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Improvement of Growth and Development of Sweet Basil (*Ocimum basilicum* L.) Through the Application of Chitosan at Different Plant Maturity Stages

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ABSTRACT

Sweet basil is one of the most popular culinary, medicinal, and fragrance herbs in Mediterranean, Asian, and Western countries. This study aims to increase the growth performance of sweet basil via different concentrations of chitosan, which is applied at three growth stages. The study was arranged in a factorial randomized complete block design with four replications. The plants were divided into three growth stages, which were the vegetative stage (S1), the reproductive stage (S2), and both the vegetative and reproductive stages (S1 + S2). Those plants were then treated with four different concentrations of chitosan (0, 2, 4, and 6 ml/L) either on S1, S2, or S1 + S2. The results indicated that plants treated with chitosan at S1 showed greater performance. Chitosan concentration of 4 ml/L produced greater plant height (55.09 ± 1.75 cm/plant), stem diameter (11.08 ± 0.89 mm/plant), and a number of leaves (296.57 ± 11.61 leaves/plant). It is also interesting to observe that the lowest chitosan concentration was non-significantly different, with 4 ml/L at S1 in some parameters. Plants in those treatments showed the highest average length of internode, number of branches, total root length, average root diameter, total root volume, and total root surface area. Besides, correlation analysis proved that all the parameters significantly correlated positively. As the concentration of 4 ml/L showed a superior effect, especially on the number of yields, thus it is recommended for growers to apply chitosan at 4 ml/L during S1.

Keywords: Chitosan, drenching, physiology, reproductive stage, vegetative stage

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INTRODUCTION

The Lamiaceae family is one of the most important and largest pharmaceutical and aromatic plants, where sweet basil (*Ocimum basilicum* L.) is the main valuable member (Mosadegh et al., 2021). Sweet basil is an annual, warm-season, and sun-loving herb (Zulfiqar et al., 2021). Sweet basil poses a valuable source of nutraceuticals such as proteins, carbohydrates, minerals, and vitamin C (Corrado et al., 2020). Due to widespread culinary, medicinal, and aromatic consumption, sweet basil is a popular herb in Mediterranean, Asian, and Western countries (Bufalo et al., 2015; Delbeke et al., 2015; Harnafi et al., 2013; Incrocci et al., 2019). Sweet basil is a naturally eatable antioxidant (Patriani et al., 2021). The antioxidant compounds present in sweet basil provide treatment for human cell cancers (Hanachi et al., 2021). Traditionally, it is used to treat inflammation and helminthic (Osei-Akoto et al., 2020).

Regarding dietary, sweet basil is used as a fresh vegetable alone or with many foods and salads (Mirzajani et al., 2015). Commonly, sweet basil is produced for the fresh market (Corrado et al., 2020; Delbeke et al., 2015; Fattahi et al., 2019; Klintham et al., 2018). Since as a medicinal herb, the whole plant is used in traditional pharmacies (Ghasemzadeh et al., 2016). The total fresh biomass is considered as yield (Scagel et al., 2019). Hence, the yield is assumed to be associated with plant growth and development. In recent years the demand for sweet basil has increased (Acharya et al., 2020; Ciriello et al., 2021; Pandey et al., 2019).

Sweet basil is the most consumed herb and is a highly demanded fresh market, but still, the production is insufficient to meet the demand. In sweet basil, the yield is associated with growth parameters. Thus, the growth and yield of sweet basil could be affected by environmental factors (Elhindi et al., 2017). One of those factors is a chitin-based biopolymer plant growth promoter named chitosan (Govindaraju & Arulselvi, 2018). Since chitosan is obtained from waste materials of seafood industries, the use of chitosan could be actively attributed to reducing pollution (El-Amerany et al., 2020). Chitosan is biodegradable, biocompatible, and ecologically friendly with low economical cost (Jiao et al., 2018). Chitosan has been tested in crops other than sweet basil and showed greater growth performance (Monfared et al., 2020; Turk, 2019). For instance, Mondal et al. (2016) suggested that *Solanum lycopersicum* be treated with 75 mg/L to obtain maximum plant growth and yield components. In addition, a concentration of 5 mg/L chitosan gave a higher number of branches, length of branches, and a number of leaves compared to other common growth regulators such as cytokinins, kinetin, and auxins in *Ipomoea purpurea* L. (Acemi et al., 2018). Similarly, Acemi (2020) suggested that chitosan could be a good alternative to other growth regulators, such as 6-benzylaminopurine and jasmonic acid, in improving plant growth and development. Also, Chamnanmanoontham et al. (2015) reported that 40 mg/L chitosan resulted in greater growth performance in terms of shoot and root in *Oryza sativa* L. plants.

Since herbal yield is associated with growth parameters as well as the number of leaves per plant, chitosan was reported to increase herbal yield in several plants such as *Thymus vulgaris* L., *Mentha* × *Piperita* L., *Nigella sativa* L., and *Lavandula officinalis* Chaix. (Fahmy & Nosir, 2021; Goudarzian et al., 2020; Ibrahim, 2020; Waly et al., 2020).

Although chitosan is considered a growth-promoting substance, its effectiveness depends on the crop species, concentration, and plant's growth stage to be treated (Heidari et al., 2020). Hence, it would be a great contribution for the producers to determine the effective concentration of chitosan and the suitable time of application for sweet basil. Therefore, in the current study, four concentrations of chitosan were tested at three different growth stages on sweet basil.

MATERIALS AND METHODS

Experiment

To evaluate four different levels of chitosan, including 0, 2, 4, and 6 ml/L at three different stages of growth, including the growing stage (S1: 45 days after sowing [DAS]), reproductive stage (S2: 65 DAS), and both (S1 + S2) on sweet basil cv. 213, the experiment was arranged in a randomized complete block design (RCBD) with four replications. The blocking was oriented against sunlight movement. Five plants were included in each replication, and the total number of observed plants was 240 (12 × 4 × 5). Furthermore, all 240 observations contributed to the data belonging to plant height, stem diameter, length of internode, number of branches, number of leaves, total root length, average root diameter, total root volume, total root surface area, and total fresh biomass.

Field Conditions and Activities

The experiment was performed in Field 15 of the research farm in the Faculty of Agriculture, Universiti Putra Malaysia. Seeds were purchased from Green World Genetics Sdn. Bhd., Malaysia. Seeds germinated in peat moss, and seedlings were produced under 50% shade and transplanted at 25 days old prior to 4 leaf stages. Plants grew in 14" polybags with a commercial soil mixture (Bio-soil, Melayu Impra™, Malaysia). Plants were oriented at 3,500 cm^2 planting density in open field conditions and treated with concentrations of 0, 2, 4, and 6 ml/L chitosan (KitosanPlus+, Malaysia) at the growing stage (S1, 45 DAS), reproductive stage (S2, 65 DAS), and both stages (S1 + S2). Finally, plants were harvested at 85 DAS.

Data Collection and Analysis

The physiological parameters such as plant height, stem diameter, and leaf number were collected prior to harvesting at 84-85 DAS in the field. Briefly, plant height was measured from the base form of the stem to the shoot tip using a ruler (100 cm stainless steel), and the average was taken (Hassnain et al., 2020). Stem diameter was measured by using an electronic digital calliper (Insize, Resolution: 0.01mm/0.0005", Accuracy: ± 0.2mm/0.1", Germany), and the average was taken. The internode length was measured using a ruler (30 cm), and the average was taken. Branches from the base

of the stem to the end were counted, and the average was taken for data on the number of branches. Leaves of the whole plant were counted, and the average was recorded as data on the number of leaves per plant. After this, plants were harvested and delivered to the laboratory for further investigation. Both shoot and root were weighed using a digital balance (Model B303-S, Mettler Toledo, Switzerland) and recorded as data of total fresh biomass. The root systems were detached from the stems and subjected to a root scanner (WinRHIZO Pro 2019a, Canada) for picture analysis. Data of root parameters such as total root length, average root diameter, total root volume, and total root surface area were taken from the output.

The data were analyzed using Statistical Analysis Software version 9.4 (SAS 9.4). Analysis of variance was performed using least significant differences (LSD) at 95% significant difference, and means were separated. Besides, Pearson correlation analysis was performed to evaluate the relationships between 10 parameters—the results of correlation analysis are presented in Table 1.

RESULTS

Plant Height

The results showed that plant height was significantly affected by the interaction of chitosan concentrations and plant maturity stages at ($p \leq 0.05$). Concentrations of 2 and 4 ml/L of chitosan applied at the vegetative stage had significantly increased plant height from 45.35 ± 2.59 to 55.08 ± 1.75 cm and decreased to 42.20 ± 2.23 cm at 6 ml/L (Figure 1). The non-treated chitosan plants produced shorter plant heights, where all the plants were less than 40 cm tall. However, applying chitosan at all concentrations in the reproductive stage was not significantly

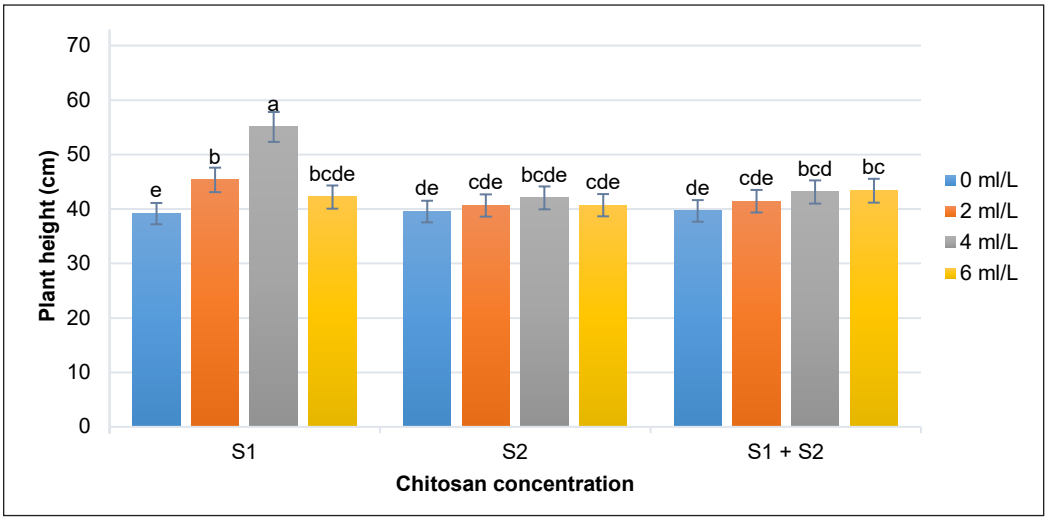

Figure 1. Effect of application of different chitosan concentrations in plant height of sweet basil. Means with the same letters are not significantly different at p ≤ 0.05, and the error bar shows the standard error
Note. S1 = 45 days after sowing; S2 = 65 days after sowing

different compared to the control. When chitosan was applied two times (vegetative and reproductive stage), only plants treated with 6 ml/L showed significantly taller compared to the control, which was 43.36 ± 1.12 cm. The differences in plant height can be seen in Figure 2.

