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Improvement of Chlorophyll, Antioxidant Properties, and Biomass Yield in Sweet Basil (*Ocimum basilicum* L.) Using Chitosan at Various Growing Stages

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Abstract

Sweet basil (*Ocimum basilicum* L.) is known for its numerous health-promising antioxidant phytochemicals and is primarily used in nutritive, medicinal, and cosmetic products. Previous attempts to increase the antioxidant content of sweet basil were associated with disadvantages, including ecological problems, reduced biomass yield, and increased cost. Alternatively, the current study aimed to improve selected antioxidants and biomass yield by using chitosan as an organic and cost-effective growth promoter. In this experiment, a total of four different concentrations of chitosan were applied (0%, 0.2%, 0.4%, and 0.6% v/v) at two different growing stages: early growth stage (GS1: 45-day-old plant), late growth stage (GS2: 65-day-old plant), and at both growth stages (known as GS3: 45 & 65-day-old plants). Results showed that plants treated with 0.4% chitosan at GS1 reached the highest chlorophyll a (4.33 mg/cm^2), chlorophyll b (2.50 mg/cm^2), total chlorophyll (6.84 mg/cm^2), total leaf area (2234.31 cm^2), total dry biomass (22.72 g per plant), total flavonoid content (33.23 mg QUE/g DE) and DPPH inhibition (92.34%) compared to other treatments. Based on the yield and phytochemical content, it is recommended to apply chitosan at 4% on the 45-day-old plant.

Keywords: flavonoids, phenolics, phytochemicals, physiology, yield

Introduction

Sweet basil (*Ocimum basilicum* L.) is one of the most significant herbs among over 150 members of the Lamiaceae family (Spence, 2024). It is a popular herb for pharmaceutical use (Gossa, 2024) and is widely used in nutritional and cosmetic products (Biswas et al., 2025; Putri et al., 2024). Sweet basil is reported to have high therapeutic potential for serious diseases such as cancer (Chintapula et al., 2024), kidney malfunctions (Gossa, 2024), neural disorders (Oyeniran et al., 2024), and heart disease (Younas et al., 2024). In herbal medicines, therapeutic properties are associated with antioxidant phytochemicals. One important group of these compounds is chlorophylls (Băbeanu et al., 2020; Schmitzer et al., 2021; Sun et al., 2024). From the pharmacological point of view, chlorophyll is used to avoid occurring cancer (Sun et al., 2024), regulate blood sugar, detoxify the liver (Alobaidi, 2024), cure heart diseases (Martel et al., 2017), and prevent obesity (Li et al., 2019). In addition, chlorophyll is used as a food ingredient and as a cosmetic agent (Krishnakripa & Thoppil, 2025). Hence, it is worth improving the chlorophyll content in edible herbs such as sweet basil. Phenolic constituents, as well as flavonoids, are the other dominant group of antioxidants in sweet basil (Yolcu & Yilmaz, 2025). As antioxidants, these phytochemicals are used in nutritional, medicinal, and cosmetic products (Afshar et al., 2022; Ibrahim et al., 2023).

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While the demand for these health-promising phytochemicals is increasing day by day, their production is directly associated with both their content and the amount of biomass produced per plant. To increase antioxidant compounds in sweet basil, several approaches were attempted, including the application of stress factors, artificial plant growth regulators, and biofortification; however, these efforts showed several drawbacks. In particular, although stress factors increased the production of antioxidant compounds, they declined biomass yield. Artificial plant growth regulators were found to be ecologically restricted, and biofortification seemed economically costly.

Alternatively, chitosan is an eco-friendly (Karamchandani et al., 2024), inexpensive (Amitaye et al., 2024), and largely available exogenous plant growth promoter (Xu et al., 2020). It is produced through deacetylation of chitin, a biopolymer primarily sourced from the exoskeleton of crustaceans such as shrimps, crabs, lobsters, and krills are regarded as a waste-product of shellfish industry (Jia et al., 2024). Foliar application of chitosan has been shown to increase chlorophyll content in several crops, including *Piper longum* L. and *Fragaria* x *ananassa* Duch. and *Brassica rapa* L. (Kocaman, 2024; Ullah et al., 2020; Vishnu et al., 2025). Thus, it could improve the accumulation of phenolic and flavonoid compounds in plants (Kahromi & Khara, 2021; Pourbeyrami-Hir et al., 2022; Stasińska-Jakubas et al., 2023). Although chitosan has been proven to be an effective compound in enhancing plant growth and development, its effectiveness is, however, dependent on the concentration and application timing. For instance, a concentration of 125 ppm chitosan resulted in maximum growth and yield in *Abelmoschus esculentus* L. (Mondal et al., 2012), where the highest growth and yield of *Basella alba* L. was observed when plants received 75 ppm chitosan (Mondal et al., 2011). On the other hand, chitosan was found to be sufficient at the early growing stage on *Solanum lycopersicum* L. (Mondal et al., 2016), while on *Solanum lycopersicum* L., chitosan showed promise at early and late growing stage frequencies (El-Amerany et al., 2022). Therefore, this study was conducted to investigate the biomass yield and antioxidant compound accumulation in sweet basil in response to chitosan at various growth stages.

Materials and Methods

Experimental and Growing Conditions

The study was conducted in a 2-factorial Randomized Complete Block Design comprising four replications and five plants per replicate under the open area of (Latitude 30° 27'S, longitude 149° 80'E) in Malaysia. The average of daily maximum and minimum temperatures was 30.5 °C and 26.56 °C, respectively. The average daily relative humidity ranged between 88.20% to 64%. Briefly, sweet basil seeds were germinated in 100% peat moss, and seedlings were transplanted into polybags containing a soil mixture (Bio-soil, brand Melayu Impra™). Plants were manually irrigated daily and fertilized with an amount of 2.5 g per plant N.P.K (50:50:50). A commercial brand of chitosan (Brand: (KitosanPlus) in water at the rate of (0%, 0.2%, 0.4%, and 0.6% v/v) at various growing stages (GS) including at early growing stage (GS1: 45-day old plant, DOP), late growing stage (GS2: 65-day old plant, DOP) and at both growing stages (GS3: 45 and 65-day old plants) were applied. Basils were harvested 95 days after sowing (DAS).

