Chitosan application for maintaining the growth of lettuce (Lactuca sativa) under drought condition

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Abstract. Drought stress is one of the most common abiotic stress in agriculture. The use of antitranspirant agents to reduce the effect of drought stress on crops has been considered as a potential method. The application of chitosan as an antitranspirant agent has been reported to be effective in several crops. This study was aimed to analyze the effect of chitosan foliar application for maintaining the growth of lettuce under drought conditions. Three concentrations of chitosan (0.2 g/L; 0.4 g/L; 0.6 g/L) were tested in this study. The parameter observed were the height of the lettuce, number of leaves, leaf length and the width, root length, as well as fresh and dry weight. The drought condition without chitosan application reduced the growth of lettuce in all parameters. Chitosan application at 0.2 g/L was able to reduce the effect of drought stress and maintain the growth of lettuce. However, higher chitosan concentration applications (0.4 g/L and 0.6 g/L) were observed to reduce the growth of lettuce. Based on this study, chitosan was reported to reduce the effect of drought stress in lettuce. As chitosan affects the transpiration process by stomatal closure, a higher concentration of chitosan may also affect the photosynthesis process leading to growth inhibition.

1. Introduction
Drought stress is one of the most common abiotic stress in agriculture. In general, drought stress occurs when the water availability on the soil is fewer, and the atmospheric condition causes continuous water loss due to transpiration or evaporation [1]. The main cause of drought is climate change. As many as 51.2% of Indonesia's territory in 2020 experienced a dry season marked by reduced rainy days and a low amount of rainfall measured on the surface [2],[3]. Most areas in East Nusa Tenggara, West Nusa Tenggara, Bali and Java have experienced consecutive days without precipitation ranging from 20-60 days [2]. Meanwhile, in 2021 it is also predicted that several regions in Indonesia have the potential to experience meteorological drought in the classification of alert (days without precipitation >31 days) to alert (days without precipitation >61 days) with an estimated chance of precipitation (<20 mm/10 days) [4].

Constant drought stress can be harmful to the farmer. A decrease in crop yield because of low water content in soil may lead to decreased photosynthesis as well as biomass production in plants is decreased [5]. Drought conditions would encourage stomatal closure to reduce water loss [6]. However, the closure of stomata may also cause the gas exchange hindering so that CO₂ concentrations decrease [7],[8]. In addition, drought stress can also reduce the synthesis of photosynthetic enzymes [8]. Decreased CO₂
concentrations and reduced photosynthetic enzymes cause the process of photosynthesis of plants to be inhibited [8], even within a certain period, which may result in the death of the plant [1].

The response of plants to drought conditions varies, depending on the severity and duration of the drought as well as the plant species and its growth stage [9]. Lettuce is one of the plants that are susceptible to drought [10]. That matter is caused by the fact that lettuce has a short rooting system [1], [10],[11]. In addition, lettuce cultivation carried out by farmers is generally done conventionally using soil, which causes lettuce to become susceptible to drought caused by the availability of water in the soil are fewer. Moreover, the uncertain dry season in Indonesia widened the drought conditions, which further reduced the availability of water in the ground. Thus, the results of lettuce yield and productivity can decrease [12].

Lettuce requires a high water content (>95% of the field capacity of the soil) to support its growth [13]. Lettuce is experiencing mild drought if the need for lettuce encounters 75-90% of the field capacity [14] and severe drought if the field capacity of the soil is <50% [15]. When lettuce is exposed to drought, lettuce will be wilted, growth will decrease, and leaves will turn yellow [16]. Based on Sayyari et al (2013) research, water deficits of 30% to 40% drastically reduce fresh weight and lettuce crop production and may increase oxidative damage [5].

Over the past few years, various cultivation techniques have been applied to overcome the water deficit in crops [17]. One strategy to reduce the impact of water deficit is by using antitranspirant compounds that could potentially induce drought tolerance in plants [18]. One of the antitranspirant compounds that can be used is chitosan [19]. Some examples of other antitranspirant compounds often used are magnesium carbonate, abscisic acid, salicylic acid, phenylmercuric acetate, and kaolin [17]. Chitosan will act as an antitranspirant because it can induce the closure of stomata in plants so that plants can reduce water loss in their bodies [17].