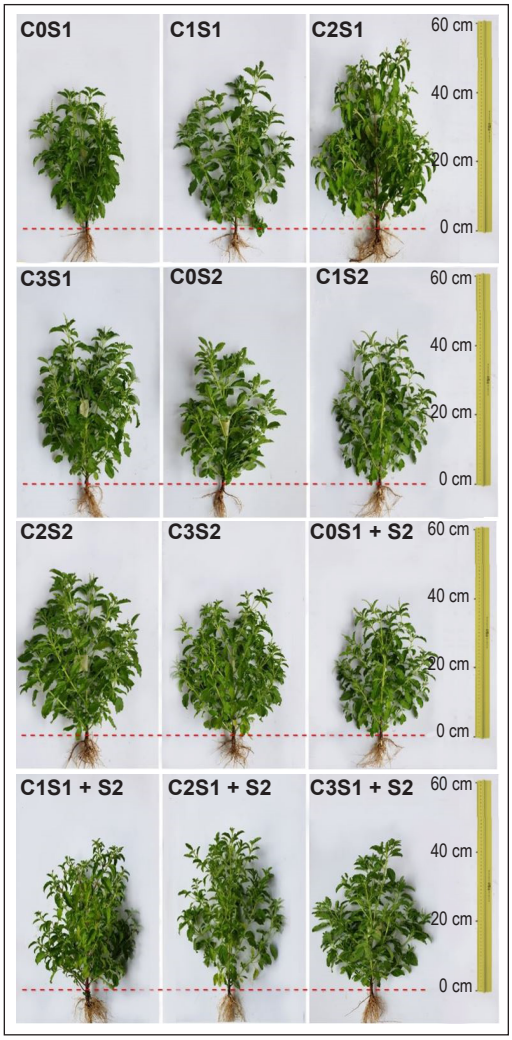

Figure 2. Representative sweet basil plants treated with different concentrations of chitosan. Where C0, C1, C2, and C3 are 0, 2, 4, and 6 ml/L chitosan, respectively
Note. S1 = 45 days after sowing; S2 = 65 days after sowing

Stem Diameter

The result showed that stem diameter was significantly affected by different concentrations of chitosan and the plant's maturity stage at $p \leq 0.5$. Plants treated with chitosan showed bigger diameters compared to non-treated plants (Figure 3). Single application of chitosan at vegetative stage increased the stem diameter from 10.04 ± 0.71 (2 ml/L of chitosan) to 11.08 ± 0.89 mm (4 ml/L of chitosan). A further increase to 6 ml/L of chitosan applied at the vegetative stage reduced the stem diameter to 9.02 ± 0.26 mm, which is still bigger than the control (8.06 ± 0.80 mm). Those plants were not significantly different from others that received 6 ml/L at both the reproductive and vegetative stages.

Applying chitosan at the reproductive stage showed all plants having less than 9 mm of stem diameter, including the control. However, those applied two times (at vegetative and reproductive stages) showed a stem diameter of more than 9 mm, with 4 ml/L recorded at 10.04 ± 0.70 mm of stem diameter, which was significantly bigger compared to other treatments at this time of application.

Length of Internode

The results showed that the internode length was significantly affected by the interaction of different concentrations of chitosan and plant maturity stages at $p \leq 0.05$. Applying chitosan at 4 ml/L at the vegetative stage significantly increased the internode length from 39.30 ± 4.30 (control) to 50.43 ± 4.30 mm per internode. It was the highest

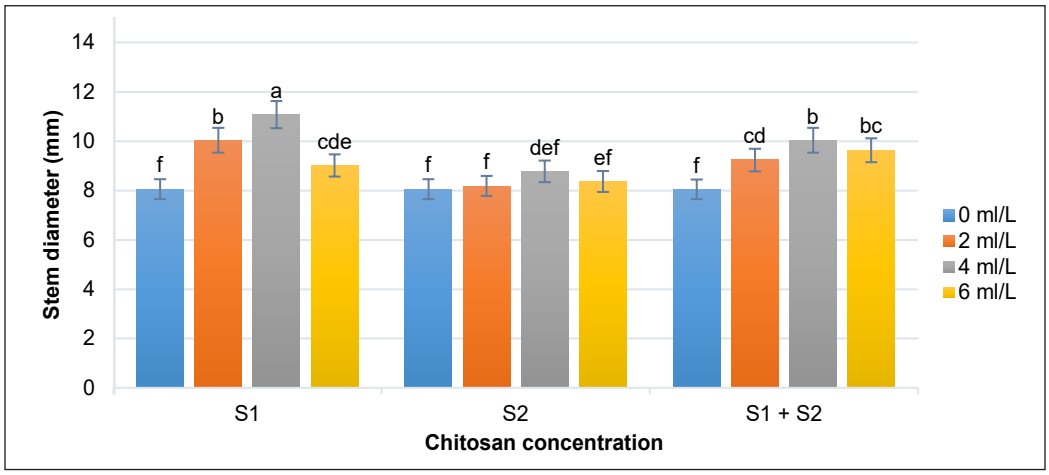

Figure 3. Effect of application of different chitosan concentrations in stem diameter of sweet basil
Note. Means with the same letters are not significantly different at $p \leq 0.05$, and the error bar shows the standard error. S1 = 45 days after sowing; S2 = 65 days after sowing

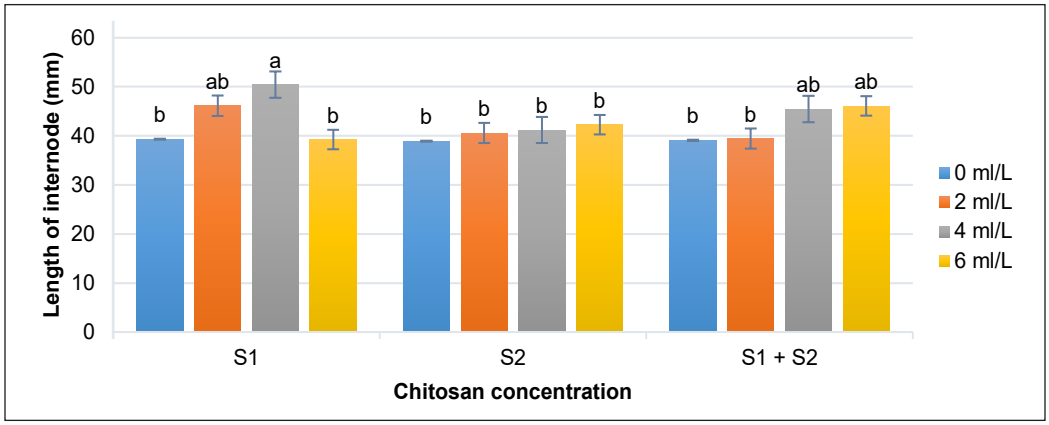

Figure 4. Effect of application of different chitosan concentrations in the length of internode of sweet basil
Note. Means with the same letters are not significantly different at $p \leq 0.05$, and the error bar shows the standard error. S1 = 45 days after sowing; S2 = 65 days after sowing

value recorded in this study's internode length. Twice application of chitosan does not effectively enhance the length of the internode (Figure 4).

Number of Branches

A number of branches were significantly affected by the interaction of chitosan concentrations and plant maturity stages at $p \leq 0.05$. Based on the results, plants treated with concentrations of 2, 4, and 6 ml/L chitosan in the vegetative stage produced 16.04 ± 0.77, 16.70 ± 0.21, and 15.16 ± 0.33 branches per plant, which are significantly higher compared to none treated plants (13.83 ± 0.96 branches). Concentrations of 2 and 4 ml/L chitosan applied on the reproductive stage showed similar results to the control,

while 6 ml/L chitosan resulted from a significantly higher number of branches (15.24 ± 0.95 branches per plant) compared to the control. Twice the application of chitosan, which was at vegetative and reproductive stages at 2 ml/L, showed similar results to control plants, where plants treated with concentrations of 4 and 6 ml/L chitosan showed a significantly higher number of branches by 15.36 ± 0.49 and 15.57 ± 0.83 branches per plant, respectively (Figure 5).

