

Effect of Concentration and Time of Chitosan Application on the Growth of Pineapple (*Ananas comosus*)

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Abstract

Pineapple (*Ananas comosus*) is a popular fruit crop in Indonesia. Pineapple crowns are one of the planting materials used in pineapple cultivation; however, their growth is slower than that of other planting materials. Therefore, the addition of chitosan is necessary, as it has the potential to stimulate growth. This study aims to determine the best concentration and application time of chitosan that can improve pineapple growth. The study was conducted in the screenhouse of the Faculty of Agriculture, Sebelas Maret University, Surakarta, during March-June 2025 with a Completely Randomized Design (CRD) with two factors, namely chitosan concentration (without chitosan, 1 ppm, 3 ppm, 5 ppm) and time of chitosan application (morning, afternoon, morning and afternoon). Each treatment was repeated 4 times, resulting in 48 experimental units. The results showed that no combination of concentration and time of chitosan application was found to increase pineapple growth simultaneously. The treatment concentrations of 1 ppm and 3 ppm yielded the best results for increasing leaf number. Concentrations of 1 ppm, 3 ppm, and 5 ppm showed the best results for increasing total leaf area and the most extended root length. The time of chitosan application, once in the morning or evening, increased the photosynthesis rate and stomatal conductance. Chitosan has the potential to serve as a biostimulant for plant growth, enhancing the physiological activity of plants.

Keywords: biostimulant; bromeliaceae; chitin; crown propagation; morning

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Introduction

Indonesia has high diversity in fruit species due to its tropical location and climate, which support the growth of various plant species. One popular fruit crop in Indonesia is the pineapple (*Ananas comosus*). People consume pineapples fresh or process them into different food products, such as jam, pickles, and chips. Pineapples have high economic potential and are a leading commodity in the tropical fruit trade. Additionally, pineapples have a high nutritional content, including the enzyme bromelain, which can boost immunity (1). Pineapples are cultivated in various regions in Indonesia. According to data from the (2), pineapples were grown in 33 provinces, with an average production of 3,156,576 tons in 2023. This pineapple production decreased by 47,199

tons compared to the previous year. Pineapple production levels highly depend on the cultivation's planting material (3). One planting material that can be used as seeds is the crown of the fruit. Pineapple crowns are often discarded despite their potential for use as seedlings. The downside of crowns is their slower growth rate compared to other shoots (4). The vegetative propagation technique can produce genetically identical seedlings with the productive mother plant (5). Pineapple crowns as seedlings can be optimized by applying plant growth regulators (PGRs) such as chitosan. Chitosan can be an alternative for enhancing pineapple vegetative growth. Chitosan production involves the processes of protein separation (deproteinization) and mineral separation (demineralization) to obtain chitin

from shrimp shells. Adeacetylation process is then used to get the chitosan product (6). Chitosan itself is a naturally acetylated derivative of chitin, which can trigger localized and systemic plant immune responses, potentially increasing plant diversity indirectly. Diversity can be caused by environmental factors (7). The factors that determine the success of chitosan application to crowns are concentration and application time. Based on the above description, it is essential to understand the potential of chitosan as a biostimulant in pineapple cultivation from crowns. The objectives of this study were to determine the optimal combination of chitosan concentration and application time for pineapple growth, the optimal chitosan concentration for pineapple growth, and the optimal application time for pineapple growth.

Material and Methods

The research was conducted at the greenhouse of the Faculty of Agriculture, Sebelas Maret University, Surakarta City, Central Java Province. The coordinates of the research location are at 7°56'14.68"S and 110°85'91.20"E, with an altitude of 97 meters above sea level. Soil fertility analysis was conducted at the Soil Chemistry and Fertility Laboratory of the Faculty of Agriculture, Sebelas Maret University. Physiological analysis was performed at the Plant Physiology and Biotechnology Laboratory of the Faculty of Agriculture, Sebelas Maret University. The research was conducted from March to June 2025. The materials used in this study included Queen pineapple crowns (Nanas Madu Pemalang), river sand, rice husk charcoal, compost, polybags, chitosan, water, and labels. The tools used for the cultivation process included plant shovels, buckets, measuring cups, and hand sprayers. The tools used for variable observations included a thermohygrometer, a lux meter, a plant photosynthetic meter, a microscope, a soil plant analysis development (SPAD), and a UV-Vis spectrophotometer.

