ORIGINAL RESEARCH ARTICLE

Current Research on Biosciences and Biotechnology **5** (2) 2024 5-11

Current Research on Biosciences and Biotechnology

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# The influence of chitosan on plant growth and β-sitosterol content in adventitious roots of *Talinum paniculatum* in NFT hydroponic cultivation system

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ABSTRACT

β-sitosterol is the most abundant type of phytosterol found in nature and it offers numerous health benefits for humans including anticancer, antidiabetic, and blood cholesterol-lowering properties. One biomass with a significant β-sitosterol content is the Javanese ginseng root (*Talinum paniculatum*). However, Javanese ginseng has not been fully exploited and is often perceived as a mere wild plant. This research was conducted by cultivating Javanese ginseng using the Nutrient Film Technique (NFT) hydroponic method along with the addition of chitosan elicitors at various concentrations: 0, 12.5, 25, and 50 ppm. The purpose of this research was to determine the optimal chitosan concentration for the growth of Javanese ginseng, the β-sitosterol content, and the productivity of β-sitosterol in Javanese ginseng roots. The research revealed that a 12.5 ppm chitosan concentration could increase the root weight by 8.785 g and the shoot weight by 88 g. The growth rate increased to 3.457 g/day and the productivity of β-sitosterol in the roots reached 25.084 mg/m2/month. On the other hand, the addition of chitosan led to a decrease in the root-to-shoot ratio, indicating an improved cultivation environment compared to cultivation without chitosan. Furthermore, the results of the research also indicated that the addition of chitosan did not affect the β -sitosterol content in the Javanese ginseng adventitious roots. Therefore, it can be concluded that the optimal chitosan concentration that positively impacts Javanese ginseng cultivated using the NFT hydroponic method is 12.5 ppm because chitosan generates Reactive Oxygen Species (ROS) which causing plant damage and cell death when threshold exceeded.

*Article history:*

Received 19 Jan 2024
Revised 21 Feb 2024
Accepted 23 Feb 2024
Available online 29 Feb 2024

*Keywords:*

β-sitosterol
chitosan
Javanese ginseng (*Talinum paniculatum*)
NFT hydroponic
plant growth

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DOI: 10.5614/crbb.2024.5.2/JH7W60YM



## 1. Introduction

β-sitosterol is a phytosterol with diverse human health benefits, including anticancer, antidiabetic, and blood cholesterol-lowering properties (Babu and Jayaraman, 2020). It is relatively abundant in nature, making it readily available (Clifton, 2002). One plant with a substantial β-sitosterol content is Javanese ginseng (*Talinum paniculatum*), especially in its root, which accounts for as much as 17.37% of the total content (Thanamool et al., 2013).

Javanese ginseng is a wild plant that grows along roadsides and ditches, making its seedlings easily accessible (Nuhamara, 2017). Furthermore, the growth of Javanese ginseng does not require specific conditions such as temperature, pH, light intensity, or air humidity (Imaniar et al., 2017). However, its utilization has thus far been largely limited to culinary purposes.

Traditionally, Javanese ginseng is cultivated in soil, but the quality of the harvest can vary due to inconsistent nutrient levels in the soil (infarm, 2020). Therefore, cultivation methods that ensure a uniform nutrient supply to plants are sought to achieve consistent quality across different crops (infarm, 2020). One such cultivation method is the Nutrient Film Technique (NFT) hydroponic system. NFT hydroponic stands out as a leading technique because it allows plant roots to receive water, nutrients, and oxygen precisely as needed. The quality and quantity of the harvest can also be improved using elicitors such as chitosan.

Chitosan is a biotic elicitor derived from chitin that supports plant growth (Malerba and Cerana, 2016). Compared to other types of elicitors, chitosan is known to help plants respond to a wider range of stresses, including both biotic and abiotic factors (Hidangmayum et al., 2019). In addition, chitosan is not harmful to either the plants themselves or the environment because of its non-toxic nature (Zahra and Slamet, 2022). Chitosan enhances the absorption of water and nutrients. Chitosan also activates phytohormones such as auxins and cytokinins, which play an active role in plant growth (Chakraborty et al., 2020). Additionally, chitosan boosts the production of antioxidant compounds to counteract the damage caused by Reactive Oxygen Species (ROS) (Chakraborty et al., 2020).

This research aims to determine the optimal chitosan concentration among four concentrations (0 as control, 12.5, 25, and 50 ppm) for Javanese ginseng growth. It also aims to evaluate β-sitosterol content and productivity in the roots of Javanese

ginseng cultivated using NFT hydroponic methods. The research aims to advance Javanese ginseng utilization beyond its wild plant status and potentially increase β-sitosterol production, which is known for its various health benefits.

