Analysis of the potential application of chitosan to improve vegetative growth and reduce transpiration rate in *Amaranthus hybridus*

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**Abstract.** Various benefits have been attributed to chitosan in agriculture. This research is aimed at identifying the optimal concentration of chitosan to increase vegetative growth of *Amaranthus hybridus* L. (green amaranth) and also to determine the effectiveness of chitosan to enable *A. hybridus* cope with drought stress by acting as an antitranspirant agent. The concentrations of chitosan used to amend the growing medium were 0, 10, 30, 50 and 70 ppm. Results showed that chitosan significantly increased the height of *A. hybridus* by up to 19.59 % and also significantly increased the number of leaves at 10 ppm. However, chitosan treatment had no effect on chlorophyll content and fresh or dry weight although dosage response was highest at 10 ppm. In addition, chitosan reduced the transpiration rate by 36.66–66.26 %. Therefore, chitosan treatment could be an alternative way to reduce drought stress.

**Keywords:** *Amaranthus hybridus*, chitosan, drought stress, vegetative growth

1. **Introduction**

Inorganic fertilizer utilization is the main problem for the cultivation of green amaranth. Excessive use of inorganic fertilizers leads to the waste of nutrients, which can have negative impacts on the environment, such as soil acidification due to the over use of nitrogen fertilizers, physical structural damage in the form of soil compaction as a result of mechanical application of fertilizers, and decreased biodiversity of soil organisms. Examples of common inorganic fertilizers are NPK (Nitrogen, Phosphate, Potassium), and potassium chloride [1-3].

The replacement of inorganic fertilizers with organic fertilizers has many challenges. Organic fertilizers are less desirable for farmers because the growth-stimulating effects on plants are slower than those of inorganic ones. Nutrients in organic fertilizers are largely associated with organic complexes; therefore, they are released slowly [1-4].

Another problem faced by farmers is increasing global temperatures and drought, which influences water availability. Green amaranth cultivation has several requirements to achieve optimum growth, one of which is water availability. Green amaranth requires as much as 4 L water/m²/d for 1 week after planting. The water requirement then increases to 8 L/m²/day until harvest time. Increasing temperature will increase a plant’s transpiration rate; therefore, water utilization is less efficient. Alteration in water usage can have negative impacts on crop productivity and yield quality [5-7].
Chitosan can have various beneficial impacts in agriculture. One such effect is to increase the growth of plants in nutrient-deficient environments by increasing the absorption of nutrients from the soil [8-10]. Chitosan treatment has been reported to increase the chlorophyll concentration in coffee plants [8], soybeans [11], peanuts [11] and *Dendrobium* species [12]. Increasing chlorophyll can accelerate the absorption of nutrients and increase photosynthetic rate under stress conditions [8, 10]. In addition, chitosan also helps plants cope with abiotic stresses, such as drought, by acting as an antitranspiration agent [8-10], and treatment with chitosan has been shown to reduce the transpiration rate in coffee [8] and pepper [13] plants.

2. Materials and method

2.1. Chemicals and plants

The plants used in this research were *Amaranthus hybridus* (green amaranth), seed of which was obtained from CV Enno & Co. Seed. Chitosan was obtained from CV CHIMULTIGUNA. Chitosan solution was prepared by dissolving 0.05 g chitosan in 1% acetic acid 10 mL and then, 10 L distilled water was added to make 5 ppm concentration. Chitosan solution 10 ppm, 15 ppm and 20 ppm was prepared by dissolving 0.1 g, 0.15 g, and 0.2 g chitosan with the same volume of acetic acid and distilled water.

2.2. Treatments and experimental design

This research was conducted in the greenhouse of the Biology Department, Faculty of Mathematics and Natural Sciences, Universitas Indonesia. Green amaranth was grown in polyethylene bags (size, 17 cm × 9 cm) with garden soil as grown medium. The study was conducted using a completely randomized design. There were two control groups and four treatment groups, each consisting of six replicates. In the first control group (K1), green amaranth plants were provided with inorganic NPK fertilizer and 300 mL water daily; in the second control group (K2), green amaranth plants were provided with composted cow manure and 300 mL water daily. For the first treatment group (P1), green amaranth plants were provided with composted cow manure, sprayed and watered with 300 mL 10 ppm chitosan solution every 5 days, and provided with 300 mL water each day between chitosan applications. The second (P2), third (P3), and fourth (P4) treatment group procedures were similar to the first treatment group, but with chitosan doses of 30, 50 and 70 ppm, respectively.

2.3. Plant analysis

The plant parameters measured were plant height, number of leaves, chlorophyll concentration, fresh weight, dry weight, and transpiration rate. Plant height was measured with a scale, from the tip to the base of the stem. Plant height and leaf number were measured every 3 days.

Chlorophyll concentration was determined using the Winterman and de Mots method [14]. Green amaranth leaves (1 g) were cut into small pieces and immersed in 96% alcohol solvent, and then, centrifuged at 1500 rpm for 10 min. An equal volume of the same solvent was added to the supernatant to make a total volume of 100 mL, and absorbance was measured at 649 nm and 665 nm wavelengths.