Assessment of Leaf Chlorophyll Content

Data on chlorophyll a, chlorophyll b and total chlorophyll were measured based on the method of Coombs et al. (1987). The samples were taken using a cork borer (1 cm^2) at three random spots on each mature leaf of 95 DOPs. The samples were then soaked in 20 ml of 80% acetone in a glass vial and incubated for 7 days in dark conditions at 25 °C (Al-Harthy, 2019). After a week, a total of 5 ml from the solution was taken and subjected to a UV Scanning Spectrophotometer (Shimadzu Model UV-3101 PC, Japan) at 664 nm and 647 nm. The

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absorbance was recorded, and the targeted parameters were calculated using the following formulas:

$$Chlorophyll\ a\ \left(\frac{mg}{cm^2}\right) = 13.19\ A_{664} - 2.57\ A_{647}$$

$$Chlorophyll\ b\ \left(\frac{mg}{cm^2}\right) = 22.10\ A_{647} - 5.26\ A_{664}$$

$$Total\ chlorophyll\ \left(\frac{mg}{cm^2}\right) = (chlorophyll\ a + chlorophyll\ b)$$

Where:
A_{664}: is the absorbance of the solution at 664 nm.
A_{647}: is the absorbance of the solution at 647 nm.

Assessment of Growth and Yield Components

To determine the average length of branches, all the branches on the main stem were carefully measured by using a ruler on each branch. Data was expressed as an average length per branch in cm. The 95 DOPs were harvested and brought to the laboratory immediately. The detached leaves were carefully measured using a leaf area meter (Model LI-3100A, Lincoln Inc., Nebraska, USA), and the recorded data were expressed as total leaf area (cm²) per plant. The leaves were then processed for further investigations. To estimate total dry biomass, all parts of plants, such as leaves, stem (including lateral branches), and roots, were separately subjected to 50 °C using an electrical oven (Schutzart DIN 40050 – IP 20, Memmert, model: ULM 500). The process continued until a constant weight was reached. Then, all parts were weighed for their dry weight and recorded as total dry biomass. The dried stems and roots were discarded, and dried leaves were stored at 25 °C for phytochemical analysis.

Extraction of Antioxidant Compounds

The dried leaves of sweet basil were ground into a powder using an electronic grinder (Culatti, No. 10318194, Model: MFC). Briefly, 10 g of dry leaf powder was placed in 250 ml conical flasks and added to 100 ml of 60% ethanol. The extraction was performed at 80 °C for 90 min in a water bath (Model 760, Schutzart DIN 40050 – IP 20, Memmert, Germany), followed by filtration using Whatman filter paper (Whatman No. 1, UK). The process was duplicated to ensure complete extraction of targeted phytochemicals from the samples. The extract solution from the first and second extractions was mixed and subjected to an Eyela rotary evaporator (Model: CCA-1111 CE, China). After the solvent evaporated from the samples, the dry extract was collected and stored at –20 °C for phytochemical analysis.

Determination of Total Phenolic Content

The total phenolic content (TPC) was determined following the protocol developed by Ghasemzadeh et al. (2016) with slight modifications. Briefly, a total of 2 mg dry extract was mixed with 1 ml of pure methanol. Then, 200 µl of the solution was transferred to a test tube, and 1 ml of Folin-Ciocalteu reagent (tenfold) was added and mixed well. The solution was incubated in total darkness at 25 °C for 10 min. After 10 min, 1 ml of sodium carbonate (7.5%) was added, mixed well, and incubated in total darkness at 25 °C for an additional 30 min. After that, the mixture was triplicated in a microplate and read at 765 nm using a spectrophotometer (Thermo Scientific, Model 1510, Fisher Scientific, Malaysia). Meanwhile, different levels of gallic acid, including (0, 25, 50, 75, 100, 125, 150 & 175 µg/ml) were measured at 765 nm using the spectrophotometer, and the data were used for establishing the calibration equation. Finally, the total phenolic content was calculated according to the method documented by Genwali et al. (2013), using the following formula, the data were presented as milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g DE).

$$TPC = \frac{(C\ x\ V)}{M}$$

Where:

C: is the concentration of gallic acid from the calibration equation (mg/ml)
V: is the volume of the sample tested (ml)
M: is the mass of the tested sample (g)

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Determination of Total Flavonoid Content

The determination of total flavonoid content (TFC) was carried out based on the method documented by Ghasemzadeh et al. (2016) with slight modifications. The 2 mg dry extract sample was mixed with 1 ml of pure methanol, transferred into a glass test tube, and then added to 4 ml of sodium nitrite solution (1:5, w/v). The mixture was incubated in total darkness at 25 °C for 6 min. Then, the solution was added to an aluminum chloride solution (1:10, w/v) at a rate of 0.3 ml and mixed well. After 6 min incubation under the abovementioned condition, 2 ml of sodium hydroxide (1M) was added and kept for another 10 min in total darkness at 25 °C. The sample was then placed on a microplate and read at 510 nm using the spectrophotometer. Thus, the calibration equation was established using nine different concentrations of quercetin, including (0, 40, 80, 120, 160, 200, 240, 280, and 320 µg/ml) under the spectrophotometric method at 510 nm. Finally, calculation of TFC was performed using the following formula, and data were presented as milligrams of quercetin equivalent per gram of dry extract (mg of QUE/g of DE).

$$TPC = \frac{(C\,x\,V)}{M}$$

Where:

C: the concentration of quercetin from the calibration equation (mg/ml)
V: the volume of the sample (ml)
M: the mass of the sample (g)

Determination of Antioxidant Activity

To determine the antioxidant activity of sweet basil's leaf extract, the method of Aadesariya et al. (2017) was used with a slight modification. Briefly, 0.1 mg of dry extract was mixed well in 1 ml of pure methanol. The solution was mixed with 1 ml of (0.1 mM) 2,2-diphenyl-1-picrylhydrazyl (DPPH) and followed by 30 min incubation in total darkness at 25 °C. Meanwhile, 1 ml of DPPH (0.1 mM) without a sample was used as a control. The tested sample and control were both read at 517 nm using the spectrophotometer, and the absorbances were recorded. Finally, the calculation of antioxidant activity of sweet basil's leaf extract was performed by using the following formula and presented as a percentage of DPPH inhibition:

$$DPPH\ Inhibition\% = \frac{(A_{control} - A_{sample})}{A_{control}}\ X\ 100$$

Where:

$A_{control}$: absorbance value of control
A_{sample}: absorbance value of the sample

Determination of Antioxidant Activity

The Statistical Analysis System (SAS) 9.4 version was employed for data analysis. The analysis of variance (ANOVA) was done by using Least Significant Differences at (p < 0.01). Means were separated, and the data were presented in table and figure form.

Results

Leaf Chlorophyll a

The results indicated that the content of chlorophyll a was significantly affected by the interaction between different chitosan levels and the plants' growing stage, with p < 0.01. Generally, plants treated with chitosan exhibited a higher chlorophyll content compared to non-treated plants. A single application of 0.2%–0.4% chitosan at GS1 raised chlorophyll a from 8.63% to a peak of 9.90% compared to controls. The amount of chlorophyll declined to 6.35% compared to the control plants at 0.6% chitosan (Figure 1). Apart from this, application of chitosan at 0.2%, 0.4%, and 0.6% at the late growing stage (G2), the chlorophyll a showed significantly higher levels by 2.53%, 7.09%, and 3.29%, respectively, compared to the control. Double dosages of chitosan application (G3) at rates of 0.2%, 0.4%, and 0.6% significantly

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increased chlorophyll a by 4.81%, 7.59%, and 4.56%, respectively, compared to the control. It appears that treating sweet basil plants with chitosan at the early growing stage is more effective than at either the late or mature stages.

Leaf Chlorophyll b

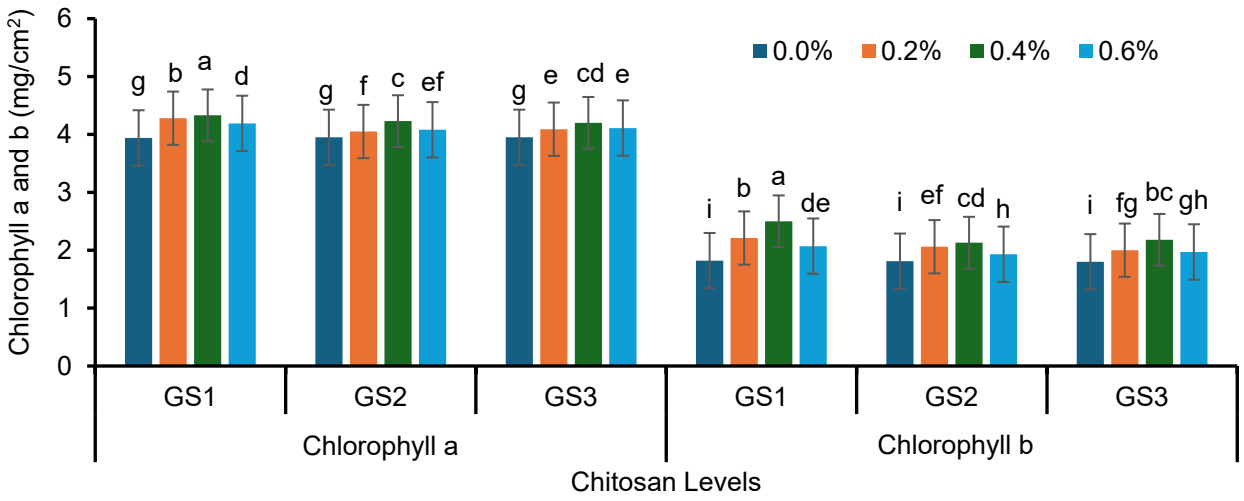
It was noticed that chlorophyll b was influenced by both factors and their interaction at a significant level ($p < 0.01$). Plants treated with chitosan showed a higher chlorophyll b content than the control (Figure 1). Based on the analysis, the application of chitosan at the early growing stage (GS1) appears more promising compared to other times. Applying 0.2% chitosan at GS1 increased the chlorophyll b content by 21.46% compared to the control. This was followed by the peak chlorophyll b value, which was 37.36% higher at the 0.4% chitosan treatment. By increasing the chitosan concentration to 0.6%, chlorophyll b tended to decline, showing a 13.74% superiority compared to the control. At GS2, compared with GS1, chitosan was less effective: the levels of 0.2%, 0.4%, and 0.6% chitosan increased chlorophyll b by 13.81%, 17.68%, and 6.63%, respectively, compared with the control plants. While plants treated with 0.4% chitosan at GS3 showed higher growth by 11.11%, 21.11%, and 9.44%, respectively, compared to the control plants.