## 2. Materials and methods

### 2.1. Materials

The materials used in this research were 4-month-old Javanese ginseng plants obtained from UPTD Balai Pengembangan Benih Hortikultura dan Aneka Tanaman, located in Jatinangor, Sumedang, West Java. The substances included AB Mix nutrient solution, chitosan from Himedia, acetic acid ($CH_3COOH$), distilled water (aquades), 96% ethanol (EtOH), Whatman No. 1 filter paper, β-sitosterol standard (MarkHerb, Bandung, Indonesia), HPLC-grade methanol (MeOH) from Supelco (Merck), HPLC-grade acetonitrile from Supelco (Merck), aquabides, PA-grade chloroform from Supelco (Merck), and 0.22 µm PTFE filters.

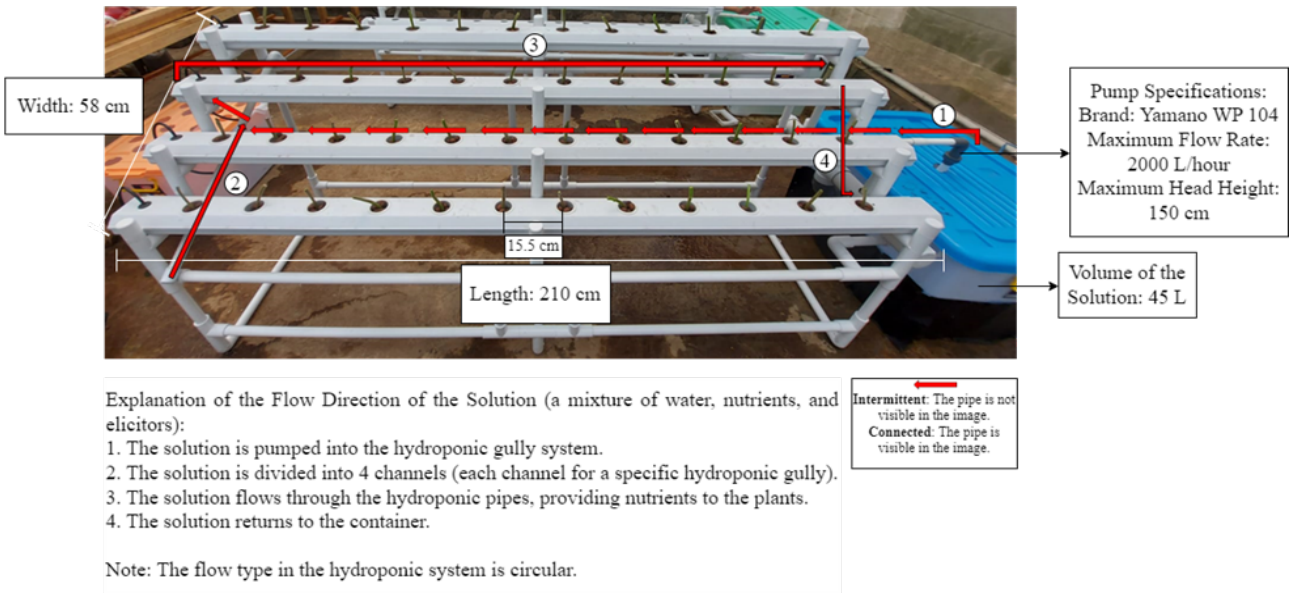


**Fig. 1.** NFT Hydroponic System

### 2.2. Methods

#### 2.2.1. Preparation of javanese ginseng stem

Stem cuttings were obtained from 4-month-old Javanese ginseng plants. The stem was cut at a 45° angle at one end using a cutter whose blade had been sterilized with 70% alcohol beforehand. After obtaining stem segments measuring 10 - 12 cm in length and 0.7 - 1 cm in diameter, 12 selected stems were weighed. Subsequently, these stems were inserted into netpots, and hydrotons ware added to ensure that the stems stood upright. The netpots were then placed in designated locations within the hydroponic gully and the hydroponic set was connected to electricity.