Chitosan can induce the closure of the stomata by creating a signal to the chloroplasts so that they can form H$_2$O$_2$ and NO as the second messenger responsible for improving the synthesis of abscisic acid (ABA) [20]. In addition, chitosan can act as an elicitor because chitosan can protect plants from various oxidative stress by inducing different enzymes and secondary metabolites in the biosynthetic or signaling pathway [21]. Chitosan can induce phytoalexin, polyphenolic, lignin, and flavonoid to increase plant immunity [22]. Chitosan is also biocompatible, biodegradable, non-toxic, and environmentally friendly and is frequently used as a substitute for pesticides because it can act as an antimicrobial agent in plants [23], [24]. Application of chitosan can be used in various ways such as foliar application, seed coating, seedling root dipping, enrichment soil, and addition of chitosan as a supplement in plant tissues [25]. A commonly used method of chitosan application is a foliar application [26]. Foliar application using chitosan is most widely used because, generally, water loss in plants occurs through stomata [26].

The application of chitosan to overcome drought stress has been reported in some crops. Spraying leaves using chitosan is said to reduce the transpiration rate of pepper plants (Capsicum sp.) by 26–43% without a reduction in crop biomass [27]. In addition, a foliar application using chitosan can also improve the parameters of growth, crop yield, and quality of cowpea (Vigna unguiculata (L) Walp) under drought conditions [28].

Chitosan application on lettuce plants has also been reported. Xu and Mou (2018) said that the application of chitosan on soil could improve growth parameters, phytochemical efficiency, and gas exchange in lettuce [29]. Meanwhile, Kurzawińska (2007) also reported that seed and root dipping in chitosan solution could increase germination and reduce pathogen attacks on lettuce [30]. However, neither study was conducted in drought conditions. Therefore, research to evaluate the effectiveness of chitosan in facing the impact of drought on lettuce plants needs to be done.

The beneficial effect of chitosan depends on the concentration, the method of application, environmental conditions, and the growth status of the plant [21]. Foliar application using chitosan with a concentration variation of (0.1–0.4 g/L) was reported to improve growth and physiology parameters and the quality of the fruit of the pepper plant [27], common bean [31], and basil [21]. Based on previous research results, the concentration of chitosan was small, so that in this study, a narrower range of chitosan concentrations will be used (0.2; 0.4; and 0.6 g/L). This study aimed to analyze the effect of
chitosan foliar application with concentration variations of (0.2; 0.4; and 0.6 g/L) for maintaining the growth of lettuce (*Lactuca sativa*) under drought conditions.

## 2. Material and Methods

### 2.1. Material

The materials used in the study were plastic tray [72x72], polybag size 20x25 cm (diameter x height), black plastic (90x120 cm), a mixture of soil: goat manure: sand (1:1:1), lettuce seeds [cv. Grand Rapid], chitosan powder, 0.5% acetic acid, 5% NaOCl, aquades, water, kitchen towel, and envelope.

### 2.2. Methods

The experiment was conducted in the greenhouse of the Department of Biology, Faculty of Mathematics and Natural Sciences, Universitas Indonesia, from November 2020 to January 2021. The research was conducted experimentally using a Completely Random Design consisting of 5 treatments. There were five treatments in this study: positive control, negative control, chitosan 0.2 g/L, chitosan 0.4 g/L, and chitosan 0.6 g/L. Each treatment consists of 5 plants, so that the total number of plants for five treatments were 25 plants.

The planting medium used was soil with a mixture of sand and goat manure by comparison (1:1:1). Lettuce seeds used were sterilized first by soaking the seeds in a 5% NaOCl solution for 5 minutes and then rinsed with water five times [32]. Seedlings that already had 4-5 leaves were transplanted to polybags [33]. Before being given drought treatment, lettuce was still watered with regular irrigation (100% field capacity) in the morning and afternoon for up to 2 weeks as an acclimatization stage [34]. The chitosan solution was made by dissolving the chitosan powder in 1000 mL of 0.5% acetic acid. This study used chitosan levels consisting of (0.2, 0.4, and 0.6 g/L).

Drought treatment was carried out after the acclimatization period was complete (2 weeks after the transplant). Negative control, chitosan 0.2 g/L, chitosan 0.4 g/L, and chitosan 0.6 g/L groups that received drought treatment were watered with a frequency of once a day in the morning with 75% field capacity [35]. Meanwhile, the treatment of positive control, which does not experience drought, remains watered with a frequency of twice a day in the morning and afternoon with 100% field capacity [35]. During the experiment, the application of chitosan was used twice over 30 days. Approximately 20 ml per plant of these solutions were sprayed at the surface of the upper and lower lettuce leaves using a hand sprayer [27].