Leaf Total Chlorophyll

Total chlorophyll content was affected by both factors: chitosan concentration and plant growth stage. Chitosan-treated plants (Figure 2) exhibited greater total chlorophyll content than control plants. From the results, plants receiving chitosan at the early growing stage (GS1) showed better performance than those in GS2 and GS3. At GS1, increasing the chitosan level from 0.2% to 0.4% significantly increased total chlorophyll content by 18.75% compared to control plants. As the plants matured (GS2), the application of chitosan at this stage was less practical than at GS1, with the maximum total chlorophyll achieved by the 0.4% chitosan treatment being only 10.59% higher than that of control plants. Apart from this, when the plants were treated twice with chitosan (GS3), there was no further improvement in total chlorophyll

Figure 1

The Effect of Different Levels of Chitosan on Chlorophyll a and Chlorophyll bat Various Growing Stages of Sweet Basil Plants



Notes. Means devoted with different letters are significantly different at ($p < 0.01$). Chitosan applications were performed when basil plants were 45 days old (GS1), 65 days old (GS2), and at both 45 and 65 days of age.

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content, with the maximum value of 6.39 mg/cm^2 only. However, it was still 10.94% higher than the control plants, where the total chlorophyll content was only 5.76 mg/cm^2.

Total Leaf Area

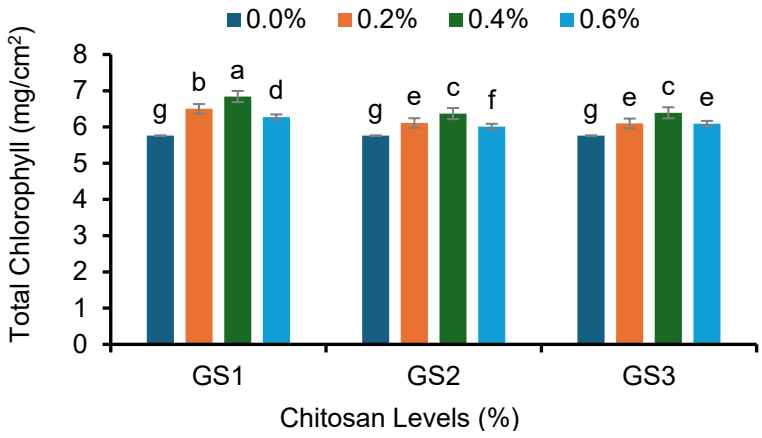
Data on total leaf area were collected from 95 DOPs and subjected to statistical analysis. The analysis of variance revealed that the interaction between chitosan levels and various growing stages had a significant effect on total leaf area at $p < 0.01$. Based on the results (Figure 3), higher total leaf area was detected when plants were treated with chitosan at all levels (0.2%, 0.4% and 0.6%), on GS1 compared with the control plants; where plants reached 1963.74, 2234.31, and 1745.90 cm^2 of total leaf area per plant, respectively. Among them, 0.4% chitosan corresponded to the highest total leaf area, which was 37.19% higher than the control plants. In the case of plants that received chitosan at GS2, the level of 0.6% showed the highest total leaf area, which was 1748.99 cm^2. As for other levels, all plants showed a non-significant difference from the control plant, with total leaf areas ranging from 1629 to 1727 cm². Considering these findings, it can be concluded that the level of chitosan applied to sweet basil reacted differently according to the plant's age, whereby a low level is more effective in enhancing higher total leaf area at GS1. However, a different pattern emerged as the plants matured. Meanwhile, the frequent application of all chitosan levels (0.2%, 0.4%, and 0.6%) on GS3 significantly increased the total leaf area by 1821.88 cm², 1966.30 cm², and 1778.47 cm², respectively, compared to the control plants.

Average Branch Length

The average length of each branch was measured to estimate the growth performance of sweet basil as affected by the treatments administered. Compared to all the parameters discussed earlier, interestingly, the length per branch was not influenced by the interaction between the two factors: the different levels of chitosan and the plants' growing stages. Based on the results (Table 1), chitosan applied at 0.2% and 0.4% showed significantly longer branches by 6.43 and 10.71% compared to the control plants, while 0.6% chitosan was similar to the control plants.

Figure 2

The Effect of Different Chitosan Levels at Various Growth Stages on Total Chlorophyll in Sweet Basil Plants



Notes. Means devoted with different letters are significantly different at ($p < 0.01$). Chitosan applications were performed when basil plants were 45 days old (GS1), 65 days old (GS2), and at both 45 and 65 days of age.

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Total Dry Biomass

The results indicated that the total dry biomass of sweet basil plants was significantly affected by the interaction between chitosan levels and plant growth stages at $p < 0.01$. As shown in Figure 4, all treated plants exhibited greater total dry biomass than the control plants, except those that received chitosan at the GS2 stage, which were similar to the control. Specifically, the application of 0.4% chitosan at GS1 yielded the highest total dry biomass among the treatments, at 22.74 g per plant. The second-highest total dry biomass was obtained at GS3 with 0.2% chitosan and 0.4%, which were 18.90 and 18.83 g per plant, respectively. Thus, there was no significant difference in total dry biomass between plants receiving 0.2% and 0.6% chitosan at GS3.

Table 1

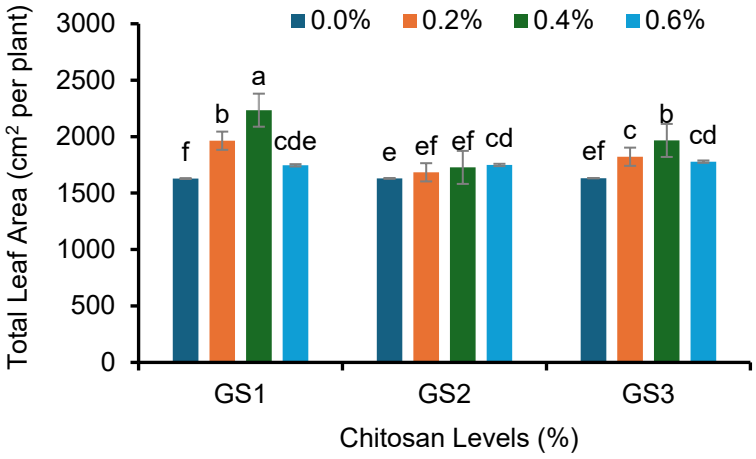
Effects of Chitosan Concentration Applied at Different Growth Stages on the Average Length per Branch in Sweet Basil

Treatments	Branch length (cm)
Chitosan (%)	
0	22.41[b]
0.2	23.85[a]
0.4	24.81[a]
0.6	23.72[ab]
GSs (DOP)	
20	24.18[ns]
40	23.20[ns]
20 + 40	23.73[ns]
Chitosan x GSs	ns

Notes. Means devoted with the same letters are not significantly different at ($p < 0.01$) by LSD test in factorial (4 x 3). ns = non-significant. GSs = growing stages, DOP = day-old plants.