This study used a Completely Randomized Design (CRD) with two factors and four replications. The first factor was the treatment with varying chitosan concentrations (without chitosan, 1 ppm, 3 ppm, 5 ppm). The second factor was the treatment of chitosan application time (morning, afternoon, and evening). Chitosan is extracted from a concentrated solution with an initial concentration of 0.5%. The method of

diluting a 0.5% concentrated chitosan solution to a lower concentration involves taking a specific volume of the concentrated solution and then adding solvent until the desired target concentration is achieved. The concentration of 1 ppm is achieved by diluting a 0.2 ml focused solution of 0.5% chitosan solution with water until the total volume of the solution reaches 1 liter. The concentration of 3 ppm is achieved by diluting a concentrated solution of 0.4 ml of a 0.5% chitosan solution with water until the total volume of the solution reaches 1 liter. The concentration of 5 ppm is achieved by diluting a concentrated solution of 0.8 ml of a 0.5% chitosan solution with water until the total volume of the solution reaches 1 liter. The research variables included leaf number, total leaf area, the most extended root length, photosynthesis rate, and stomatal conductance. The data obtained were then analyzed using Analysis of Variance (ANOVA). If significant differences exist, the analysis is continued with Duncan's Multiple Range Test (DMRT) at a 5% significance level.

Results and Discussion

Leaf Number

The analysis of variance revealed that the concentration of chitosan had a significant effect on the number of pineapple leaves. The increase in the number of leaves with varying concentrations and application times of chitosan is evident in Table 1.

Table 1. Leaf number of pineapple at 12 WAP with different concentrations and application times of chitosan

Treatment Level	Leaf Number $\pm$ Standard Deviation
<i>Chitosan concentration (ppm)</i>	
Without chitosan	10.00 $\pm$ 0.25 b1
1 ppm	14.08 $\pm$ 1.66 a
3 ppm	13.25 $\pm$ 1.30 a
5 ppm	10.25 $\pm$ 2.78 b
<i>Chitosan application time</i>	
Morning	11.31 $\pm$ 2.88
Evening	12.38 $\pm$ 3.14
Morning and Evening	12.00 $\pm$ 1.49

Notes: The result of the number followed by the equal alphabet in each experiment indicates that there is no significant difference in the DMRT α 5% test.

Based on Table 1, the concentration of chitosan increased the number of pineapple leaves. A concentration of 1 ppm was the same as a concentration of 3 ppm, but different from a concentration of 5 ppm, without the addition of chitosan. Economically, a concentration of 1 ppm increased the number of pineapple leaves. This finding aligns with the research of (8), which demonstrated that administering oligochitosan at a concentration of 100 ppm increased the number of leaves in corn plants. Chitosan acts as a biological signal that stimulates the production of plant growth hormones, including gibberellins, auxins (IAA), and cytokinins. These hormones play a role in cell division, differentiation, and elongation, which contribute to the formation of new leaves. In this research, chitosan was applied via foliar spray. Chitosan's absorption through the leaves enables nutrients and active ingredients to quickly penetrate the plant, resulting in a more effective response in leaf growth.

Total Leaf Area

The variance analysis revealed that the chitosan concentration had a significant effect on the pineapple leaf area. Application time and the interaction between chitosan concentration and application time did not significantly affect leaf area. The leaf area with different chitosan concentrations and application times is presented in Table 2.