#### 2.2.2. Cultivation of javanese ginseng plants

For each treatment, the cultivation period of Javanese ginseng plants was 28 days in the Nutrient Film Technique (NFT) hydroponic system, as depicted in Fig. 1. The breakdown of the cultivation period consisted of the following phases: 7 days of acclimatization, 7 days with 50% nutrient solution, 7 days with 100% nutrient solution, and 7 days of elicitation with chitosan. During the acclimatization phase, 45 liters of water was used to irrigate the plants. In the subsequent 50% nutrient phase, the AB Mix nutrient was introduced, resulting in a solution that irrigated the plants with a mixture of nutrient A (135 mL), nutrient B (135 mL), and water to reach a total volume of 45 liters. In the 100% nutrient phase, the solutions used to irrigate the plants were nutrient A (270 mL), nutrient B (270 mL), and water to achieve a total volume of 45 liters. During the final elicitation phase, chitosan, previously dissolved in 0.1 M acetic acid at a 1:1 (w/v) ratio, was added. This modified the composition of the solution that flowed to the plants, which consisted of nutrient A (270 mL), nutrient B (270 mL), chitosan solution (56.25 mL for a concentration of 12.5 ppm, 112.5 mL for a concentration of 25 ppm, and 225 mL for a concentration of 50 ppm), or acetic acid solution (225 mL for the control), and water to achieve a total volume of 45 liters. Routine pH checks were conducted every 2 days during the elicitation phase, and pH adjustments were made as needed by adding 10% KOH (if the condition was too acidic) or 0.1 M $CH_3COOH$ (if the condition was too basic).

#### 2.2.3. Harvesting javanese ginseng plants

After 28 days, the Javanese ginseng plants were harvested. Roots and shoots were then separated and weighed. From the obtained weights, the shoot and root weight increments were calculated, as well as the growth rate, using the respective equations provided in Eq. (1), (2), and (3) (Carberry, 2022).

$$Shoot\ Weight\ Increase\ (g) = FS(g) - IS(g) \quad \text{Eq. (1)}$$

$$Root\ Weight\ Increase\ (g) = R(g) \quad \text{Eq. (2)}$$

$$Growth\ Rate\ \left(\frac{g}{day}\right) = \frac{(FS(g)+R(g)-IS(g)}{t\ cultivation\ (day)} \quad \text{Eq. (3)}$$

where IS is the initial shoot weight, FS is the final shoot weight, R is the root weight, and t cultivation is the cultivation duration.

Next, both the shoots and roots were dried at 50°C for 48 hours and then weighed. The weighing results could be used to calculate the root-to-shoot ratio using Eq. (4) (Roger et al., 1996).

$$Root - to - Shoot\ Ratio\ \left(\frac{g\ dry\ root}{g\ dry\ shoot}\right) = \frac{DRW}{DSW} \quad \text{Eq. (4)}$$

where DRW is the dry weight of the root and DSW is the dry weight of the shoot.

### 2.2.4. Extraction of β-sitosterol from the adventitious roots of javanese ginseng plants

A total of 2 g of dried Javanese ginseng roots were finely ground in a blender. Powdered ginseng roots were then subjected to an extraction process using 50 mL of 96% ethanol as the solvent. Extraction was carried out by sonication for 60 minutes. The extraction process was followed by centrifugation for 20 minutes at 5000 rpm to obtain the liquid filtrate and solid residue. Subsequently, both were separated using filter paper. The solid residue obtained from the previous step was subjected to another round of sonication, using an additional 50 mL of 96% ethanol, and subjected to the same process of centrifugation and filtration as in the previous step. The filtrate obtained from the second round of extraction was combined with the filtrate from the initial extraction. The combined filtrates were then concentrated using a rotary vacuum evaporator. This process was conducted at 60°C with a rotation speed of 75 rpm to remove any residual water and solvent from the extract. The extract was dried in an oven at 50°C to remove any remaining water and solvent.

### 2.2.5. Analysis of β-sitosterol using HPLC

In this analysis, 10 mg of β-sitosterol standard was dissolved in a few drops of PA-grade chloroform and then supplemented with HPLC-grade methanol to reach a final volume of 10 mL. This solution was then prepared as a stock solution at a concentration of 1000 ppm, and further dilutions were made as needed using HPLC-grade methanol until the desired concentrations were achieved. All solutions with varying concentrations were filtered through a 0.22 µm PTFE filter before being injected into the HPLC system. HPLC analysis was performed at a flow rate of 1 mL/minute, using a mobile phase composed of methanol:acetonitrile (9:1, v/v), C18 stationary phase, and a wavelength of 202 nm to detect β-sitosterol (Khonsa et al., 2022). After obtaining the peak areas from the HPLC analysis, a graph was constructed by plotting the concentrations of the standard β-sitosterol solutions (x-axis) against the corresponding peak areas (y-axis). The resulting line equation became the standard curve for β-sitosterol.

For the analysis of β-sitosterol content in the dry extract of Javanese ginseng roots, the extract was initially dissolved in 5 mL HPLC-grade methanol. Subsequently, the extract solution was filtered through a 0.22 µm PTFE filter into an HPLC vial, reaching a final volume of 1.5 mL. This solution was then injected into the HPLC instrument using the same HPLC settings as those used for the analysis of standard β-sitosterol solutions of various concentrations. The peak area obtained from HPLC analysis was used as the y-value in the standard curve equation to determine the concentration of β-sitosterol in the sample as the x-value.