Figure 3

Effects of Chitosan Concentration Applied at Different Growth Stages on Total Leaf Area in Sweet Basil Plants



Notes. Means devoted with different letters are significantly different at ($p < 0.01$). Chitosan applications were performed when basil plants were 45 days old (GS1), 65 days old (GS2), and at both 45 and 65 days of age.

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Total Phenolic Content (TPC)

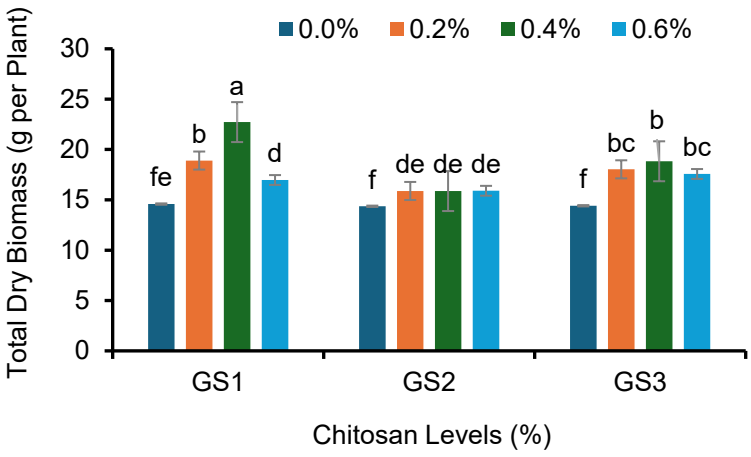
In sweet basil, phytochemicals are the most important yield components; therefore, a few selected phytochemicals, as well as TPC, were investigated in this study. The result indicated that TPC was significantly affected ($p < 0.01$) by the interaction between chitosan levels and plant growth stages in sweet basil leaf extract. Plants treated with chitosan had a significantly greater content of phenols compared to control plants, except for the frequent application of chitosan at the highest level (Figure 5). The analysis also showed that the optimal time for applying chitosan is at GS1, followed by GS2 and GS3, where plants exhibited TPC values of 79.17, 73.85, and 68.40 mg of GAE/g of DE, respectively, when treated with 0.2%, 0.4%, and 0.6% chitosan. The highest phenol content was obtained with chitosan at 0.4%, followed by 0.2% at GS1. The plant extracts showed TPC values of 88.67 and 84.16 mg GAE/g DE, respectively. These are 42.99% and 35.72% higher than the control plants (62.01 mg of GAE/g of DE). When the plants were treated with a higher level of chitosan (0.6%) at GS1, the TPC was significantly reduced to 81.86 mg of GAE/g of DE but still was higher compared to the untreated plants (62.01 mg GAE/g DE). All chitosan levels (2%, 4% & 0.6%) at GS2 significantly improved TPC to 78.22, 78.39, and 77.05 mg of GAE/g of DE, respectively, compared to the control plants (61.73 mg of GAE/g of DE). Conversely, no significant difference was found between 0.2% and 0.4% which were applied at GS2. Similarly, 0.2% and 0.4% chitosan were applied to GS3, significantly increasing TPC to 82.70 and 81.87 mg of GAE/g of DE, respectively, compared with the control plants (61.55 mg of GAE/g of DE). However, both are significantly similar to each other. In contrast, the overapplication of chitosan was found to be toxic to sweet basil plants, with 0.6% at GS3 significantly reducing TPC to 47.48 mg of GAE/g of DE compared to the untreated plants. This decrease is 29.63% lower when compared with the control plants.

Total Flavonoid Content (TFC)

TFC is one of the important compounds in sweet basil. In this study, the amount of TFC was enhanced by the application of chitosan at a suitable stage of plant growth. A significant interaction was found between different chitosan levels and the plants' growing stage at $p < 0.01$. Plants that received 0.4% chitosan at

Figure 4

Effects of Chitosan Concentration Applied at Different Growth Stages on Total Dry Biomass in Sweet Basil Plants



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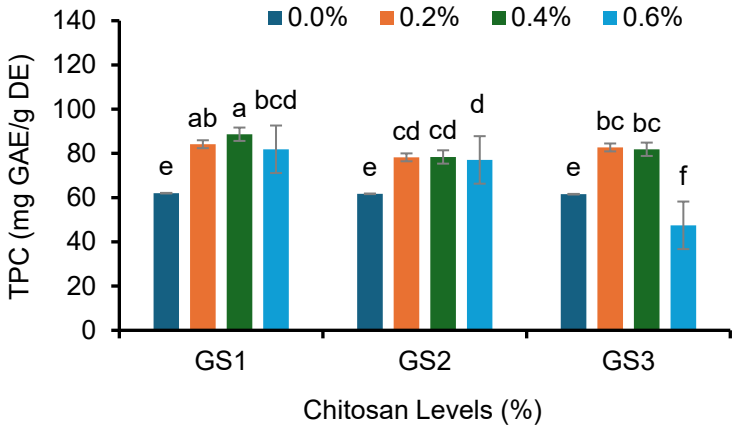
GS1 accumulated the maximum amount of TFC (33.2 mg QUE/g DE) compared to other treatments, where 0.6% chitosan at this stage resulted in a decrease in the content of flavonoid constituents to 29.8 mg QUE/g DE (Figure 6). When chitosan was applied to plants at GS2, it resulted in a similar TFC value to the control plants, where the TFC was accumulated as 24 to 25 mg QUE/g DE only. Based on this result, it can be specified that late application of chitosan not only increased the production budget, but also does not give any positive impact on the amount of TFC. Applications of 0.2% and 0.4% chitosan at GS3 showed a significant increment in the content of flavonoid by 11.73 and 17.79%, respectively, whereas applying 0.6% chitosan at this stage resulted in a significant reduction of TFC by 7.16% compared with the control plants.