Table 2. Total leaf area (cm²) of pineapple at 12 WAP with different concentrations and application times of chitosan

Treatment Level	Total Leaf Area ± Standard Deviation (cm ²)
<i>Chitosan concentration (ppm)</i>	
Without chitosan	156.66±21.51 b1
1 ppm	223.28±48.86 a
3 ppm	206.94±59.58 ab
5 ppm	234.94±27.18 a
<i>Chitosan application time</i>	
Morning	188.59±32.69
Evening	231.80±67.11
Morning and Evening	195.98±36.50

Note: The result of the number followed by the equal alphabet in each experiment indicates that there is no significant difference in the DMRT α 5% test

Based on Table 2, chitosan concentration can increase pineapple leaf area. A concentration of 5 ppm is equivalent to 1 ppm,

but distinct from 3 ppm, and does not involve the addition of chitosan. Economically, a concentration of 1 ppm can be chosen as the best concentration because it can increase the pineapple leaf area. This aligns with research by (9), which states that applying chitosan at 3 ppm can produce the best average leaf width in orchid plants. Regarding plant hormones, the increase in leaf number and area upon chitosan application may involve interactions with hormones such as gibberellin and cytokinin, which regulate leaf formation and expansion. Chitosan at low concentrations likely stimulates physiological responses related to leaf growth, including enhanced photosynthetic activity, chlorophyll synthesis, and cell expansion. These processes align with the known effects of gibberellins and cytokinins, which promote leaf initiation and development. According to (10), chitosan supports vegetative plant growth by increasing leaf area. Chitosan has a high nitrogen content of 6% to 9% (11). Nitrogen is an essential element that supports the synthesis of proteins and amino acids. Plants that receive an adequate nitrogen supply will exhibit increased chlorophyll content in their leaves, resulting in a higher rate of photosynthesis and broader, more numerous leaf growth.

Longest Root Length

The roots of a plant are an essential part of plant growth, indicating that the plants are growing steadily (12). The analysis of variance revealed that the chitosan concentration had a significant effect on the longest root length of pineapple. The application time and the interaction between chitosan concentration and application time did not significantly affect the most extended root length. This means that chitosan concentration can increase the pineapple's longest root length. The most extended root length of pineapple with different concentrations and application times of chitosan is shown in Table 3.

Table 3. Length of the longest root (cm) of pineapple at 12 WAP with different concentrations and application times of chitosan

Treatment Level	Longest Root Length $\pm$ Standard Deviation (cm)
<i>Chitosan concentration (ppm)</i>	
Without chitosan	16.87 $\pm$ 1.84 b1
1 ppm	22.42 $\pm$ 2.10 a
3 ppm	18.82 $\pm$ 0.51 ab
5 ppm	17.39 $\pm$ 2.14 ab
<i>Chitosan application time</i>	
Morning	17.63 $\pm$ 3.79
Evening	19.38 $\pm$ 3.03
Morning and Evening	19.62 $\pm$ 1.31

Note: The result of the number followed by the equal alphabet in each experiment indicates that there is no significant difference in the DMRT α 5% test

Based on Table 3, the concentration of 1 ppm chitosan is the same as that of 3 ppm and 5 ppm, but different from that without chitosan. Economically, applying 1 ppm chitosan can increase the length of pineapple roots. This finding aligns with the research of (13), which demonstrated that using 12 ml of chitosan/plant increased the average most extended root length of Liberica coffee plants. This is because chitosan can activate the auxin hormone in plants, as stated in (14), which notes that auxin content plays a crucial role in stimulating the growth of root primordia cells, thereby facilitating root elongation. (15) added that the cell elongation process in plants is greatly influenced by the auxin hormone, both that which is naturally synthesized by plants (endogenous) and that which is given to plants in the form of growth regulators (exogenous). Chitosan also promotes auxin accumulation in the apex of plant roots (16).

Photosynthetic Rate

The analysis of variance revealed that the application time had a significant impact on the rate of pineapple photosynthesis. The concentration and interaction between the concentration and time of chitosan application did not significantly affect the rate of pineapple photosynthesis. This means that the time of chitosan application can increase the rate of pineapple photosynthesis. These results align with the research of (17), which states that applying chitosan in the morning or evening can increase the rate of photosynthesis of matoa plants compared to those without chitosan. The photosynthesis rate of pineapple with varying

concentrations and application times of chitosan is presented in Table 4.