The concentration of β-sitosterol was then converted into the β-sitosterol content in the root extract using Eq. (5) (Fuentes-Arderiu, 2013).

$$\beta - sitosterol\ Content\left(\frac{mg\ \beta - sitosterol}{g\ dry\ root\ weight}\right) = \frac{C\ (SIT)(ppm)\ x\ Vs\ (mL)}{RPW\ (g) x\ 1000} \quad \text{Eq. (5)}$$

where C(SIT) represents the concentration of β-sitosterol, Vs is the solvent volume (HPLC-grade methanol), and RPW is the weight of root powder used for one-sample replication. Furthermore, the β-sitosterol productivity of the roots was calculated using Eq. (6) (Mangoli, 2020). In calculating the β-sitosterol productivity from the roots, we assumed the dimensions of the hydroponic gully to be 2.1 m x 0.58 m (length x width) with a total of 48 plants in each hydroponic set.

$$\beta - sitosterol\ Productivity\left(\frac{mg\ \beta - sitosterol}{\frac{m^2}{month}}\right) = \frac{Content\ (SIT)\ x\ avg\ DRW\ (g)\ x\ Number\ of\ Plants}{Hydroponic\ Gully\ Area\ (m^2)/\ t\ cultivation\ (month)} \quad \text{Eq. (6)}$$

where Content (SIT) is the β-sitosterol content, average DRW is the mean dry root weight per plant, and t cultivation is the cultivation duration.

### 2.2.6. Statistical analysis

The research design in this study used a completely randomized design (CRD) with four variations of chitosan concentrations: 0, 12.5, 25, and 50 ppm, along with 12 replications (for cultivation data) or 3 replications (for HPLC data). ANOVA (Analysis of Variance) One-Way test was conducted to determine the presence of differences among the data groups. If differences were found, ANOVA was followed by Tukey's test (at a significance level of 0.05) to assess the significance of the effects of chitosan concentration on variables such as root weight increase, shoot weight increase, growth rate, root-to-shoot ratio, β-sitosterol content in the roots, and β-sitosterol productivity from the roots. All statistical analyses in this research were conducted using Minitab 19 software.

## 3. Results and discussion

### 3.1. Plant growth

Cultivation period is a crucial phase of plants growth. Growth can be influenced by various factors, including biotic factors such as the presence of pests and weeds as well as abiotic factors such as sunlight intensity, environmental temperature, air humidity, and pH levels (Imaniar et al., 2017). Therefore, the NFT hydroponic cultivation method was used in this research to control environmental factors, ensuring that the plants had consistent conditions across treatments.

During the cultivation period from the first to the third week, there were no discernible differences in the physical appearance of Javanese ginseng plants among the various treatments. Small leaves and roots began to emerge by the end of the first week. By the end of the second week, there was an increase in both the size and quantity of roots and leaves, and leaf stems began to appear. By the end of the third week, the quantity and size of the leaves, leaf stems, and roots continued to increase. In addition, the stems grew longer.

Differences in the physical appearance of Javanese ginseng plants cultivated using the NFT hydroponic method were evident only after the elicitation phase. Based on Fig. 2A and Fig. 2D, it can be observed that both groups displayed similar physical characteristics, such as fragile, soft, dark brown roots, which were relatively short, and short stems with small and few leaves. However, the root structure in the control treatment was superior to that in the chitosan 50 ppm treatment. Furthermore, as shown in Fig. 2B and Fig. 2C, it can be observed that plants from these different treatments also exhibited similar physical characteristics, including thick, rigid, long, light brown roots, long stems, and leaf stems, with large and numerous leaves. However, the physical appearance in the chitosan 12.5 ppm treatment was superior in all aspects compared to the chitosan 25 ppm treatment. Therefore, it can be concluded that the plants treated with chitosan 12.5 ppm exhibited the most favorable physical appearance among the four treatments.

In Fig. 2, it can be observed that the roots formed do not take on the typical tuberous shape of Javanese ginseng roots but appear as long fibrous adventitious roots. This is because the roots that develop during the cultivation period do not grow from the radicle, but rather from the base of the stem in response to the wound (Gogna et al., 2022). In this research, stem cuttings were used to

cultivate Javanese ginseng plants. Stem cuttings create wounds in the form of damaged tissue at the cut ends of stems. The presence of damaged plant tissue triggers a signal that enhances auxin production as a repair response to the damaged tissue (Hoermayer et al., 2020). Auxin stimulates the formation of adventitious roots (Pop et al., 2011).