The criteria for lettuce that can be harvested was when lettuce reached the age of 2-3 months (35-60 days) after transplantation [36]. Harvesting was done in the morning by removing all parts of lettuce and its roots [36]. The parameter observed were divided into 2 aspects, environmental and growth parameters. Environmental parameters observed were temperature and humidity. Meanwhile, growth parameters observed included the plant height, leaves number, leaf length, and width, root length as well as fresh and dry weight of the plant.

### 2.3. Statistical analysis

The result data were tested with a one-way variant analysis test (ANOVA) with a significance of 95% to determine the effect of chitosan application on the growth of lettuce plants. All statistical analysis was done using Statistical Product and Service Solution (SPSS) ver. 24.

## 3. Result and Discussion

### 3.1. Environmental Parameters

Environmental parameters measured included temperature and humidity. The measurement results of environmental parameters were presented in the form of an average table during planting to harvesting (Table 1). The average temperature in greenhouse tends to be high beyond the optimal limit of lettuce (25±2°C) [37], especially during the day (33.38±3.36°C) (Table 1). In contrast to temperature, the
The highest humidity occurs in the morning (72±10.08%) (Table 1). Along with the increase in temperature, the humidity decreases. From the result, we know that the average temperature of a greenhouse was not ideal for supporting lettuce growth. That condition worsened the dry treatment for lettuce growth.

### Table 1. Environmental Parameters in the Greenhouse

| Time       | Temperature (°C) | Humidity (%) |
|------------|------------------|--------------|
| Morning    | 29.69±2.12       | 72±10.08     |
| Noon       | 33.38±3.36       | 60±11.62     |
| Afternoon  | 32.08±2.97       | 62±14.09     |

#### 3.2. Growth Parameters

Growth parameters measured included the plant height, leaves number, leaf length, and width, root length as well as fresh and dry weight of the plant. The positive control group showed better growth on all parameters than the negative control group (Table 2). This matter indicates that drought stress can reduce the parameters of lettuce growth. However, the positive control group tends to be not differed from the negative control group (Table 2). During the experiment, the temperature inside the greenhouse was higher than the suitable temperature required for lettuce to grow optimally. Thus, we assume that lettuce in the positive control group also suffers from drought stress like negative control.

Based on this research, the application of chitosan 0.2 g/L was able to increase the height of lettuce, the leaves number, leaf length and width, root length, as well as fresh and dry weight of the lettuce than the negative control (Table 2). Meanwhile, chitosan 0.4 and 0.6 g/L was only able to increase the height of lettuce compared to the negative control (Table 2). The number of the leaves, the leaves size, root length, and produce biomass on chitosan 0.4 and 0.6 g/L was tended to be not differed from the negative control group (Table 2).

### Table 2. Growth Parameters

| Treatment    | Plant height (cm) | Leaves Number | The Leaf Length (cm) | The Leaf Width (cm) | Root Length (cm) | Fresh Weight (g) | Dry Weight (g) |
|--------------|-------------------|---------------|----------------------|--------------------|------------------|------------------|----------------|
| Positive Control | 8.1±1.64          | 7.4±1.52      | 9.05±1.56            | 4.1±0.58           | 8.9±1.95         | 3.79±1.41        | 0.17±0.05      |
| Negative Control | 7.02±1.18         | 6.6±1.14      | 8.95±1.08            | 4.05±0.48          | 8±3.02           | 2.08±1.05        | 0.11±0.06      |
| Chitosan 0.2 g/L | 11.83±4.37        | 7.25±2.50     | 9.88±2.26            | 5.06±1.33          | 9.13±2.95        | 4.02±3.06        | 0.19±0.05      |
| Chitosan 0.4 g/L | 9.3±2.77          | 5±1.41        | 8.2±2.83             | 4.6±1.52           | 7.3±2.36         | 2.31±2.36        | 0.12±0.13      |
| Chitosan 0.6 g/L | 9.6±4.14          | 4±2.07        | 7.55±2.78            | 3.45±1.64          | 6.3±1.48         | 1.96±1.51        | 0.09±0.07      |

Lettuce treated with a chitosan treatment can grow higher under drought conditions compared to the negative control group. Uthairatanakij et al (2007) reported chitosan application can increase plant growth by increasing the synthesis of auxin hormone through the independent pathway of tryptophan [38]. According to Saharan and Pal (2016), the growth increase is due to the activation of the expression of the AX 11 gene, a gene that plays a role in signaling the synthesis of auxin hormone [39]. However, the mechanism of increasing the synthesis of these hormones is not yet known [40].