Figure 5

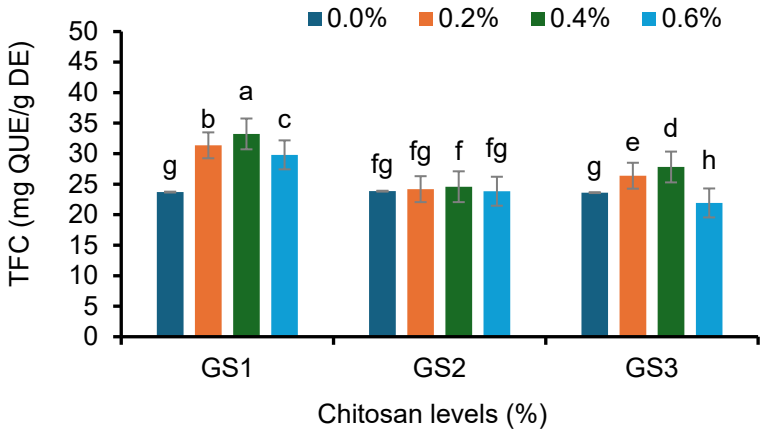
Effects of Chitosan Concentration Applied at Different Growth Stages on Total Phenolic Content in Sweet Basil Plants



Notes. Means devoted with different letters are significantly different at ($p < 0.01$). Chitosan applications were performed when basil plants were 45 days old (GS1), 65 days old (GS2), and at both 45 and 65 days of age.

Figure 6

Effects of Chitosan Concentration Applied at Different Growth Stages on Total Flavonoid Content (TFC) in Sweet Basil Plants



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Antioxidant Activity (AA)

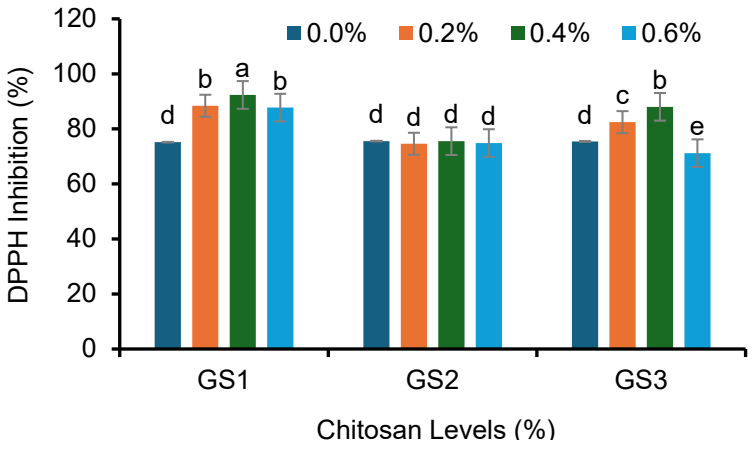
Based on the results from this study, the AA of sweet basil's leaf extract was significantly influenced (p < 0.01) by the interaction of different levels of chitosan and the plants' growing stages. Plants given 0.4% chitosan at GS1 had 92.34% AA, followed by 0.2% (88.41%) and 0.6% (87.74%) (Figure 7). The control plants produced AA at approximately 75%–76%. Surprisingly, the control plants did not show a significant difference among all treated plants at GS2. When plants received chitosan at GS3, significant changes were revealed compared to the control plants. At this stage, plants treated with 0.2% chitosan showed an 82.45% AA. When the level of chitosan reached 0.4%, the AA value increased to 88.02%, followed by a sharp decrease (71.19% AA) when the level of chitosan reached 0.6%.

Discussions

In the current study, chitosan was found to be a potential plant growth promoter, enhancing both chlorophyll pigment and chlorophyll a in sweet basil plants. This is supported by the earlier reports saying that chitosan had increased the content of chlorophyll a in *Zea mays* (Reis et al., 2019) and *Robusta coffee* (Van et al., 2013). There are some factors that reveal the possibility for chlorophyll enhancement in plants due to chitosan treatment. For instance, the biosynthesis of chlorophyll a is stimulated by the accumulation of cytokinins, while application of chitosan may increase endogenous level of the hormone (Reis et al., 2019). Thus, chitosan can improve chlorophyll content to improve the plant defense system, which ensures well-performed growth (Choudhary et al., 2017). Also, it is assumed that chitosan could increase the light utilization capacity of plants and lead to an increase in chlorophyll content. For this reason, chlorophyll a is well known for converting solar energy to its chemical form in the photosynthesis process, leading to primary metabolites (Richardson et al., 2002). Chlorophyll possesses antioxidant and anti-inflammatory potential, which is used in conventional and phyto-medicine (Subramoniam et al., 2012; Uchendu et al., 2024). Hence, the increment of chlorophyll a in sweet basil herb could be a great contribution to both plant productivity and its pharmaceutical potential.

Figure 7

Effects of Chitosan Concentration Applied at Different Growth Stages on Antioxidant Activity (AA) in Sweet Basil Plants



Notes. Means devoted with different letters are significantly different at (p < 0.01). Chitosan applications were performed when basil plants were 45 days old (GS1), 65 days old (GS2), and at both 45 and 65 days of age.