Number of Leaves

Results showed that number of leaves was significantly affected by the interaction of chitosan concentrations and plant maturity stages at $p \leq 0.05$. Treated plants with chitosan concentrations significantly increased the number of leaves compared to the control (Figure 6). From the result, the application of chitosan at the vegetative stage positively influenced the number of leaves per plant, followed by the frequent

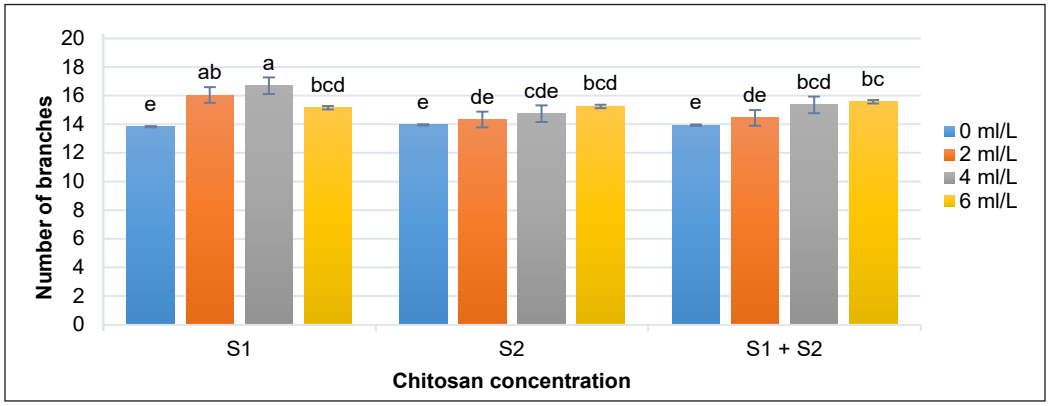

Figure 5. Effect of application of different chitosan concentrations in the number of branches of sweet basil
Note. Means with the same letters are not significantly different at $p \leq 0.05$, and the error bar shows the standard error. S1 = 45 days after sowing; S2 = 65 days after sowing

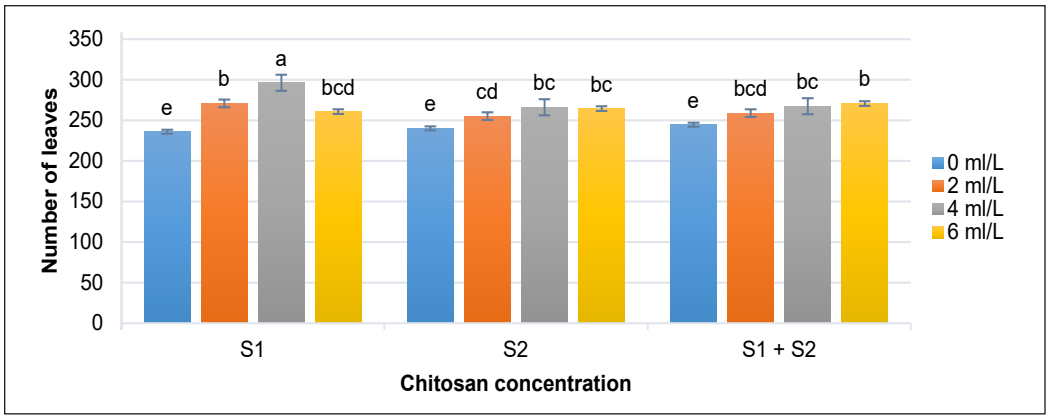

Figure 6. Effect of application of different chitosan concentrations in the number of leaves of sweet basil
Note. Means with the same letters are not significantly different at $p \leq 0.05$, and the error bar shows the standard error. S1 = 45 days after sowing; S2 = 65 days after sowing

application at vegetative and reproductive stages compared to at reproductive stage. Increasing the chitosan concentration from 2 to 4 ml/L increased the number of leaves from 270.90 ± 3.01 to 296.57 ± 11.61 per plant. Further increased to 6 ml/L showed a reduction in the number of leaves where the plants only produced 260.71 ± 8.85 leaves per plant. However, those in control showed fewer leaves produced, only 235.91 ± 10.17 per plant.

Applying chitosan at the reproductive stage showed no significant difference among different concentrations of chitosan. However, those plants were still producing a higher number of leaves compared to the control. The same pattern was also found when chitosan was applied at both vegetative and reproductive stages, where all chitosan-treated plants showed non-significant differences in the number of leaves but were still higher than the control.

Total Root Length

The underground part of sweet basil plants was also investigated. Results indicated that the interaction of different concentrations of chitosan with plant maturity stages significantly affected total root length at $p \leq 0.05$. Plants treated with chitosan with a single application at the vegetative stage and twice application at the vegetative and reproductive stages showed significantly higher root lengths compared to non-treated plants (Figure 7). Total root length was increased with the increase in chitosan concentration until it reached 4 ml/L (454.90 ± 16.35 cm) and reduced at 6 ml/L (405.26 ± 33.87 cm) for plants treated at the vegetative stage. However, control plants have only 339.02 ± 27.02 cm of total root length. It showed that chitosan effectively enhanced root growth and development in sweet basil. However, a different pattern was found when chitosan was applied at the reproductive stage of plant growth. All

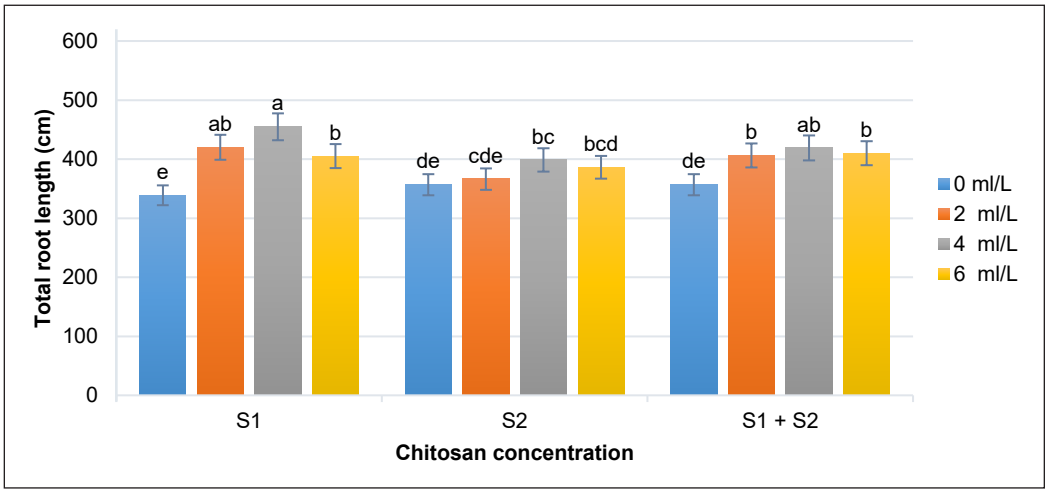

Figure 7. Effect of application of different chitosan concentrations in total root length of sweet basil
Note. Means with the same letters are not significantly different at $p \leq 0.05$, and the error bar shows the standard error. S1 = 45 days after sowing; S2 = 65 days after sowing

plants treated with a single application of chitosan at the reproductive stage showed no effectiveness in enhancing root growth in sweet basil, whereas all plants under this treatment showed a total root length of less than 400 cm. Thus, it is confirmed that chitosan can only help root development when applied at the vegetative stage and not at the reproductive stage. Furthermore, when chitosan was applied twice during the vegetative and reproductive stages, the total root length for all plants was more than 400 cm. Despite different concentrations being applied in this treatment, the root length was not significantly different from each other. The differences in root appearance as well as root length can be seen in (Figure 8).

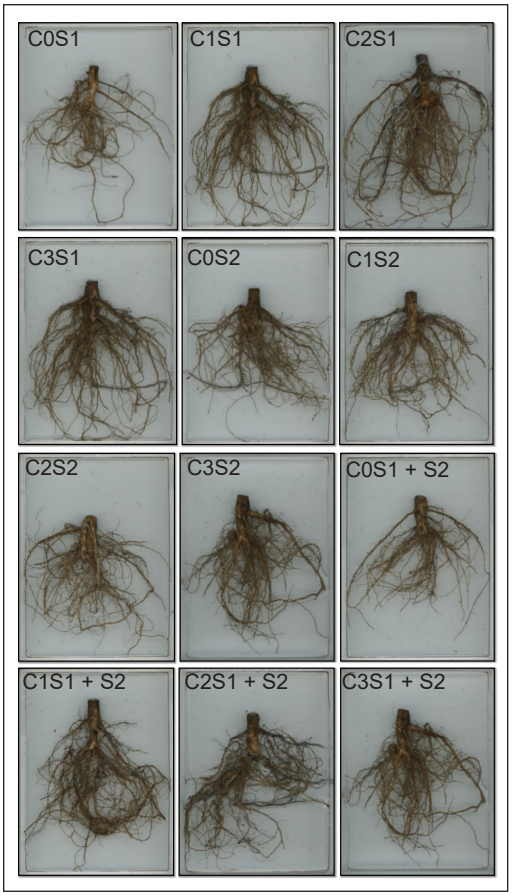

Figure 8. Roots of representative sweet basil plants treated with different concentrations of chitosan at different plant maturity stages in 15 cm × 20 cm containers. Where C0, C1, C2, and C3 are 0, 2, 4, and 6 ml/L chitosan, respectively

Note. S1 = 45 days after sowing; S2 = 65 days after sowing

Root Average Diameter

Root average diameter was investigated to qualify the root system of sweet basil plants. Results indicated that the interaction of chitosan concentrations and plant maturity stages at $p \leq 0.05$ significantly affected average root diameter. Chitosan-treated plants showed significantly higher average root diameters than non-treated plants, except those treated once at the reproductive stage.