Table 4. Photosynthetic rate ($\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$) of pineapple at 12 WAP with different concentrations and application times of chitosan

Treatment Level	Photosynthetic rate $\pm$ standard deviation ($\mu\text{mol CO}_2 \text{ m}^{-2} \cdot \text{s}^{-1}$)
<i>Chitosan concentration (ppm)</i>	
Without chitosan	0,54 $\pm$ 0,11
1 ppm	0,50 $\pm$ 0,07
3 ppm	0,46 $\pm$ 0,05
5 ppm	0,56 $\pm$ 0,05
<i>Chitosan application time</i>	
Morning	0,57 $\pm$ 0,07 a
Evening	0,51 $\pm$ 0,08 ab
Morning and Evening	0,45 $\pm$ 0,05 b

Note: The result of the number followed by the equal alphabet in each experiment indicates that there is no significant difference in the DMRT α 5% test

Chitosan can increase chlorophyll, which helps absorb light used in the process of photosynthesis. The time of the morning application is the same as the time of the afternoon application, but it differs from the time of the morning and afternoon applications. The morning or afternoon application time can be chosen as the best time because the environmental conditions support plant absorption of chitosan. According to (18), the effectiveness of plant growth regulators is greatly influenced by the time of application. The low temperature and light intensity at that time prevented chitosan from readily evaporating and being absorbed well by plants.

Stomatal Conductance

The analysis of variance revealed that the time of chitosan application had a significant effect on the stomatal conductance of pineapples. The concentration and interaction between concentration and time of chitosan application did not significantly affect the stomatal conductance of pineapple. This means that the time of chitosan application can increase stomatal conductance. Pineapple stomatal conductance with different concentrations and application times of chitosan is presented in Table 5.

Table 5. Stomatal conductance ($\text{mmol H}_2\text{O m}^{-2}\cdot\text{s}^{-1}$) of pineapple at 12 WAP with different concentrations and application times of chitosan

Treatment Level	Stomatal Conductance $\pm$ standard deviation ($\text{mmol H}_2\text{O m}^{-2}\cdot\text{s}^{-1}$)
<i>Chitosan concentration (ppm)</i>	
Without chitosan	0,021 $\pm$ 0,002
1 ppm	0,019 $\pm$ 0,008
3 ppm	0,033 $\pm$ 0,022
5 ppm	0,017 $\pm$ 0,008
<i>Chitosan application time</i>	
Morning	0,021 $\pm$ 0,007 ab
Evening	0,029 $\pm$ 0,019 a
Morning and Evening	0,015 $\pm$ 0,005 b

Note: The result of the number followed by the equal alphabet in each experiment indicates that there is no significant difference in the DMRT α 5% test

Based on Table 5, the application of chitosan increased stomatal conductance over time. These results align with the research of (19), which states that the combination of inorganic fertilizer and chitosan increased stomatal conductance values in tea plants. The time of afternoon application was the same as the morning application, but different from the morning-evening application. This suggests that applying chitosan in the morning or evening may be the optimal time for chitosan application. (20) stated that the stomatal guard cells still contain starch in the morning, triggering the stomata to open. Meanwhile, (21) explained that fertilizer application to CAM plants needs to be adjusted to the time the stomata open, namely in the afternoon. Chitosan elevates ABA activity and regulates the stomatal aperture (22).

Conclusion

The conclusion that can be drawn is that no combination of chitosan concentration and application time can increase pineapple growth. Applying chitosan at 1 ppm and 3 ppm can increase the number of leaves, while applying chitosan at 1 ppm, 3 ppm, and 5 ppm can increase the total leaf area and the length of the longest pineapple root. Chitosan application is sufficient to be done only once in the morning or afternoon to increase the rate of photosynthesis and pineapple stomatal conductance. Based on the research conducted, a concentration of 1 ppm and morning chitosan application are recommended for pineapple

cultivation, as they can increase the number of leaves, total leaf area, most extended root length, photosynthesis rate, and pineapple stomatal conductance. Furthermore, additional research is required to investigate the impact of chitosan on the reproductive period of pineapple plants and its effect on post-harvest quality.

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