seen that the dry weight of treatment A1 (SP-36) is 11,800 grams and is not significantly different at the 5% level against the administration of biological agents in combination or independently in treatments A2 (Bacillus subtilis), A3 (CMA), A4 (SP-36 + Bacillus subtilis), A5 (SP-36 + CMA), and A6 (Bacillus subtilis + CMA). SP-36 fertilizer has the advantage of a fairly high P content and is more readily available for absorption by plant roots. Although it has difficult to dissolve nature, the more phosphorus is given, the more phosphorus is bound by soil colloids. This condition will increase the total P of the soil. Total P is the accumulation of dissolved phosphorus and insoluble phosphorus in the soil, but has the potential to become an available form (Afriani et al., 2021). This result is following the research of Faizin et al., (2015) The treatment of giving 25 g/polybag phosphorus fertilizer was able to increase the available P by 527.66 in the soil through the mechanism of phosphorus release from the adsorption complex so that the availability of phosphorus elements increased and was able to increase the height of Acacia seedlings by 9.46 cm compared to the control, which was 5.73. H

Treatment A6 (Bacillus subtilis + CMA) at 19,427 grams and Treatment A7 (SP-36 + Bacillus subtilis + CMA) at 23,253 grams showed no significant difference, but both treatments were significantly different from the control treatment (A0) at a 5% significance level. Both BPF and CMA, when applied independently, were able to interact with organic matter as a basic fertilizer in soil processing. Organic matter influences the population and activity of phosphate-solubilizing bacteria, leading to increased phosphate dissolution in the soil. Phosphate-solubilizing bacteria utilize organic matter as an energy source for their metabolic activities. Research by Setyawan and Santoso (2021) demonstrated that combining cow manure and BPF at a concentration of 6 ml/l can enhance soybean plant growth rate by 2.6% and phosphorus absorption by 0.08%.

AMF symbiosis expands the root surface through external and internal hyphae structures, enhancing nutrient and water absorption by plants. Increased phosphorus absorption by AMF improves root and shoot growth, boosting photosynthesis rates. Phosphorus absorbed by AMF also supports cell formation in root and shoot tissues during plant growth and production. Research by Suwarniati (2018) indicated that AMF application significantly affected seedling dry weight. The best combination in terms of dry weight in this study was Treatment A7 (SP-36 + Bacillus subtilis + AMF) at 23,253 grams. This finding aligns with research by Asih & Wartapa (2022), which suggested that the combination of BPF and AMF can increase peanut pod contents and enhance germination power and growth potential.

3.2. Net Assimilation Rate

The assimilation rate describes the ability of plants to produce dry matter per unit leaf area per unit time. Assimilation rate is linearly related to leaf area and plant dry weight. The weighted average net assimilation rate is presented in Table 2.

Based on Table 2, treatments A1 (SP-36), A2 (Bacillus subtilis), A3 (CMA), A4 (SP-36 + Bacillus subtilis), A5 (SP-36 + CMA), and A6 (Bacillus subtilis + CMA) were not significantly different from treatments A0 (Control) and A7 (SP-36 + Bacillus subtilis + CMA). However, treatment A0 (Control), which is 0.7600, significantly differs from treatment A7 (SP-36 + Bacillus subtilis + CMA), which is 2.7950 at the 5% level. The interaction between the provision of P fertilizer, BPF (Bacillus subtilis) and CMA can increase the net assimilation rate of plants. P fertilizer applied to alfisol land can be dissolved by Bacillus subtilis bacteria by producing phosphatase enzymes. Phosphatase enzymes are formed if phosphate availability in the soil is low. During the mineralization process of organic matter, organic phosphate compounds are broken down into inorganic phosphate forms that plants can access with the help of phosphatase enzymes. (Arifin et al., 2023) The symbiosis between BPF and CMA can maximize the P element in the soil or other nutrients that allow plants to immediately absorb less available nutrients so that they are fulfilled to stimulate growth. In his research, Rahman et al. (2015) stated that the application of BPF and Mycorrhiza to chili plants had a real effect.

Table 2. Weighted average net assimilation rate

Treatment	NAR
A0=Control	0.7600±0.72[b]
A1=SP-36	1.3500±0.32[ab]
A2=*Bacillus subtilis;*	1.1300±0.48[ab]
A3=CMA;	1.3500±0.98[ab]
A4=SP-36 + *Bacillus subtilis;*	1.2500±1.02[ab]
A5=SP-36 + CMA	1.8000±0.78[ab]
A6=*Bacillus subtilis* + CMA	2.2700±0.19[ab]
A7=SP-36 + *Bacillus subtilis* + CMA.	2.79500.02[a]

Notes: The numbers in the same column followed by the same letter are not significantly different (P>0.05) in the 5% BNT test.