Application of chitosan when plants are at the vegetative stage and concentrations of 2, 4, and 6 ml/L increased the average root diameter by 1.16 ± 0.01, 1.20 ± 0.01, and 1.15 ± 0.08 mm significantly, compared to control (1.05 ± 0.05 mm). Surprisingly, all plants treated with chitosan at the reproductive stage showed a similar average of root diameter to control. This study's finding proves that the time of application of chitosan is important to sweet basil, as it only influences root development, particularly the enlargement of the root when applied at the vegetative stage. The result from plants treated at vegetative and reproductive stages (two times) again proved this: the average root diameter increased by 1.16 ± 0.03, 1.13 ± 0.01, and

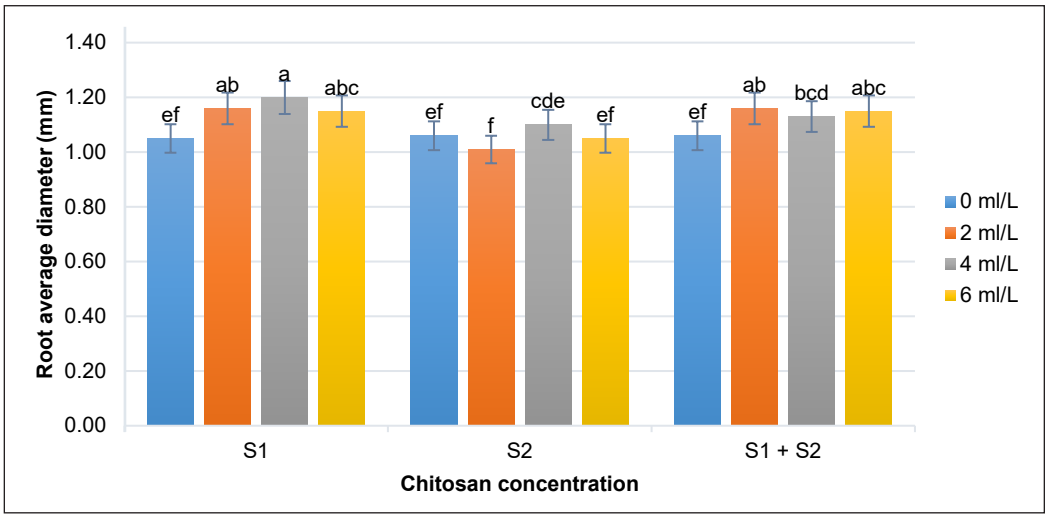

Figure 9. Effect of application of different chitosan concentrations in average root diameter of sweet basil
Note. Means with the same letters are not significantly different at $p \leq 0.05$, and the error bar shows the standard error. S1 = 45 days after sowing; S2 = 65 days after sowing

1.15 ± 0.02 mm, respectively, increase in concentrations compared to control (Figure 9). It is then assumed that the increase in diameter for those plants was caused by the application at the vegetative stage and not at the reproductive stage. Thus, a single application at the vegetative stage only is needed to enlarge the diameter of the sweet basil root.

Total Root Volume

In sweet basil plants, root volume was significantly and positively affected when plants were treated with different concentrations of chitosan at different plant maturity stages ($p \leq 0.05$). Treated plants showed greater root volume compared to non-treated plants, except those treated with 2 and 4 ml/L chitosan in the reproductive stage (Figure 10). Based on single-factor analysis, the difference between concentrations of chitosan was insignificant, where application at the vegetative stage showed a greater value of root volume compared to the application at the reproductive stage and both vegetative and reproductive stages. The interaction of both factors showed that the highest root volume (4.76 ± 0.34 and 5.09 ± 0.38 cm^3 per plant) were observed at concentrations of 2 ml/L and 4 ml/L, respectively, where higher concentration (6 ml/L) resulted in 4.02 ± 0.27 cm^3 per plant, which is still higher compared to control (3.13 ± 0.20 cm^3 per plant). Applying 6 ml/L chitosan during the reproductive stage significantly increased root volume from 3.16 ± 0.25 to 4.57 ± 0.54 cm^3, where there was no significant difference between 2 and 4 ml/L with control. When plants were treated two times on vegetative and reproductive stages with concentrations of 2, 4, and 6 ml/L chitosan, the root volume significantly increased by 4.17 ± 0.58, 4.66 ± 0.26, and 4.00 ± 0.14

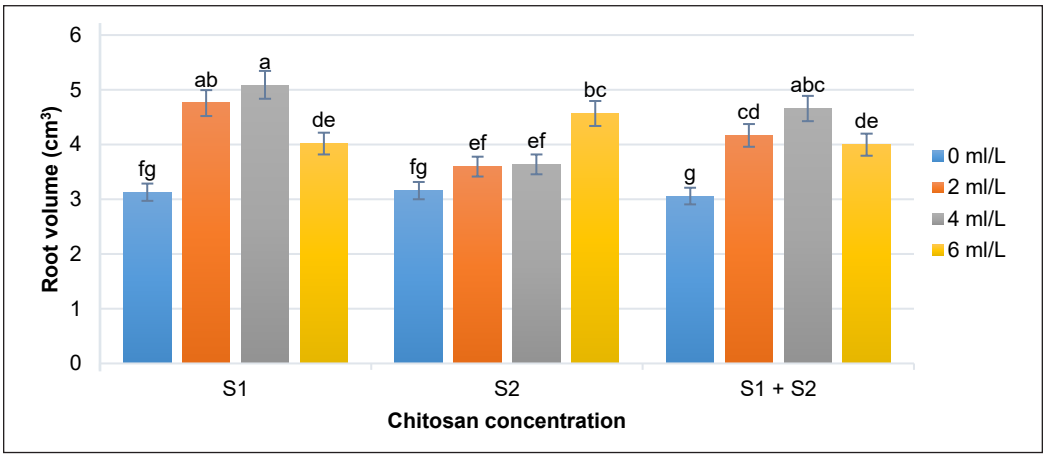

Figure 10. Effect of application of different chitosan concentrations in total root volume of sweet basil
Note. Means with the same letters are not significantly different at $p \leq 0.05$, and the error bar shows the standard error. S1 = 45 days after sowing; S2 = 65 days after sowing

cm^3, respectively compared to control. This result shows that the highest root volume is 62.62% greater than the control.

Total Root Surface Area

Analysis of variances for root surface area showed a significant difference in the interaction of chitosan concentrations and plant maturity stages at $p \leq 0.05$. Plants treated with chitosan showed significantly higher root surface area compared to non-treated plants, except for those treated with 2 ml/L at the reproductive stage (Figure 11). A concentration of 2 ml/L applied at the

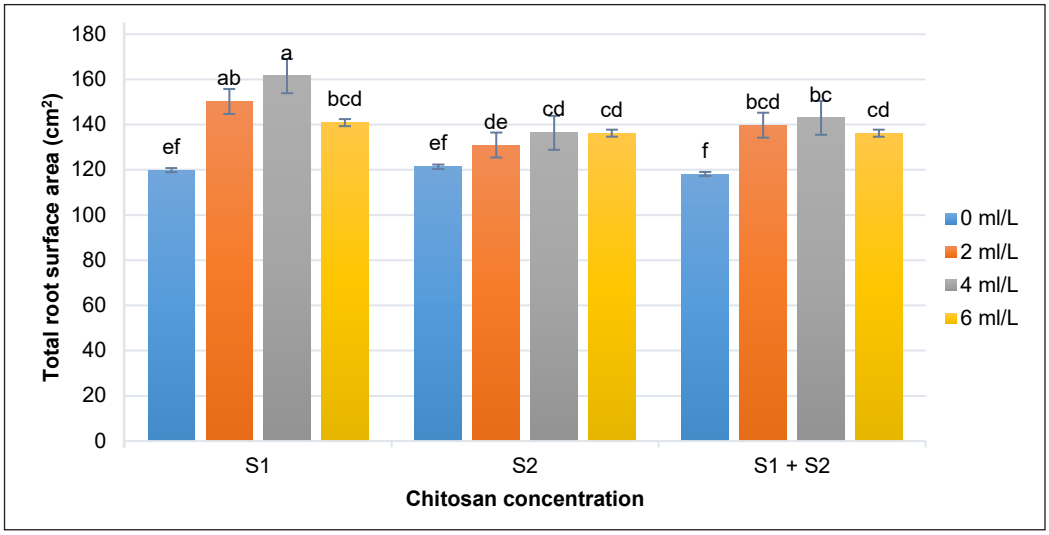

Figure 11. Effect of application of different chitosan concentrations in total root surface area of sweet basil
Note. Means with the same letters are not significantly different at $p \leq 0.05$, and the error bar shows the standard error. S1 = 45 days after sowing; S2 = 65 days after sowing

reproductive stage resulted in 130.97 ± 8.33 cm^2 of total root surface area, which was non-significant with control (121.32 ± 4.39 cm^2). At the same time, 4 and 6 ml/L chitosan concentrations showed significantly higher root surface area by 136.34 ± 13.54 and 136.19 ± 9.56 cm^2, respectively. Applying chitosan at both vegetative and reproductive stages seems ineffective, as all concentrations showed non-significantly differences with chitosan applied at the reproductive stage. The peak value of root surface area was found in those treated at the vegetative stage, where a concentration of 4 ml/L was 34.79% higher compared to the control. It showed that a specific chitosan concentration enhances the root surface area when applied at the correct plant's age.

Total Fresh Biomass

The data on total fresh biomass showed a significant difference at $p \leq 0.05$ between a combination of chitosan concentrations and plant maturity stages. All concentrations of chitosan applied at the vegetative stage showed greater value compared to the control (Figure 12). The highest total fresh biomass (109.49 ± 2.01 g per plant) was obtained when plants were treated with 4 ml/L at the vegetative stage, followed by the second highest (103.42 ± 2.59 g per plant) at 2 ml/L. A higher concentration of chitosan applied at the vegetative and reproductive stages or both vegetative and reproductive stages showed total fresh biomass ranging from 93.65 ± 5.31 to 94.85 ± 2.93 g per plant, still higher compared to the control. Those plants treated with low concentrations (2 or 4 ml/L) at the reproductive stage or both vegetative and reproductive stages showed total fresh biomass ranging from 89.43 ± 1.9 to 94.85 ± 2.9 g per plant, which are similar to the control.