**Fig. 2.** The physical appearance of Javanese ginseng plants treated with chitosan concentrations (A) 0 ppm (control), (B) 12.5 ppm, (C) 25 ppm, and (D) 50 ppm after 28 days of cultivation

The emergence of adventitious roots in this research was a result of using stem cuttings and the choice of the growing medium. To obtain tuberous Javanese ginseng roots, cultivation should ideally be initiated from seedlings rather than stem cuttings (Pusat Kajian Hortikultura Tropika Institut Pertanian Bogor, 2018). Moreover, the medium used should be one that allows for greater spacing between individual plants, such as perlite and vermiculite, rather than a medium with close spacing such as hydroton (Dinas Pertanian dan Pangan Kabupaten Badung, 2018). This is because the tuberous roots growth requires ample space.

Reactive oxygen species (ROS) exist in the form of $H_2O_2$ and NO as a result of the interaction between chitosan and plant cells (Pichayangkura and Chadchawan, 2015). $H_2O_2$ and NO support plant growth. $H_2O_2$ promotes root growth, resulting in a robust plant root system in plants (Hameed et al., 2004). It also increases the absorption area and enhances the mineral uptake (Nurnaeimah et al., 2020). In contrast, NO supports root elongation induces the formation of adventitious roots and increases leaf number (Belligni and Lamattina, 2001). Therefore, the addition of chitosan can improve the physical characteristics of plants, as shown in Fig. 2. However, excess ROS can cause damage and even cell death (National Cancer Institute [NCI], n.d.).

As the concentration of chitosan increased, the production of $H_2O_2$ and NO also increased, leading to higher levels of ROS within cells. Damage to the cells in the roots and shoots of Javanese ginseng plants results in suboptimal plant growth. This can be observed in the physical appearance of the Javanese ginseng plants in Fig. 2D. Based on these observations, it can be concluded that the addition of chitosan at concentrations of 12.5 and 25 ppm supports plant growth, as evidenced by the better physical appearance of the plants in Fig. 2B and Fig. 2C compared with the control treatment.

## 3.2. Influence of chitosan concentration on the javanese ginseng roots and shoots weight increase

The effect of chitosan concentration on the increase in weight of Javanese ginseng roots and shoots is shown in Fig. 3. The greatest increase in root weight was observed in the treatment with 12.5 ppm chitosan (8.785 g), followed by the treatment with 25 ppm chitosan (5.204 g). The treatments with 50 ppm chitosan and the control showed relatively similar values (2.017 and 2.399 g, respectively). It was also known that the concentration of 12.5 ppm chitosan resulted in the highest increase in shoot weight (88 g). The second-highest increase in shoot weight was observed in the treatment with 25 ppm chitosan (52.553 g). Similar to the increase in root weight, the treatments with 50 ppm chitosan and the control showed values that were relatively similar (27.763 g and 22.809 g, respectively).

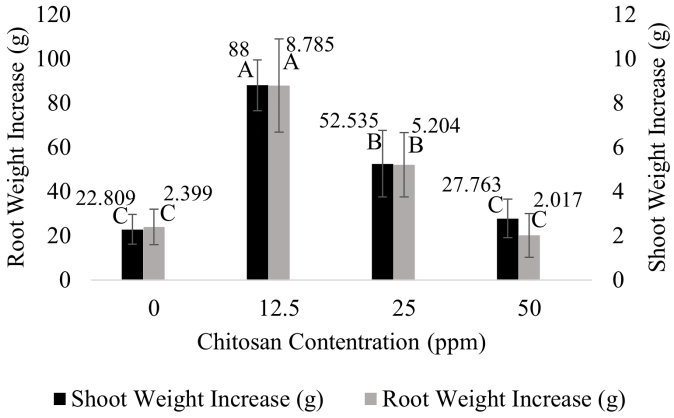


**Fig. 3.** Influence of chitosan concentration on the root and shoot weight increase

Based on the graph in Fig. 3, it can be observed that the optimal chitosan concentration for increasing the weight of Javanese ginseng roots and shoots in hydroponic NFT cultivation is 12.5 ppm. Chitosan is a biotic elicitor that supports plant growth (Malerba and Cerana, 2016). The interaction between the positively charged amino groups of chitosan and the negatively charged phospholipids in plant cells leads to the formation of $H_2O_2$ and JA (jasmonic acid) through the octadecanoid pathway and the generation of NO (Pichayangkura and Chadchawan, 2015). $H_2O_2$ and NO can promote root growth (Beligni and Lamattina, 2001; Hameed et al., 2004). NO can also increase the number of leaves (Beligni and Lamattina, 2001). In contrast, JA supports root elongation (Han et al., 2023).