Based on the result, the application of chitosan 0.2 g/L increased the leaf number, leaf length and width, and root length of the lettuce than chitosan 0.4 and 0.6 g/L treatment although not significantly. This is supported by Abu-Muerrifah (2013) that chitosan 0.2 g/L increased the leaf number and leaf area of common bean (*Phaseolus vulgaris* L.) under drought stress than chitosan 0.4 g/L [31]. Shehzad et al
(2020) also report that the application of chitosan 0.2 g/L increased the root system development and improved the water absorption capability in sunflowers (*Helianthus annuus* L.) [41].

Drought stress causes increased transpiration rates, which cause the water content of the cell to fall below optimum. Physiological processes are not to be running optimally because the water content in cells decrease so that plants will reduce biomass production and reduce growth [5]. However, based on this result the application of chitosan 0.2 g/L was able to increase the fresh and dry weight of the plant under drought conditions. Abu-Muerifah (2013) also reports similarly that chitosan 0.2 g/L can increase the yield and quality of the common bean [31].

Chitosan as an antitranspirant is known to decrease transpiration rate by inducing the closure of stomata in plants. Bittelli et al. (2001) reported that spraying leaves with chitosan could reduce the concentration of potassium ions in guard cells [27]. If the concentration of potassium ions in guard cells is low, then the stomata will close and vice versa [42].

In addition to the decrease in potassium ions, the reduction in transpiration rate is also caused by the abscisic acid (ABA) hormone [20]. Chitosan can bind to the cell membrane through interactions between positively charged amine groups of chitosan with negatively charged phospholipids [30]. The interaction induces secondary messenger signals in cells so that in chloroplasts, hydrogen peroxide (H$_2$O$_2$) is formed via the octadecanoid pathway and forms NO [20]. The emergence of H$_2$O$_2$ compounds stimulates the accumulation of Reactive Oxygen Species (ROS) and the synthesis of ABA hormones [20]. Meanwhile, NO creates phosphoric acid through the phospholipase D (PLD), phospholipase C (PLC), and diacylglycerol kinase (DGK) pathways [20]. Phosphoric acid plays a role in increasing the work of ABA by inhibiting the ABI1 gene, which is a negative regulator of ABA [20]. Therefore, ABA can induce the closure of stomata in plants.

In addition to providing a positive effect on plant growth, the application of chitosan can also have a negative impact. Davenport et al (1969) stated that giving chitosan with too high concentrations can cause damage to the surface of the leaves [26]. Giving chitosan at concentrations that are too high can also decrease plant productivity. If the concentration of chitosan given is too high, carbon assimilation in plants decreases because water absorption is suppressed [19]. Based on this result, if the stomata close for a long time may lead to a reduction in photosynthetic activity that plays an essential role in plant growth [42]. That matter causes concentrations of chitosan 0.4, and 0.6 g/L cannot increase lettuce growth compared to chitosan 0.2 g/L. Therefore, the concentration of chitosan plays an essential role in determining the effectiveness of chitosan.

Chitosan 0.2 g/L was confirmed to increase lettuce growth even if not significantly. Similarly, it was also reported by Abu-Muerifah that 0.2 g/L of chitosan showed the best growth in the common bean [31]. Meanwhile, higher chitosan concentration (0.4 and 0.6 g/L) was confirmed to reduce the effectiveness of chitosan. This was evidenced by all the observed parameters, lettuce growth with chitosan treatment of 0.4 and 0.6 g/L was lower than chitosan 0.2 g/L and did not even present a significant difference when compared to the negative controls.

Based on the previous study, the application of chitosan 0.4 g/L reportedly did not have a significant effect on sunflower (*Helianthus annuus* L.) [41] and sage (*Salvia officinalis* L.) [44]. However, the application of chitosan 0.4 g/L and 0.6 g/L can increase the growth parameter in basil (*Ocimum basilicum*) [45] and stevia (*Stevia rebaudiana* Bertoni) [46] significantly. That study shows that the application of chitosan 0.4 g/L and 0.6 g/L still has a significant effect on plant growth. This indicates that optimal concentrations may vary depending on plant species and chitosan concentration.

In addition to chitosan concentration and plant species, the influence of environmental conditions also allows for different results. Temperature conditions in the greenhouse were higher than the suitable temperature required for lettuce to grow optimally [33],[36]. Higher temperatures can increase the evaporation and transpiration net in the plant [47].

Based on this research, the concentration of chitosan that showed the best results for maintaining the growth of lettuce plants was chitosan 0.2 g/L.
4. Conclusion
Chitosan application at 0.2 g/L was able to reduce the effect of drought stress and maintain the growth of lettuce. However, higher chitosan concentration applications (0.4 and 0.6 g/L) were observed to reduce the growth of lettuce.

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