Correlation Between Growth and Yield Components

The Pearson correlation analysis showed a significant positive correlation between

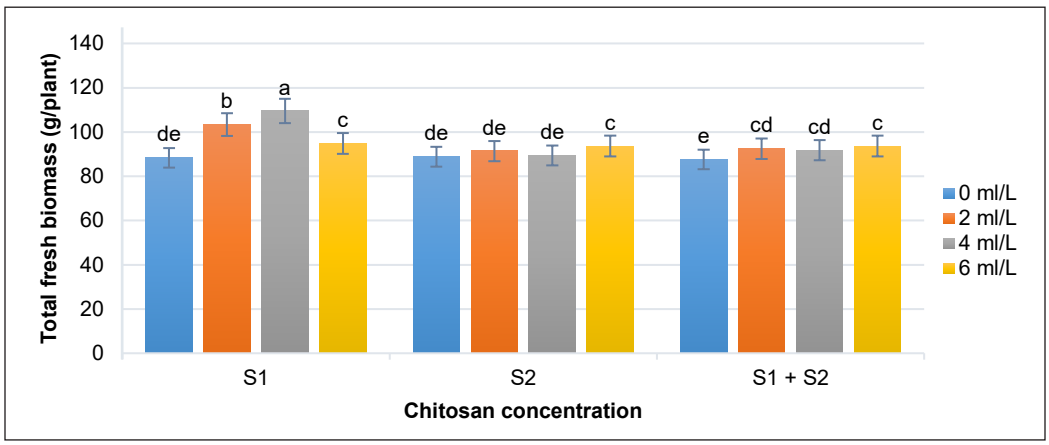

Figure 12. Effect of application of different chitosan concentrations in total fresh biomass of sweet basil
Note. Means with the same letters are not significantly different at $p \leq 0.05$, and the error bar shows the standard error. S1 = 45 days after sowing; S2 = 65 days after sowing

all growth parameters and biomass yield (Table 1). Based on the results, plant height has a strong positive and intermediate correlation with stem diameter and length of internode at r = 0.79 and r = 0.71, respectively. Similar to this, a positive intermediate correlation was found between plant height and the number of branches (r = 0.47). Thus, a positive intermediate correlation was found between the number of leaves with plant height and the number of branches at r = 0.54 and r = 0.58, respectively. In addition, total root length showed a positive intermediate correlation with plant height, stem diameter, length of internode, number of branches, and number of leaves at r = 0.63, 0.60, 0.58, 0.42, and 0.59, respectively. The total fresh biomass positively correlated with aerial parts such as plant height, stem diameter, length of internode, and the number of branches and leaves at r = 0.61, 0.77, 0.54, 0.62, and 0.68, respectively. Similarly, a significant and positive correlation was found between total fresh weight and the root parameters such as total root length, average root diameter, total root volume, and total root surface area at r = 0.66, 0.72, 0.70, and 0.57, respectively.

DISCUSSION

The results show that specific chitosan concentration acts differently according to the plant's growth stage in enhancing plant height. Besides, without a doubt, applying chitosan led to enhancing plant height. It agrees with Mukta et al. (2017), who found that a concentration of 250 mg/L chitosan was applied on *Fragaria* × *ananassa* Duch, produced the tallest plant height (19 cm) compared to the control, which was only 17.30 cm. Similarly, Rahman et al. (2018) reported that foliar application of chitosan at a concentration of 500 mg/L on *Fragaria*

Table 1
Correlation matrix between yield components of sweet basil

Variable	1	2	3	4	5	6	7	8	9	10
PH	1									
SD	0.79***	1								
LOI	0.71**	0.72**	1							
NOB	0.47**	0.55**	0.71**	1						
NOL	0.54**	0.65**	0.67**	0.58**	1					
TRL	0.63**	0.60**	0.58**	0.42*	0.59**	1				
RAD	0.71**	0.70**	0.59**	0.48*	0.69**	0.60**	1			
TRV	0.73**	0.74**	0.72**	0.61**	0.59**	0.76**	0.68**	1		
TRSA	0.61**	0.61**	0.71**	0.55**	0.59**	0.85***	0.47*	0.80***	1	
TFB	0.61**	0.77***	0.54**	0.62**	0.68**	0.66**	0.72**	0.70**	0.57**	1

Note. PH = Plant height; SD = Stem diameter; LOI = Length of internode; NOB = Number of branches; NOL = Number of leaves; TRL = Total root length; RAD = Root average diameter; TRV = Total root volume; TRSA = Total root surface area; TFB = Total fresh biomass
** and *** = Significant intermediate correlation and significant strong correlation, respectively

× *ananassa* Duch. plants resulted in higher plant height (25.10 cm) compared to the control (19.50 cm). The mechanism behind chitosan's effectiveness in increasing plant height is regulated by gibberellic acid inside the plant tissue (Lopez-Moya et al., 2019). Gibberellic acid can be found in all plants and is responsible for incrementing the number and length of cells in the plant's tissues (Guttridge & Thompson, 1959).

Similarly, the increment of stem diameter under the application of chitosan agrees with the previous reports of Choudhary et al. (2017), Dwyer et al. (1995), and Ullah et al. (2020), who confirmed the ability of chitosan to increase stem diameter. A bigger stem diameter can lead to a strong and stable stem, which can help the plants support a greater number of leaves and protect the plants from strong wind situations. Besides, stems with large diameters transport materials essential for plant growth and development as well as plant leaf biomass and contribute to biomass yield (Sun et al., 2019). Therefore, a bigger stem diameter in sweet basil is assumed to contribute to yield, which is leaf biomass.

Since chitosan regulates the accumulation of gibberellic acid inside the plants (Lopez-Moya et al., 2019), gibberellic acid was proven responsible for incrementing internode length (Mahmoody & Noori, 2014). This result agrees with Avestan et al. (2017), who reported the increment of internode length in chitosan-treated apple cuttings. This increment first appears in the length of internodes and then contributes to the increment of plant height (Atait & Qureshi, 2020; Brian, 1958; Wang et al., 2017).

Plants treated with 4 ml/L chitosan in the vegetative stage showed the best performance in relation to the number of branches. The increment of lateral branches under the application of chitosan agreed with Salehi et al. (2017), who reported the enhancement of a number of branches under the application of chitosan in *Satureja hortensis* plants. Similarly, Mondal et al. (2016) also stated that chitosan at a concentration of 100 mg/L can increase the number of branches in *Solanum lycopersicum* L. plants by 38.81%. In the case of sweet basil, a higher number of branches will bring benefits whereby it gives more surface for the leaves to develop and thus increasing its yield.

Also, chitosan enhanced the number of leaves in sweet basil plants. Similar to the results of the current study, early reports confirmed the increment of the number of leaves under the application of chitosan in *Curcuma longa* L., *Oryza sativa*, *Amaranthus hybridus*, *Hordeum vulgare*, and *Stevia rebaudiana* (Anusuya & Sathiyabama, 2016; Berliana et al., 2020; Divya et al., 2019; Hafez et al., 2020). For instance, chitosan at the rate of 75 ml/L applied as seed soaking increased the number of leaves from 14 to 16 per plant in *Capsicum frutescens* Linn. (Sari et al., 2020). Chitosan was claimed to be related to stimulating plant nutrition, where it has been reported that chitosan provides essential nutrients for plants (Sharif et al., 2018) to enhance plant growth and

development and produce leaves. Besides, soil application of chitosan was said to increase the absorption of essential nutrients for plants by increasing the number of beneficial soil microorganisms (Boonlertnirun et al., 2008). Water is assumed to transfer nutrients from the soil to different organs. Interestingly, chitosan increases water absorption in plants by increasing the growth and development of the root system (Hidangmayum et al., 2019). Thus, applying chitosan at the vegetative stage resulted in more leaves than in the reproductive stage. It is supported by Oosterhuis (1990), which stated that the early growing stage (after the seedling stage) is the time for *Gossypium hirsutum* plant to develop a leaf canopy. It is also assumed that plants are more active at an early growth stage and could easily respond to chitosan concentrations. At the late growing stage, plants may not absorb enough chitosan or cannot fully respond. However, more investigation is needed to clarify the mechanism of the physiological response of sweet basil plants to chitosan at different growth stages.

Since nitrogen is a mobile element in the soil and can be easily leached deeper, plants should provide long roots to search for this essential element. Therefore, reports say plants often produce long roots when facing nitrogen deficit only (Gruber et al., 2013). The results do not agree with this since the literature says that chitosan provides essential nutrition for plants as well as nitrogen elements (Xu & Mou, 2018). Thus, the results showed increased total root length because of chitosan treatment. It is supported by Sathiyabama and Parthasarathy (2016), who reported an increment of total root length to 31.25% in *Cicer arietinum* seedlings under the application of 0.10% chitosan. It is near the best treatment, resulting in a 34.18 % higher total root length in sweet basil. Similarly, Khan et al. (2011) observed an increment of total root length by 35% in *Arabidopsis thaliana* plants under the application of chitosan at a concentration of 10 µM. Furthermore, Iglesias et al. (2019) mentioned that applying chitosan could increase auxin accumulation in the plant's root system. At the same time, auxin is well-known as a root growth hormone (Tanimoto, 2005; Went, 1935). Therefore, the enhancement of root length in this study could be related to the potential of chitosan in stimulating the biosynthesis of auxin, which functioned in the root system. Longer root growth and development can help plants to reach more minerals to support their growth, besides increasing the anchoring of the plants to the soil, which in return is more stable.

From the results, sweet basil's growing stage was able to influence the effects of chitosan on the root system as well as total root length. The early growth stage was more efficient than the late growing stage for sweet basil plants to respond to treatment applications. Although, there was no significant difference between the vegetative and at both vegetative and reproductive stages of growing to apply chitosan on sweet basil plants based on single factor analysis.

The application of chitosan at reproductive phase has no effect on the root development, particularly on the root length. Furthermore, the effectiveness of chitosan at frequent application could be mostly related to the effectiveness at the vegetative stage. It is well supported by Ljung et al. (2001), which declared that auxin as root growth hormone could be synthesized at a maximum rate when the plant is at the early growth stage. To interoperate this, at the early growing stage of sweet basil plants, chitosan can improve the accumulation of auxin, where this hormone optimizes its function and increases root growth as well as prolonging of roots.

Root diameter is another important parameter of the root system (Bouma et al., 2000). Plants with thicker root diameters could survive longer and easily (Baddeley & Watson, 2005). It could be helpful for plants in finding water and nutrient elements from the soil. In addition, roots with higher diameters can absorb and transport a large amount of water and nutrient solution over the plants (Hutchings & John, 2003). Therefore, the thick root diameter in the root system could contribute to the overall growth of the plant canopy. Root diameter was improved due to applying chitosan at the vegetative stage. The result from this study agrees with Khan et al. (2011), who reported an increment of root diameter under the application of chitosan at a concentration of 100 µM chitosan in *A. thaliana* plants. The increment in average root diameter could be related to the potential of chitosan in manipulating auxin in the root system (Iglesias et al., 2019).