The chlorophyll contents were calculated by the following equation:

\[
\text{Chlorophyll total (mg/L) = (20.0} \times A_{649}) + (6.1 \times A_{665})
\]

\[
\text{Chlorophyll a = (13.7} \times A_{665}) - (5.76 \times A_{649})
\]

\[
\text{Chlorophyll b = (25.8} \times A_{649}) - (7.7 \times A_{665})
\]

Fresh weights were measured immediately after harvesting, and the same plants were then dried in an oven at 40–50 °C for 5 days for dry weight measurement.
Transpiration rate measurement was performed using cobalt chloride paper. A 3 cm × 1 cm strip of cobalt chloride paper was affixed to the adaxial surface of the leaf with the help of a glass and a paper clip. The time it took for the color of the cobalt chloride paper to change from blue to pink was noted. The area of cobalt chloride paper used was divided by the time required, to calculate the transpiration rate.

2.4. Statistical analysis

Statistical analysis was performed using the nonparametric Kruskal–Wallis test.

3. Results and discussion

3.1. Effect of chitosan on plant height and leaf number

Chitosan has shown a significant effect on plant height (figure 1) and the number of leaves (table 1) based on the nonparametric Kruskal–Wallis test. The plant height and leaf number was maximum for plants treated with 10 ppm chitosan (P1). The lowest values for both parameters in the four treatments were exhibited by plants treated with the highest chitosan dosage, 70 ppm. Supplying nutrients in organic form (K2) resulted in taller plants with more leaves than when the nutrients were supplied in inorganic forms (K1).

According to Uthairatanakij et al., chitosan can increase signaling through gibberellins. These plant hormones work in conjunction with auxin to play a role in stem extension and leaf growth [15]. Hormones operate at very low concentrations; if the concentration is too high it will have a negative effect on plant growth, which may explain the negative effects of chitosan dosages greater than 10 ppm on both parameters.

![Figure 1](image)

**Figure 1.** Effect of chitosan on the plant height.

| Table 1. Effect of chitosan dosage on leaf number of *A. hybridus*. |
|---------------------------------------------------------------|
| Treatment | Number of leaves |
|------------|-----------------|
| K1         | 11–19           |
| K2         | 14–18           |
| P1         | 14–20           |
| P2         | 12–18           |
| P3         | 10–17           |
| P4         | 9–14            |
Boonlertnirum et al. reported that chitosan acted as a carbon source for soil microbes and accelerated the mineralization of organic compounds into inorganic compounds, which can more easily be utilized by plants [16]. In addition, chitosan can increase the activity of nitrate reductase, glutamine synthetase, and protease enzymes to help nitrogen absorption and metabolism, and thereby, increase vegetative growth of plants [18-20].

3.2. Effect of chitosan on fresh and dry weight of A. hybridus

The highest fresh weight value was at 10 ppm chitosan (P1) and the lowest value was at 0 ppm chitosan (K1) (figure 2). This indicates that chitosan can increase the growth of the plant, but that the dosage response is not linear. There was no significant effect of chitosan on plant fresh weight. Fresh weight consists of approximately 95% water and can be affected by many endogenous or exogenous factors, including temperature and light intensity, and it is generally accepted that the dry weight is a more reliable measure than fresh weight [20].

There was a significant effect of the treatments on plant dry weight. This result indicated that 10 ppm chitosan can increase plant biomass. Figure 3 shows that although the overall responses of fresh and dry weight to chitosan concentration were similar, clear differences were apparent. Treatment P4 (70 ppm chitosan) resulted in plants with the lowest fresh weight (figure 2) of all the P treatments, but plants with a dry weight greater than that in P2 (figure 3), suggesting that the water content in P2 was more abundant than in P4.

3.3. Effect of chitosan on chlorophyll content

There were no significant differences in the chlorophyll concentrations between the different treatments. The highest concentration of chlorophyll a was in P1 and the lowest value in P4. Similarly, the highest concentration of chlorophyll b was in P1 and the lowest concentration was in P4 (figure 4). The concentration of chlorophyll a is greater than that of chlorophyll b, suggesting that energy demand in the vegetative phase for antenna complexes in photosynthesis is greater [21]. Chitosan were already observed as a one of chemical which can enhance and promote the growth of adventitious roots [22].

3.4. Effect of chitosan on transpiration rate

Increasing chitosan concentration had an inverse effect on the transpiration rate (figure 5), suggesting that chitosan may have the potential to help plants cope with drought stress by reducing transpiration rate and achieving more efficient water utilization in plants.

Foliar application of chitosan plays a role in the stomatal-opening mechanism by increasing the abscisic acid hormone (ABA) in plants. ABA accumulates in the leaves, causing the closure of stomata,
Figure 4. Effect of chitosan on chlorophyll concentrations of A. hybridus.

Figure 5. Effect of chitosan dosage on the transpiration rate in A. hybridus.

thereby reducing transpiration and preventing plants from losing water under drought conditions [23, 24]. However, ABA is an antagonist for growth hormones, such as auxins and gibberellins. Abnormally high ABA concentrations can inhibit growth. Therefore, it is worth considering the balance of the hormone ratio of ABA and growth hormones, such as auxin and gibberellin, to produce optimal growth in green amaranth.

4. Conclusion
Chitosan improved the growth of green amaranth under greenhouse conditions reflected as taller and heavier plants with more leaves. In addition, application of chitosan decreased the transpiration rate of the leaves. Therefore, chitosan has the potential to increase the productivity of plants, particularly under drought stress conditions.

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