NO induces PA formation, which in turn inhibits ABA1 by activating abscisic acid (ABA) (Pichayangkura and Chadchawan, 2015). ABA can promote the growth of adventitious roots (Abou-Mandour and Hartung, 1980). In addition, ABA supports shoot growth in plants (Brookbank et al., 2021).

$H_2O_2$ and NO are ROS that can cause damage and even cell death when their levels are excessively high within plant cells (NCI,

n.d.). As the chitosan concentration increased, the levels of ROS within plant cells also increase. This leads to damage to both root and shoot cells of Javanese ginseng plants. As a result, root and shoot growth were inhibited. The weights of the roots and shoots of Javanese ginseng plants will only significantly increase up to a certain maximum chitosan concentration. Beyond the maximum concentration, the increase in weight did not differ significantly from that of the control treatment.

In addition to an increased level of ROS, the production of ABA and JA should also increase as the chitosan concentration increases. Therefore, shoot and root growth should theoretically be improved with higher chitosan concentrations. However, the opposite was observed. This could be due to the shift in the focus of ABA and JA from supporting root and shoot growth to reducing damage caused by excessive ROS produced by plant cells. Therefore, plant growth at higher chitosan concentrations was not as favorable as that at lower concentrations. This mechanism is related to metabolic pathway shifts in plants that are influenced by chitosan (Fatima et al., 2021). Chitosan that enters the plant is degraded into fructose. Fructose then enters the glycolytic pathway, which is important for growth. However, excessive ROS signals in plants can redirect fructose to other pathways, such as the formation of secondary metabolites that protect the plant from potential ROS-induced damage. To protect the plant from potential ROS-induced damage, fructose resulting from chitosan degradation is directed towards compounds that function as antioxidants, including flavonoids (Speisky et al., 2022)

### 3.3. Influence of chitosan concentration on the growth rate of javanese ginseng

The effect of chitosan concentration on the growth rate of Javanese ginseng is shown in Fig. 4. The fastest growth rate was observed in the treatment with 12.5 ppm chitosan (3.457 g/day), followed by the treatment with 25 ppm chitosan (2.063 g/day). The growth rate in the treatment with 50 ppm chitosan (1.064 g/day) did not significantly differ from that in the control treatment (0.9 g/day). Based on the graph presented in Fig. 4, it can be determined that the optimal chitosan concentration for increasing the growth rate of Javanese ginseng in hydroponic NFT cultivation is 12.5 ppm.

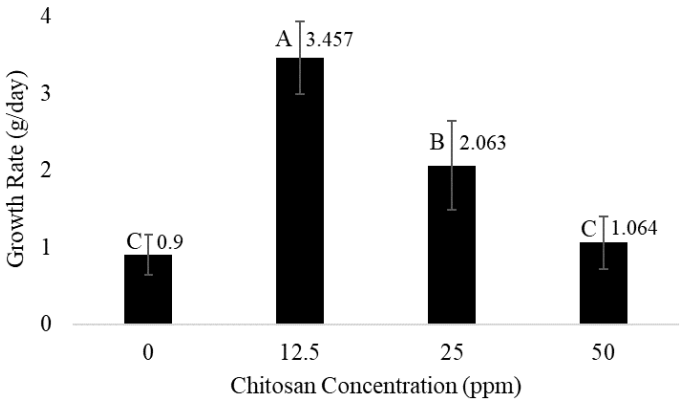


**Fig. 4.** Influence of chitosan concentration on the growth rate

The growth rate represents the rate of plant weight gain over a specific period of time (Kriedemann et al., 1999). Therefore, it can be stated that the increase in shoot and root weight is related to the growth rate. Observations from Fig. 3 and Fig. 4 indicate a direct correlation between the magnitude of root and shoot weight increments and speed of growth. This phenomenon is attributed to the ability of chitosan to enhance water and nutrient absorption in plants, facilitating a quicker supply of the raw materials required for sustaining photosynthesis, thus, promoting increased growth rates (Chakraborty et al., 2020). However, this enhanced absorption capability is only effective up to a certain chitosan concentration. Beyond this threshold, the metabolic pathways influenced by chitosan tend to prioritize reducing the potential damage caused by reactive oxygen species (ROS), which escalate with higher chitosan concentrations, rather than supporting plant growth (Fatima et al., 2021).

In addition to its ability to increase water and nutrient uptake, chitosan can activate hydrolytic enzymes to break down macromolecules for easier absorption by plants. Furthermore, chitosan can activate phytohormones, such as auxin and cytokinin, which play a role in growth. These factors contribute to the faster growth rate of Javanese ginseng when chitosan is added compared to Javanese ginseng without chitosan addition (Chakraborty et al., 2020).