According to Davis and Jacobs (2005), root volume is an important parameter in the quality assessment of the root system. The higher root volume is said to provide larger contact with soil and a chance for more nutrient uptake by the plant (Haase & Rose, 1994). The root length, root diameter, and density of the roots contribute data for the root volume. Based on the results of this study, it was proven that the drench application of chitosan at a suitable stage could increase root volume in sweet basil plants. The increment of root volume is directly linked to the enhancement of nutrient uptake by the plants (Marschener, 1998). At the same time, Farouk et al. (2011) reported a positive correlation between root volume and nutrient uptake in *Raphanus sativus* L. var. *sativus* plants. Hence, an increment in root volume may contribute to growth and development.

Chitosan could also increase root surface area, which agreed with Guo et al. (2020), who reported the increment of root surface area in *Eleutherococcus senticosus*. According to Tagliavini et al. (1993), the increment of root surface area was influenced by the increment of root length and average diameter. Moreover, the effectiveness of water and nutrient uptake from the soil was attributed to the large root surface area (Marschener, 1998; Yang et al., 2009). For instance, Genc et al. (2007) reported that the increment of root surface area had positively influenced zinc uptake by *Hordeum vulgare* plants. In trees such as *Larix gmelinii*, the increment of leaf biomass was related to the increment in root

surface area (Meng et al., 2018). Therefore, as the application of chitosan increased the root surface area in this study, it has been proven beneficial to sweet basil.

The optimum total fresh biomass yield per plant (109.49 ± 2.01 g) obtained in this study is 23.93% higher than control plants, equal to 21.14 g per plant. Normally, the conventional cultivation density of sweet basil is 140,000 plants/ha (Guerrero-Lagunes et al., 2020). Considering this, treating sweet basil plants with 4 ml/L chitosan will increase the biomass yield by 2959.60 kg/ha. It could be a great contribution to the producers. In sweet basil plants, it is assumed that the yield is associated with growth and development, and several parameters may contribute to the increment of total fresh biomass. However, the increment in yield of sweet basil, as well as total fresh biomass due to chitosan, which was applied at the vegetative stage, agrees with the previous report by Mondal et al. (2011), who confirmed the increment of fresh biomass in *Basella alba* L. plants under 75 mg/L at the early stage of growth. It is 21.56% higher when compared with the control.

From the results of correlation analysis, plant height has a strong positive correlation and a positive intermediate correlation with stem diameter and length of the internode. It is assumed that a big stem could provide sufficient water and organic materials transportation to allow the plant to grow higher (Sun et al., 2019). Furthermore, the stem cell enlargement led to an increase in the internode length, which directly influences plant height. A positive intermediate correlation exists between plant height and the number of branches. In sweet basil, branches are produced from the nodes, which proves that in addition to the length of the internode, the number of nodes also contributes to the height of sweet basil plants. A positive intermediate correlation of the number of leaves with plant height and the number of branches revealed that the increment of plant height and the number of branches provided a larger surface for the development of leaves through the canopy of sweet basil plants. In addition, there is notably a relationship between sweet basil plants' underground and aerial parts. In plants, to be facilitated growth and development, it is assumed that the root system provides mechanical support, water, and essential nutrients for the shoot to produce organic materials through photosynthesis and distribute overall the plant. Thus, the previous report confirmed a positive correlation between shoots and roots in *A. thaliana* plants (Bouteillé et al., 2012). Considering this, all the aerial and underground parameters strongly contributed to yield performance. The current study's finding proves it, whereas the total fresh biomass had a significantly positive correlation with both aerial and underground parts in sweet basil plants.

CONCLUSION

A field experiment was carried out to evaluate the performance of sweet basil under the application of chitosan at different plant maturity stages. Results showed

that application of chitosan improved growth parameters, contributing to yield performance. Based on the findings, sweet basil plants should be treated with chitosan at one time at the vegetative stage compared to the reproductive stage or both. Therefore, it is recommended for sweet basil plants be treated with 4 ml/L chitosan at the vegetative stage. The use of commercial growing media could be a limitation of this study, and minor differences may occur if growers use field soil. Future study is needed to investigate phytochemicals as well as their postharvest quality.

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REFERENCES

Acemi, A. (2020). Chitosan versus plant growth regulators: A comparative analysis of their effects on *in vitro* development of *Serapias vomeracea* (Burm. f.) Briq. *Plant Cell, Tissue and Organ Culture*, *141*, 327-338. https://doi.org/10.1007/s11240-020-01789-3

Acemi, A., Bayrak, B., Çakır, M., Demiryürek, E., Gün, E., El Gueddari, N. E., & Özen, F. (2018). Comparative analysis of the effects of chitosan and common plant growth regulators on *in vitro* propagation of *Ipomoea purpurea* (L.) Roth from nodal explants. *In Vitro Cellular & Developmental Biology-Plant*, *54*, 537-544. https://doi.org/10.1007/s11627-018-9915-0

Acharya, T. P., Reiter, M. S., Welbaum, G., & Arancibia, R. A. (2020). Nitrogen uptake and use efficiency in sweet basil production under low tunnels. *HortScience*, *55*(4), 429-435. https://doi.org/10.21273/HORTSCI14515-19

Anusuya, S., & Sathiyabama, M. (2016). Effect of chitosan on growth, yield and curcumin content in turmeric under field condition. *Biocatalysis and Agricultural Biotechnology*, *6*, 102-106. https://doi.org/10.1016/j.bcab.2016.03.002

Atait, M., & Qureshi, U. S. (2020). Efficacy of different primers on growth and yield of tulip (*Tulipa gesneriana* L.). *World Journal of Biology and Biotechnology*, *5*(2), 31-35.

Avestan, S., Naseri, L., & Barker, A. V. (2017). Evaluation of nanosilicon dioxide and chitosan on tissue culture of apple under agar-induced osmotic stress. *Journal of Plant Nutrition*, *40*(20), 2797-2807. https://doi.org/10.1080/01904167.2017.1382526

Baddeley, J. A., & Watson, C. A. (2005). Influences of root diameter, tree age, soil depth and season on fine root survivorship in *Prunus avium*. *Plant and Soil*, *276*, 15-22. https://doi.org/10.1007/s11104-005-0263-6

Berliana, A. I., Kuswandari, C. D., Retmana, B. P., Putrika, A., & Purbaningsih, S. (2020). Analysis of the potential application of chitosan to improve vegetative growth and reduce transpiration rate in *Amaranthus hybridus*. In *IOP Conference Series: Earth and Environmental Science* (Vol. 481, No. 1, p. 012021). IOP Publishing. https://doi.org/10.1088/1755-1315/481/1/012021

Boonlertnirun, S., Boonraung, C., & Suvanasara, R. (2008). Application of chitosan in rice production. *Journal of Metals, Materials and Minerals*, *18*(2), 47-52.

Bouma, T. J., Nielsen, K. L., & Koutstaal, B. A. S. (2000). Sample preparation and scanning protocol for computerised analysis of root length and diameter. *Plant and Soil*, *218*, 185-196. https://doi.org/10.1023/A:1014905104017

Bouteillé, M., Rolland, G., Balsera, C., Loudet, O., & Muller, B. (2012). Disentangling the intertwined genetic bases of root and shoot growth in *Arabidopsis*. *PLOS One*, *7*(2), e32319. https://doi.org/10.1371/journal.pone.0032319

Brian, P. W. (1958). Gibberellic acid: A new plant hormone controlling growth and flowering. *Journal of the Royal Society of Arts*, *106*(5022), 425-441.

Bufalo, J., Cantrell, C. L., Astatkie, T., Zheljazkov, V. D., Gawde, A., & Boaro, C. S. F. (2015). Organic versus conventional fertilization effects on sweet basil (*Ocimum basilicum* L.) growth in a greenhouse system. *Industrial Crops and Products*, *74*, 249-254. https://doi.org/10.1016/j.indcrop.2015.04.032

Chamnanmanoontham, N., Pongprayoon, W., Pichayangkura, R., Roytrakul, S., & Chadchawan, S. (2015). Chitosan enhances rice seedling growth via gene expression network between nucleus and chloroplast. *Plant Growth Regulation*, *75*, 101-114. https://doi.org/10.1007/s10725-014-9935-7

Choudhary, R. C., Kumaraswamy, R. V., Kumari, S., Sharma, S. S., Pal, A., Raliya, R., Biswas, P., & Saharan, V. (2017). Cu-chitosan nanoparticle boost defense responses and plant growth in maize (*Zea mays* L.). *Scientific Reports*, *7*, 9754. https://doi.org/10.1038/s41598-017-08571-0

Ciriello, M., Formisano, L., El-Nakhel, C., Corrado, G., Pannico, A., De Pascale, S., & Rouphael, Y. (2021). Morpho-physiological responses and secondary metabolites modulation by pre-harvest factors of three hydroponically grown Genovese basil cultivars. *Frontiers in Plant Science*, *12*, 671026. https://doi.org/10.3389/fpls.2021.671026

Corrado, G., Chiaiese, P., Lucini, L., Miras-Moreno, B., Colla, G., & Rouphael, Y. (2020). Successive harvests affect yield, quality and metabolic profile of sweet basil (*Ocimum basilicum* L.). *Agronomy*, *10*(6), 830. https://doi.org/10.3390/agronomy10060830

Davis, A. S., & Jacobs, D. F. (2005). Quantifying root system quality of nursery seedlings and relationship to out planting performance. *New Forests*, *30*(2), 295-311. https://doi.org/10.1007/s11056-005-7480-y

Delbeke, S., Ceuppens, S., Jacxsens, L., & Uyttendaele, M. (2015). Microbiological analysis of pre-packed sweet basil (*Ocimum basilicum*) and coriander (*Coriandrum sativum*) leaves for the presence of *Salmonella* spp. and Shiga toxin-producing *E. coli*. *International Journal of Food Microbiology*, *208*, 11-18. https://doi.org/10.1016/j.ijfoodmicro.2015.05.009