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The ability of chitosan to increase the content of chlorophyll b in sweet basil plants is well understood in the current study. This is supported by Xu et al. (2020), who reported significantly higher chlorophyll b (0.91 mg/g) when *Prunus davidiana* plants were treated with 2 g/L chitosan by foliar application. This increment was 44.9% higher when compared with the control plants (0.628 mg/g chlorophyll-b). To compare this with the results of the current study, it is 7.54% higher than the current study. However, they had used a larger amount of chitosan with the specification of 90% deacetylation degree and 80% substitution degree. Unlikely, according to a report of Farouk and Amany (2012), chitosan at 120 to 500 mg/L concentrations applied on *Vigna unguiculata* L. plants resulted in no significant difference in the content of chlorophyll-b compared to the control plants, with chlorophyll-b ranging from 0.3 to 0.24 (mg/g of Fresh Weight). These differences in the results could be due to crop species, chitosan specification, application time, or method of application. Chlorophyll b is the second abundant pigment in green plants and has a vital function in a plant's physiological mechanism (Silva-Ferreira & Sant-Anna, 2017). It serves as a light-harvesting pigment in chloroplasts in order to facilitate photosynthesis (Tanaka & Ito, 2025). Due to this, the increment in the content of chlorophyll b could have highly contributed to the plant's productivity.

It was also revealed that the drench application of chitosan at the rate of 0.4% applied at GS1 enhanced the development of total chlorophyll content in sweet basil plants. This is in line with other authors who reported the increment of total chlorophyll content in several crops under the application of chitosan (Cao et al., 2025; Choudhary et al., 2017; Vishnu et al., 2025). The rise in chlorophyll following the application of chitosan could be attributed to four possibilities. The first possibility could be related to the ability of chitosan to enhance photosynthesis, which increases organic synthesis in the leaf. Second, the potential of chitosan in the restoration of the complex protein part of pigment in chloroplasts that could protect photosynthetic association from oxidative destruction of proteins and fatty acids (Lai et al., 2007). Third, it could be related to the extra amino group of chitosan, which contributes to the multiplication of chloroplasts per cell, which helps the production of chlorophyll (Muley et al., 2019). The fourth possibility could be due to the ability of chitosan in inducing plants to uptake more minerals from soil, where the presence of minerals such as Magnesium and Iron enhances the synthesis of chlorophyll (Turan, 2019).

Not only chlorophyll, but also chitosan showed potential in producing larger leaves in sweet basil plants. Previous report was found to support this study, where there was an increase in leaf area of several plants, such as *Oryza sativa* L., *Capsicum annum* L., and *Zea mays* L. when treated with chitosan (Ekinci et al., 2024; Inam et al., 2024; Rahman et al., 2025). For instance, Hassnain et al. (2020) reported that 100 mg/L chitosan was able to increase leaf area from 68.64 to 81.05 cm^2 in *Lycopersicon esculentum* L. plants. This is an increment of 18.08% compared to the control plants. In comparison with the current study, the maximum increment in total leaf area was 19.11%. The improvement of leaf area is connected to cell division, cell elongation, and the rate of photosynthesis (Hassnain et al., 2020). The gibberellins play a vital role in plant physiology as well as in the increment of cell division and elongation (Sprangers et al., 2020). Where this hormone could be regulated by chitosan (Khan et al., 2024; Saadat et al., 2023; Stùtzel & Hanafy, 2020). Moreover, the increase in the number and size of the cells in plants may require a larger amount of food storage. In this regard, it is also confirmed that chitosan could accelerate the photosynthesis process by increasing photosynthetic pigments (Liu et al., 2020; Safahani, 2020; Sharma et al., 2020). To explain this, chitosan provides essential elements for plants, which leads to an increased content of chlorophyll in leaves. While chlorophyll harvests light and facilitates the photosynthesis process. Thus, it is assumed that at GS1, the rate of cell division, cell elongation, and degree of photosynthesis are higher than at GS2. Therefore, the increase in total leaf area in sweet basil plants treated with chitosan at GS1

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could be interpreted as chitosan's properties in cell division, elongation, and photosynthesis maximization. On the other hand, plants need nutrition elements and nitrite enzymatic reactions in leaves, where both factors could be stimulated by chitosan (Malerba & Cerana, 2018; Mondal et al., 2012; Mondal et al., 2013). Therefore, growth enhancement as well as increment of total leaf area in sweet basil could be attributed to the ability of chitosan in providing nitrogen element, contributing to its metabolism and accelerating the photosynthesis process (Xu & Mou, 2018). In sweet basil plants, the total leaf area could directly contribute to the fresh and dry biomass yield. Since sweet basil is used in both fresh and dry form, the total leaf area is considered an important parameter.

Chitosan applied at various growth stages, however, did not influence branch length, whereas chitosan alone showed a positive effect on branch elongation. The increase in branch length due to the application of chitosan agreed with Hussain et al. (2019), who reported longer branches in *Solanum lycopersicum* L. plants under the application of chitosan. It appears that longer branches offer more surface area for leaf development and contribute to biomass production.

In the current study, the total dry biomass of sweet basil was increased by 55.83% under the best treatment. The dry form of sweet basil is used in the nutritive (Spence, 2024), pharmaceutical, and cosmetic industries (Yaldiz et al., 2022). It is therefore well understood that the increase in total dry biomass positively contributes to sweet basil production and helps meet the aforementioned demand. Apart from this, it is essential to understand the mechanism by which chitosan enhances dry biomass. In plants, it is assumed that biomass production is affected by the number and size of cells, as well as the accumulation of phytochemicals in the form of solid particles within plant tissues. Chitosan can increase total dry biomass by enhancing cytokinin activation (Chakraborty et al., 2020; Dzung et al., 2011; Zhang et al., 2018), a process well known for promoting both cell division and elongation in plants. Another possibility is that chitosan can provide essential nutrients for plant growth and development by regulating osmotic pressure in plant cells (Krupa-Małkiewicz & Fornal, 2018). This could facilitate the production of both primary and secondary metabolites, which led to an increase in total dry biomass.