### 3.4. Influence of chitosan concentration on the root-to-shoot ratio of javanese ginseng

The effect of chitosan concentration on the root-to-shoot ratio of Javanese ginseng is shown in Fig. 5. In ascending order, the root-to-shoot ratios of Javanese ginseng are as follows: the control treatment (0.015), chitosan 25 ppm treatment (0.008), chitosan 12.5 ppm treatment (0.0069), and chitosan 50 ppm treatment (0.0067). Based on Fig. 5, it can be observed that the control treatment had a significantly different impact compared to the other three treatment on the root-to-shoot ratio of Javanese ginseng cultivated using the NFT hydroponic method.

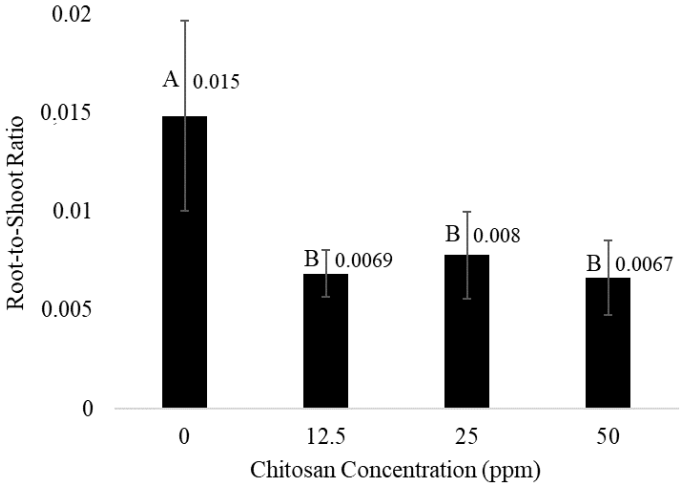


**Fig. 5.** Influence of chitosan concentration on the root-to-shoot ratio

The root-to-shoot ratio was calculated using the dry weight of both the shoots and roots to determine the effect of chitosan on nutrient absorption by the plant for organ growth (Chakraborty et al., 2020). Based on the results obtained, it can be observed that all four treatment variations have a larger proportion of shoots compared to roots. Therefore, it can be said that the growth of Javanese ginseng tends to focus on the shoot, such as stem elongation, leaf widening, and an increase in the number of leaves, rather than on the root, such as root elongation.

These results indicated that the addition of chitosan had an impact on plant growth, especially on shoots. Furthermore, the results also show that chitosan supports plant growth by enhancing nutrient absorption (Chakraborty et al., 2020). Both of these are reflected in the smaller root-to-shoot ratio values in the chitosan treatments compared with the control treatment. A smaller root-to-shoot ratio indicates a higher shoot dry weight value when the same dry weight of the root is used as the basis. Dry weight represents the condition of the plant organ without moisture content, leaving

only the components including the absorbed nutrients during the cultivation period (Groen Kennisnet, 2022).

A good root-to-shoot ratio was achieved when the value was low. A smaller root-to-shoot ratio indicated that the cultivation environment was suitable. This suggests that the addition of chitosan makes the cultivation environment (NFT hydroponic system) more ideal for growing Javanese ginseng than a cultivation environment without chitosan (Harris, 1992). This is attributed to the ability of chitosan to support plant growth by generating ROS, which can aid in root and shoot development and expand the absorption area (Nurnaeimah et al., 2020).

### 3.5. Influence of chitosan concentration on the β-sitosterol content in javanese ginseng roots

The effect of chitosan concentration on the β-sitosterol content in Javanese ginseng roots are presented in Fig. 6. The highest β-sitosterol content was observed in the control treatment (1.684 mg/g dry weight), followed by the 50 ppm chitosan treatment (1.629 mg/g dry weight), the 12.5 ppm chitosan treatment (1.562 mg/g dry weight), and the 25 ppm chitosan treatment (1.524 mg/g dry weight). Based on Fig. 6, it can be observed that the addition of chitosan did not have a significant impact on the β-sitosterol content in the Javanese ginseng roots cultivated using the NFT hydroponic method. This lack of effect may be attributed to the absence of specific stress factors induced by chitosan, which could stimulate β-sitosterol production, resulting in no additional β-sitosterol formation compared to plants without chitosan addition.