Divya, K., Vijayan, S., Nair, S. J., & Jisha, M. S. (2019). Optimization of chitosan nanoparticle synthesis and its potential application as germination elicitor of *Oryza sativa* L. *International Journal of Biological Macromolecules*, *124*, 1053-1059. https://doi.org/10.1016/j.ijbiomac.2018.11.185

Dwyer, P. J., Bannister, P., & Jameson, P. E. (1995). Effects of three plant growth regulators on growth, morphology, water relations, and frost resistance in lemonwood (*Pittosporum eugenioides* A. Cunn). *New Zealand Journal of Botany*, *33*(3), 415-424. https://doi.org/10.1080/0028825X.1995.10412968

El-Amerany, F., Rhazi, M., Wahbi, S., Taourirte, M., & Meddich, A. (2020). The effect of chitosan, *Arbuscular mycorrhizal* fungi, and compost applied individually or in combination on growth, nutrient uptake, and stem anatomy of tomato. *Scientia Horticulturae*, *261*, 109015. https://doi.org/10.1016/j.scienta.2019.109015

Elhindi, K. M., Al-Amri, S. M., Abdel-Salam, E. M., & Al-Suhaibani, N. A. (2017). Effectiveness of salicylic acid in mitigating salt-induced adverse effects on different physio-biochemical attributes in sweet basil (*Ocimum basilicum* L.). *Journal*

of Plant Nutrition, *40*(6), 908-919. https://doi.org/10.1080/01904167.2016.1270311

Fahmy, A. A., & Nosir, W. S. (2021). Influence of chitosan and micronutrients (Fe + Zn) concentrations on growth, yield components and volatile oil of lavender plant. *Scientific Journal of Flowers and Ornamental Plants*, *8*(1), 87-100. https://doi.org/10.21608/sjfop.2021.155941

Farouk, S., Mosa, A. A., Taha, A. A., & El-Gahmery, A. M. (2011). Protective effect of humic acid and chitosan on radish (*Raphanus sativus* L. var. *sativus*) plants subjected to cadmium stress. *Journal of Stress Physiology and Biochemistry*, *7*(2), 99-116.

Fattahi, B., Arzani, K., Souri, M. K., & Barzegar, M. (2019). Effects of cadmium and lead on seed germination, morphological traits, and essential oil composition of sweet basil (*Ocimum basilicum* L.). *Industrial Crops and Products*, *138*, 111584. https://doi.org/10.1016/j.indcrop.2019.111584

Genc, Y., Huang, C. Y., & Langridge, P. (2007). A study of the role of root morphological traits in growth of barley in zinc-deficient soil. *Journal of Experimental Botany*, *58*(11), 2775-2784. https://doi.org/10.1093/jxb/erm142

Ghasemzadeh, A., Ashkani, S., Baghdadi, A., Pazoki, A., Jaafar, H. Z., & Rahmat, A. (2016). Improvement in flavonoids and phenolic acids production and pharmaceutical quality of sweet basil (*Ocimum basilicum* L.) by ultraviolet-B irradiation. *Molecules*, *21*(9), 1203. https://doi.org/10.3390/molecules21091203

Goudarzian, A., Pirbalouti, A. G., & Hossaynzadeh, M. (2020). Menthol, balance of menthol/menthone, and essential oil contents of *Mentha* × *Piperita* L. under foliar-applied chitosan and inoculation of *Arbuscular mycorrhizal* fungi. *Journal of Essential Oil Bearing Plants*, *23*(5), 1012-1021. https://doi.org/10.1080/0972060X.2020.1828177

Govindaraju, S., & Arulselvi, P. I. (2018). Effect of cytokinin combined elicitors (l-phenylalanine, salicylic acid and chitosan) on *in vitro* propagation, secondary metabolites and molecular characterization of medicinal herb *Coleus aromaticus* Benth (L). *Journal of the Saudi Society of Agricultural Sciences*, *17*(4), 435-444. https://doi.org/10.1016/j.jssas.2016.11.001

Gruber, B. D., Giehl, R. F., Friedel, S., & von Wirén, N. (2013). Plasticity of the *Arabidopsis* root system under nutrient deficiencies. *Plant Physiology*, *163*(1), 161-179. https://doi.org/10.1104/pp.113.218453

Guerrero-Lagunes, L. A., Ruiz-Posadas, L. M., Rodríguez-Mendoza, M. N., & Soto-Hernández, M. (2020). Quality and yield of basil (*Ocimum basilicum* L.) essential oil under hydroponic cultivation. *Agro Productividad*, *13*(9), 89-94. https://doi.org/10.32854/agrop.vi.1616

Guo, S., Zhang, S., Jia, L., Xu, M., & Wang, Z. (2020). Root growth of eleuthero (*Eleutherococcus senticosus* [Rupr. & Maxim.] Maxim.) seedlings cultured with chitosan oligosaccharide addition under different light spectra. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *48*(2), 626-635. https://doi.org/10.15835/nbha48211634

Guttridge, C. G., & Thompson, P. A. (1959). Effect of gibberellic acid on length and number of epidermal cells in petioles of strawberry. *Nature*, *183*, 197-198. https://doi.org/10.1038/183197b0

Haase, D. L., & Rose, R. (1994). Effects of soil water content and initial root volume on the nutrient status of 2+0 Douglas-fir seedlings. *New Forests*, *8*, 265-277. https://doi.org/10.1007/BF00025372

Hafez, Y., Attia, K., Alamery, S., Ghazy, A., Al-Doss, A., Ibrahim, E., Rashwan, E., El-Maghraby, L., Awad, A., & Abdelaal, K. (2020). Beneficial effects of biochar and chitosan on antioxidative capacity, osmolytes accumulation, and anatomical characters of water-stressed barley

plants. *Agronomy*, *10*(5), 630. https://doi.org/10.3390/agronomy10050630

Hanachi, P., Fakhrnezhad, F. R., Zarringhalami, R., & Orhan, I. E. (2021). Cytotoxicity of *Ocimum basilicum* and *Impatiens walleriana* extracts on AGS and SKOV-3 cancer cell lines by flow cytometry analysis. *International Journal of Cancer Management*, *14*(3), e102610. https://doi.org/10.5812/ijcm.102610

Harnafi, H., Ramchoun, M., Tits, M., Wauters, J. N., Frederich, M., Angenot, L., Aziz, M., Alem, C., & Amrani, S. (2013). Phenolic acid-rich extract of sweet basil restores cholesterol and triglycerides metabolism in high fat diet-fed mice: A comparison with fenofibrate. *Biomedicine and Preventive Nutrition*, *3*(4), 393-397. https://doi.org/10.1016/j.bionut.2013.03.005

Hassnain, M., Alam, I., Ahmad, A., Basit, I., Ullah, N., Alam, I., Ullah, I., Khalid, M. A., Shair, M., & Ain, N. (2020). Efficacy of chitosan on performance of tomato (*Lycopersicon esculentum* L.) plant under water stress condition. *Pakistan Journal of Agricultural Research*, *33*(1), 27-41. https://doi.org/10.17582/journal.pjar/2020/33.1.27.41

Heidari, J., Amooaghaie, R., & Kiani, S. (2020). Impact of chitosan on nickel bioavailability in soil, the accumulation and tolerance of nickel in *Calendula tripterocarpa*. *International Journal of Phytoremediation*, *22*(11), 1175-1184. https://doi.org/10.1080/15226514.2020.1748564

Hidangmayum, A., Dwivedi, P., Katiyar, D., & Hemantaranjan, A. (2019). Application of chitosan on plant responses with special reference to abiotic stress. *Physiology and Molecular Biology of Plants*, *25*(2), 313-326. https://doi.org/10.1007/s12298-018-0633-1

Hutchings, M. J., & John, E. A. (2003). Distribution of roots in soil, and root foraging activity. In H. de Kroon & E. J. W. Visser (Eds.), *Root ecology* (Vol. 168, pp. 33-60). Springer. https://doi.org/10.1007/978-3-662-09784-7_2

Ibrahim, M. F. (2020). The role of vermicompost and chitosan nanoparticles as foliar application to enhancing growth, yield and oil of black cumin (*Nigella sativa* L.) plants. *Archives of Agriculture Sciences Journal*, *3*(2), 205-223. https://doi.org/10.21608/aasj.2020.178054

Iglesias, M. J., Colman, S. L., Terrile, M. C., Paris, R., Martín-Saldaña, S., Chevalier, A. A., Álvarez, V. A., & Casalongué, C. A. (2019). Enhanced properties of chitosan microparticles over bulk chitosan on the modulation of the auxin signaling pathway with beneficial impacts on root architecture in plants. *Journal of Agricultural and Food Chemistry*, *67*(25), 6911-6920. https://doi.org/10.1021/acs.jafc.9b00907

Incrocci, L., Carmassi, G., Maggini, R., Poli, C., Saidov, D., Tamburini, C., Kiferle, C., Perata, P., & Pardossi, A. (2019). Iodine accumulation and tolerance in sweet basil (*Ocimum basilicum* L.) with green or purple leaves grown in floating system technique. *Frontiers in Plant Science*, *10*, 1494. https://doi.org/10.3389/fpls.2019.01494

Jiao, J., Gai, Q.-Y., Wang, X., Qin, Q. P., Wang, Z.-Y., Liu, J., & Fu, Y.-J. (2018). Chitosan elicitation of *Isatis tinctoria* L. hairy root cultures for enhancing flavonoid productivity and gene expression and related antioxidant activity. *Industrial Crops and Products*, *124*, 28-35. https://doi.org/10.1016/j.indcrop.2018.07.056

Khan, W., Costa, C., Souleimanov, A., Prithiviraj, B., & Smith, D. L. (2011). Response of *Arabidopsis thaliana* roots to lipo-chitooligosaccharide from *Bradyrhizobium japonicum* and other chitin-like compounds. *Plant Growth Regulation*, *63*(3), 243-249. https://doi.org/10.1007/s10725-010-9521-6

Klintham, P., Tongchitpakdee, S., Chinsirikul, W., & Mahakarnchanakul, W. (2018). Two-step washing with commercial vegetable washing solutions, and electrolyzed oxidizing microbubbles water to decontaminate sweet basil and Thai mint: A

case study. *Food Control*, *94*, 324-330. https://doi.org/10.1016/j.foodcont.2018.07.025

Ljung, K., Bhalerao, R. P., & Sandberg, G. (2001). Sites and homeostatic control of auxin biosynthesis in *Arabidopsis* during vegetative growth. *The Plant Journal*, *28*(4), 465-474. https://doi.org/10.1046/j.1365-313X.2001.01173.x

Lopez-Moya, F., Suarez-Fernandez, M., & Lopez-Llorca, L. V. (2019). Molecular mechanisms of chitosan interactions with fungi and plants. *International Journal of Molecular Sciences*, *20*(2), 332. https://doi.org/10.3390/ijms20020332

Mahmoody, M., & Noori, M. (2014). Effect of gibberellic acid on growth and development plants and its relationship with abiotic stress. *International Journal of Farming and Allied Sciences*, *3*, 717-721.