Chitosan is also classified as an elicitation agent in plants, where it can interfere with gene expression and the synthesis pathway of plants' antioxidant compounds (Emami-Bistgani et al., 2017; Mahboub et al., 2024; Qiu et al., 2021). Furthermore, chitosan has been shown to activate genes involved in metabolite regulation in plants (Divya & Jisha, 2018). Particularly, Fooladi Vanda and Razavizadeh (2019) explored the induction of phenylalanine ammonia-lyase (PAL), catalase (CAT), guaiacol peroxidase (GPX) and lipoxygenase (LOX) activities along with the expression of *PAL1*, *TAT* and *RAS* genes, which were responsible for improvement of phenolic compound in chitosan treated lemon balm (*Melissa officinalis* L.) shoots. Similar to that, Chitosan was able to upregulate APX, SOD, PAL genes and trigger the antioxidant and defense response system, which led to reversing the adverse effects of salinity stress in rapeseed (*Brassica napus* L.) plants (Bigham Soostani et al., 2025). For that reason, the increase in TPC could be linked to chitosan's ability to modulate gene expression, which was applied once or twice at lower chitosan concentrations. To further explain this, the enhancement of phenolic constituents in sweet basil resulting from chitosan application is related to genes that promote the synthesis pathway. On the other hand, chitosan at higher concentrations was found to have adverse effects, including lowering TPC in this study. This decrease could be attributed to increased oxidative stress and its adverse effects, leading to reduced accumulation of phenolic constituents (Abdel-Aziz, 2019). To illustrate this, chitosan stimulates the synthesis of antioxidants to counteract oxidative stress and increase phenolic compound production, thereby reversing the harmful effects of stress factors. When the synthesis of phenolic compounds reaches its maximum, further

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increases in oxidative stress tend to decrease phenolic compound levels. This is supported by Shams Peykani and Farzami Sepehr (2018) who observed notably reduction in peroxidase enzyme activity as a result of high chitosan level application in *Triticum aestivum* L. seedlings. Taking this into account, the results showed that a higher content of phenolic compounds could be achieved when plants were treated with chitosan at GS1, followed by GS2 and GS3 (at a lower level only). It seems that GS1 is an important period for sweet basil to produce phenolic compounds compared to GS2 and GS3. Moreover, this study recorded higher TPC in sweet basil (88.67 mg GAE/g DE) than the result reported in sweet basil by Izadiyan and Hemmateenejad (2016), which was 83 mg GAE/g DE under optimum extraction conditions. Other organic treatments have also been reported to be sufficient for improving phenolic content in sweet basil. According to Koca and Karaman (2015), application of 1.0 mM spermine + 0.5 mM methyl jasmonate in the form of seed pre-sowing treatment showed the greatest TPC (6.72 mg GAE/g) in sweet basil plants. When comparing this with the result of recommended treatment in the current study, chitosan is 1.53 times superior to spermine + methyl jasmonate. This increase in phenolic constituents may have a significant impact on pharmaceutical manufacturing, as it is a valuable source of antioxidants with high therapeutic effects on chronic diseases, such as cancer (Ghasemzadeh et al., 2016).

The findings in this study agree with Vosoughi et al. (2018), who also observed that chitosan was able to increase TFC in *Salvia officinalis* L. plants. The increased availability of flavonoids as a source of antioxidants (Landi et al., 2014) could also be linked to a chitosan-induced property of gene expression. In addition, secondary metabolites are derived from primary metabolites (Alvarez, 2014). Chitosan, which also has fertilizing potential, was able to boost the manufacturing of primary metabolites (Choudhary et al., 2017) and might enhance the accumulation of flavonoid constituents. However, the current study also found that repeated application of chitosan at higher levels significantly decreased flavonoid content. This indicates that a high level of chitosan causes harmful stress in sweet basil plant cells. The bioactivity of flavonoids enabled these constituents to be included in the list of highly beneficial compounds (Koca & Karaman, 2015), such as protection of cells from damage and cancer development (Mith et al., 2016). Therefore, an increase in flavonoid content could enhance the medicinal properties of sweet basil leaf extract, benefiting the pharmaceutical industry.

The antioxidant activity of sweet basil leaf extract might be related to the presence of antioxidants, such as phenolics and flavonoids, in this study. Chitosan may have encouraged the synthesis of these compounds, thereby enabling plants to interact with unfavorable environmental conditions by strengthening their resistance systems (Malerba & Cerana, 2016). Chitosan molecules can be inserted into plant cells, and the plant's resistant receptors, present in the plant cell membrane, recognize chitosan as pathogenic fungi and activate the defense mechanism. As a result, the plant triggers the initiation of genes responsible for the synthesis of antioxidants (Jiao et al., 2018). The reduction in antioxidant activity might be further explained by the adverse effects of higher chitosan levels on the synthesis of antioxidant constituents in plant cells (Divya & Jisha, 2018).

The study was conducted in the tropical climate of Malaysia. Changes in the results may be observed if the recommended treatment is applied under different climatic conditions. Future study is encouraged to assess the chemical profile of sweet basil under the recommended treatment.

Conclusions

The impacts of drenching with chitosan at various stages of plant growth were evaluated in the field. The results indicated that chitosan during the early growth stage improved chlorophyll pigment content, biomass yield, and the levels of selected antioxidant compounds. Based on the findings, it is recommended to apply 0.4% chitosan to 45-day-old sweet basil plants using the drenching method.

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