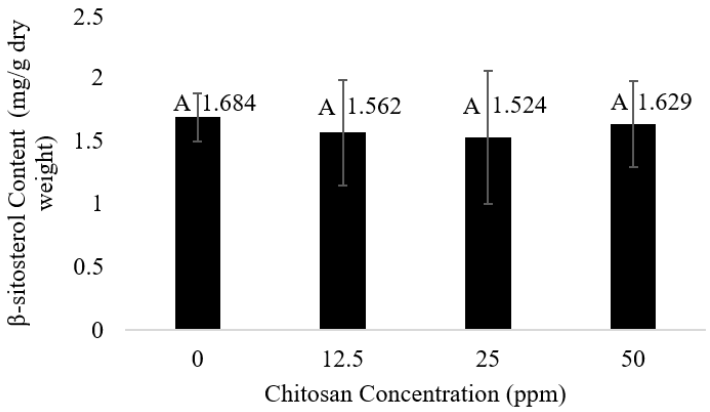


**Fig. 6.** Influence of chitosan concentration on the β-sitosterol content in roots

Drought is a stress factor capable of inducing β-sitosterol formation (Li et al., 2019). In this research, a hydroponic growth medium was used, which precluded the possibility of drought occurrence. Additionally, chitosan enhances water absorption in plants, preventing them from experiencing drought conditions (Chakraborty et al., 2020). Another stress factor known to induce β-sitosterol production is heat from excessively high temperatures (Rossi and Huang, 2019). The temperature range during the research was maintained between 25 °C and 27 °C, whereas the literature suggests that the temperature threshold for inducing such stress can reach up to 35 °C (Rossi and Huang, 2019). In other words, there was no heat stress due to the high temperatures used in this research.

### 3.6. Influence of chitosan concentration on the β-sitosterol productivity in javanese ginseng roots

The effect of chitosan concentration on the productivity of β-sitosterol in Javanese ginseng roots is shown in Fig. 7. β-sitosterol productivity is influenced by the β-sitosterol content and the weight of the Javanese ginseng plant roots, where this productivity value depicts the average production of β-sitosterol by Javanese ginseng roots per unit area of NFT hydroponic gully in 1 month (28 days). The highest productivity of β-sitosterol was observed in the treatment with 12.5 ppm chitosan (25.084 mg/m²/month), followed by the 25 ppm chitosan treatment (9.936 mg/m²/month), control treatment (9.145 mg/m²/month), and 50 ppm chitosan treatment (7.655 mg/m²/month). Based on Fig. 7, it can be inferred that the optimal chitosan concentration for enhancing the productivity of β-sitosterol in the roots of Javanese ginseng cultivated using the NFT hydroponic method was 12.5 ppm.

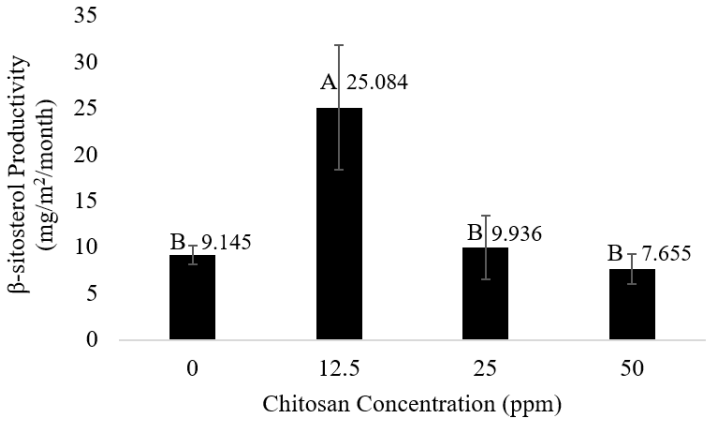


**Fig. 7.** Influence of chitosan concentration on β-sitosterol productivity in roots

The productivity of β-sitosterol is influenced by the β-sitosterol content and weight of Javanese ginseng roots. Even though the β-sitosterol content in the Javanese ginseng roots with 12.5 ppm chitosan was not the highest, it was not a concern because there was no significant difference in the β-sitosterol content values among the four treatments. Moreover, the 12.5 ppm chitosan concentration was the most optimal for significantly increasing root weight. The increased root weight ultimately contributes to the total yield of β-sitosterol from Javanese ginseng roots treated with 12.5 ppm chitosan. Therefore, 12.5 ppm chitosan concentration is considered the optimum concentration for enhancing the productivity of β-sitosterol in Javanese ginseng roots.

## 4. Conclusion

From the research conducted, it can be concluded that the optimum chitosan concentration, which influences the increase in the weight of both the shoot and the root, growth rate, and the productivity of β-sitosterol from the roots of Javanese ginseng cultivated using the NFT hydroponic method, was 12.5 ppm. The addition of chitosan also reduced the root-to-shoot ratio, although there was no optimum chitosan concentration for this parameter. However, the addition of chitosan did not affect the β-sitosterol content in the adventitious roots of Javanese ginseng cultivated using the NFT hydroponic method.

## Acknowledgements

This research was supported by PPMI ITB [research grant for the year 2023]. We thank Ela Kamelia Saleh for helping us with the HPLC instrument.

## Conflict of interest

The authors declare there is no conflict of interest in this research.

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