3.3. Crop Growth Rate (CGR)

Plant growth rate is the speed or rate at which a plant increases in height, width, weight, or size over a certain period of time. The analysis of variance results show that treatments A1 (SP-36), A2 (Bacillus subtilis), A3 (CMA), A4 (SP-36 + Bacillus subtilis), A5 (SP-36 + CMA), and A6 (Bacillus subtilis + CMA) are not significantly different from treatments A0 (Control) and A7 (SP-36 + Bacillus subtilis + CMA). Treatment A0 (Control) with a growth rate of 2.660 is significantly different from treatment A7 (SP-36 + Bacillus subtilis + CMA) with a growth rate of 9.790 at the 5% level. The average plant growth rates are presented in Table 3.

Table 3. Crop Growth Rate (CGR) analyst results

Treatment	CGR
A0=Control	2.660±0.72[b]
A1=SP-36	4.710±0.32[ab]
A2=*Bacillus subtilis;*	3.950±0.48[ab]
A3=CMA;	4.705±0.98[ab]
A4=SP-36 + *Bacillus subtilis;*	4.360±1.02[ab]
A5=SP-36 + CMA	6.300±0.78[ab]
A6=*Bacillus subtilis* + CMA	7.505±0.19[ab]
A7=SP-36 + *Bacillus subtilis* + CMA.	9.790±0.02[a]

Notes: The numbers in the same column followed by the same letter are not significantly different (P>0.05) in the 5% BNT test.

In Table 3, through the BNT test, the highest CGR results were in the A7 treatment (SP-36 + Bacillus subtilis + CMA), while the low CGR results were in the A0 = Control treatment. The condition of alfisol land is low in P elements, so administering SP-36 fertilizer combined with the biological agent Bacillus subtilis + CMA can support vegetative growth regarding plant height and the number of leaves on alfisol land. Photosynthesis increases with more leaves, which means plants grow faster. Phosphorus makes up fat, protein, and cell nuclei and affects photosynthesis and plant growth and development, such as during cell elongation in vegetative vases (Afriani et al., 2021). So, phosphate-solubilizing bacteria are very important for increasing plant growth.

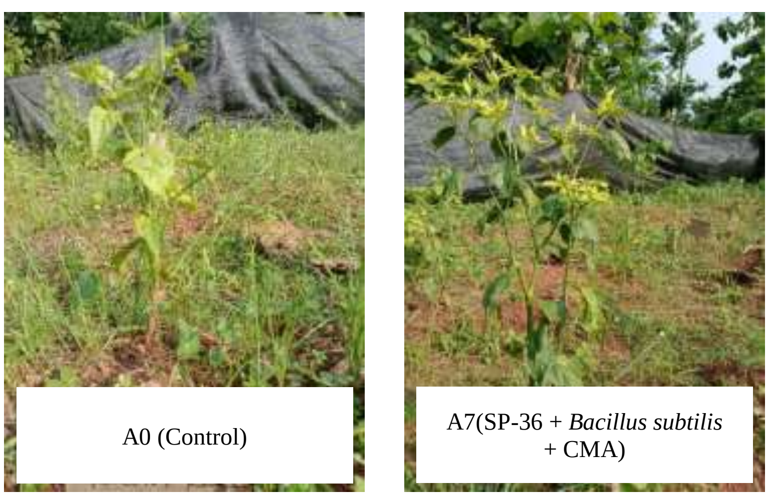

Figure 2. Chili Pepper condition after 12 hours after treatment (HST)

Leaf growth, the primary organ for photosynthesis, is influenced by nutrients such as N, Mg, K2O, P2O5, Zn, and Fe. The addition of Bacillus subtilis Plant Growth Promoting Rhizobacteria (BPF) in this study supplements these nutrients (Minarsih et al., 2020). Additionally, the inclusion of Arbuscular Mycorrhizal Fungi (AMF) acts as a symbiont that forms root hyphae to aid in nutrient absorption (Birnadi, 2012). These nutrients can stimulate cell development and increase the chlorophyll content in leaves. Elevated chlorophyll levels enhance photosynthesis rates, increasing photosynthate production and, subsequently, the relative growth rate (RGR). Bacteria, whether free-living or symbiotic with fungi are crucial in promoting plant growth by producing chemicals that mimic plant-produced phytohormones, such as auxin. Auxin is a natural plant hormone that plays a vital role in various physiological processes, including stress mitigation, growth, differentiation, and cell division (Sutrisno, 2021).

The results of the study revealed significant differences among all treatments, particularly in the A0 (control) and A7 (SP-36 + Bacillus subtilis + CMA) treatments at 12 hours after treatment (HST), as depicted in Figure 2.

4. Conclusion

Based on the research results, the A7 treatment (SP-36 + Bacillus subtilis + CMA) is a beneficial combination for alfisol soil to enhance the growth of cayenne pepper. This is evident from the BNT test results which showed a dry weight of 23.253 grams, a Net Assimilation Rate of 2.7950, and a Crop Growth Rate of 9.790.

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