Marschener, H. (1998). Role of root growth, *Arbuscular mycorrhiza*, and root exudates for the efficiency in nutrient acquisition. *Field Crops Research*, *56*(1-2), 203-207. https://doi.org/10.1016/S0378-4290(97)00131-7

Meng, S., Jia, Q., Zhou, G., Zhou, H., Liu, Q., & Yu, J. (2018). Fine root biomass and its relationship with aboveground traits of *Larix gmelinii* trees in Northeastern China. *Forests*, *9*(1), 35. https://doi.org/10.3390/f9010035

Mirzajani, Z., Hadavi, E., & Kashi, A. (2015). Changes in the essential oil content and selected traits of sweet basil (*Ocimum basilicum* L.) as induced by foliar sprays of citric acid and salicylic acid. *Industrial Crops and Products*, *76*, 269-274. https://doi.org/10.1016/j.indcrop.2015.06.052

Mondal, M. M. A., Rana, M. I. K., Dafader, N. C., & Haque, M. E. (2011). Effect of foliar application of chitosan on growth and yield in Indian spinach. *Journal of Agroforestry and Environment*, *5*(1), 99-102.

Mondal, M., Puteh, A. B., & Dafader, N. C. (2016). Foliar application of chitosan improved morphophysiological attributes and yield in summer tomato (*Solanum lycopersicum*). *Pakistan Journal of Agricultural Sciences*, *53*(2), 339-344.

Monfared, B. B., Noormohamadi, G., Rad, A. H. S., & Hervan, E. M. (2020). Effects of sowing date and chitosan on some characters of canola (*Brassica napus* L.) genotypes. *Journal of Crop Science and Biotechnology*, *23*, 65-71. https://doi.org/10.1007/s12892-019-0177-0

Mosadegh, H., Trivellini, A., Maggini, R., Ferrante, A., Incrocci, L., & Mensuali, A. (2021). *In-vivo in-vitro* screening of *Ocimum basilicum* L. ecotypes with differential UV-B radiation sensitivity. *Horticulturae*, *7*(5), 101. https://doi.org/10.3390/horticulturae7050101

Mukta, J. A., Rahman, M., Sabir, A. A., Gupta, D. R., Surovy, M. Z., Rahman, M., & Islam, M. T. (2017). Chitosan and plant probiotics application enhance growth and yield of strawberry. *Biocatalysis and Agricultural Biotechnology*, *11*, 9-18. https://doi.org/10.1016/j.bcab.2017.05.005

Oosterhuis, D. M. (1990). Growth and development of a cotton plant. In W. N. Miley & D. M. Oosterhuis (Eds.), *Nitrogen nutrition of cotton*: *Practical issues* (pp. 1-24). American Society of Agronomy. https://doi.org/10.2134/1990.nitrogennutritionofcotton.c1

Osei-Akoto, C., Acheampong, A., Boakye, Y. D., Naazo, A. A., & Adomah, D. H. (2020). Anti-inflammatory, antioxidant, and anthelmintic activities of *Ocimum basilicum* (sweet basil) fruits. *Journal of Chemistry*, *2020*, 2153534. https://doi.org/10.1155/2020/2153534

Pandey, P., Singh, S., & Banerjee, S. (2019). *Ocimum basilicum* suspension culture as resource for bioactive triterpenoids: Yield enrichment by elicitation and bioreactor cultivation. *Plant Cell,*

Tissue and Organ Culture, *137*, 65-75. https://doi.org/10.1007/s11240-018-01552-9

Patriani, P., Hellyward, J., Hafid, H., & Apsari, N. L. (2021). Application of sweet basil (*Ocimum basilicum*) on physical and organoleptic qualities of chicken meatballs. In *IOP Conference Series: Earth and Environmental Science* (Vol. 782, No. 2, p. 022083). IOP Publishing. https://doi.org/10.1088/1755-1315/782/2/022083

Rahman, M., Mukta, J. A., Sabir, A. A., Gupta, D. R., Mohi-Ud-Din, M., Hasanuzzaman, M., Miah, G., Rahman, M., & Islam, M. T. (2018). Chitosan biopolymer promotes yield and stimulates accumulation of antioxidants in strawberry fruit. *PLOS One*, *13*(9), e0203769. https://doi.org/10.1371/journal.pone.0203769

Salehi, S., Rezayatmand, Z., & Pirbalouri, A. G. (2017). The effect of foliar application of chitosan on yield and essential oil of savory (*Satureja isophylla* L.) under salt stress. *Journal of Medicinal Herbs*, *8*(2), 101-108.

Sari, S. G., Selvia, E., Nisa, C., & Junaidi, A. B. (2020). Pengaruh pemberian komposit kitosan asap cair terhadap pertumbuhan cabai rawit merah *Capsicum frutescens* Linn. [The effect of liquid smoke and chitosan composition on the growth of red cayenne pepper *Capsicum frutescens* Linn.]. *Biotropika Journal of Tropical Biology*, *8*(1), 8-12. https://doi.org/10.21776/ub.biotropika.2020.008.01.02

Sathiyabama, M., & Parthasarathy, R. (2016). Biological preparation of chitosan nanoparticles and its *in vitro* antifungal efficacy against some phytopathogenic fungi. *Carbohydrate Polymers*, *151*, 321-325. https://doi.org/10.1016/j.carbpol.2016.05.033

Scagel, C. F., Lee, J., & Mitchell, J. N. (2019). Salinity from NaCl changes the nutrient and polyphenolic composition of basil leaves. *Industrial Crops and Products*, *127*, 119-128. https://doi.org/10.1016/j.indcrop.2018.10.048

Sharif, R., Mujtaba, M., Ur Rahman, M., Shalmani, A., Ahmad, H., Anwar, T., Tianchan, D., & Wang, X. (2018). The multifunctional role of chitosan in horticultural crops; A review. *Molecules*, *23*(4), 872. https://doi.org/10.3390/molecules23040872

Sun, J., Wang, M., Lyu, M., Niklas, K. J., Zhong, Q., Li, M., & Cheng, D. (2019). Stem diameter (and not length) limits twig leaf biomass. *Frontiers in Plant Science*, *10*, 185. https://doi.org/10.3389/fpls.2019.00185

Tagliavini, M., Veto, L. J., & Looney, N. E. (1993). Measuring root surface area and mean root diameter of peach seedlings by digital image analysis. *American Society for Horticultural Science*, *28*(11), 1129-1130. https://doi.org/10.21273/HORTSCI.28.11.1129

Tanimoto, E. (2005). Regulation of root growth by plant hormones — Roles for auxin and gibberellin. *Critical Reviews in Plant Sciences*, *24*(4), 249-265. https://doi.org/10.1080/07352680500196108

Turk, H. (2019). Chitosan-induced enhanced expression and activation of alternative oxidase confer tolerance to salt stress in maize seedlings. *Plant Physiology and Biochemistry*, *141*, 415-422. https://doi.org/10.1016/j.plaphy.2019.06.025

Ullah, N., Basit, A., Ahmad, I., Ullah, I., Shah, S. T., Mohamed, H. I., & Javed, S. (2020). Mitigation the adverse effect of salinity stress on the performance of the tomato crop by exogenous application of chitosan. *Bulletin of the National Research Centre*, *44*, 181. https://doi.org/10.1186/s42269-020-00435-4

Waly, A. A., El-Fattah, A., Hassan, M. A. E., El-Ghadban, E. M., & Abd Alla, A. S. (2020). Enhancing growth, productivity and essential oil percentage of *Thymus vulgaris* L. plant using seaweeds extract, chitosan and potassium silicate in sandy soil. *Scientific Journal of Flowers and Ornamental Plants*, *7*(4), 549-562. https://doi.org/10.21608/sjfop.2020.148056

Wang, Y., Zhao, J., Lu, W., & Deng, D. (2017). Gibberellin in plant height control: Old player, new story. *Plant Cell Reports*, *36*(3), 391-398. https://doi.org/10.1007/s00299-017-2104-5

Went, F. W. (1935). Auxin, the plant growth-hormone. *The Botanical Review*, *1*(5), 162-182.

Xu, C., & Mou, B. (2018). Chitosan as soil amendment affects lettuce growth, photochemical efficiency, and gas exchange. *American Society of Horticultural Science*, *28*(4), 476-480. https://doi.org/10.21273/HORTTECH04032-18

Yang, J., Kloepper, J. W., & Ryu, C.-M. (2009). Rhizosphere bacteria help plants tolerate abiotic stress. *Trends in Plant Science*, *14*(1), 1-4. https://doi.org/10.1016/j.tplants.2008.10.004

Zulfiqar, F., Chen, J., Finnegan, P. M., Younis, A., Nafees, M., Zorrig, W., & Hamed, K. B. (2021). Application of trehalose and salicylic acid mitigates drought stress in sweet basil and improves plant growth. *Plants*, *10*(6), 1078. https://doi.org/10.3390/plants10061078