Evaluation of the Effect of *Sargassum angustifolium* Extract on Growth and Yield Indices of *Lactuca sativa* Under Drought Stress Conditions

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Abstract

Lettuce (*Lactuca sativa*) represents a major horticultural crop in Iran and worldwide. Lettuce requires a high amount of water to grow well and is adversely affected by drought. Regarding the reports of the positive effect of seaweed extract on increasing the resistance of plants to abiotic stresses, the present study aimed to evaluate the effect of the seaweed *Sargassum angustifolium* extract on growth and yield indices of lettuce under drought stress. This study was conducted as a factorial experiment with three treatments of algae extracts concentrations and two treatments of drought stress, and non-stress treatment with three replications. To evaluate the effects of algae extract on lettuce under drought stress conditions, a combination of morphological and physiological characteristics including plant height and dry weight, photosynthetic pigment content, and antioxidant activities were measured. Results showed that treatment of seaweed extract significantly increased plant resistance to drought stress and improved morphological and physiological indices of lettuce ($p < 0.05$). The best results were obtained in the 1.5 g/l treatment of seaweed extract.

Keywords: Lettuce, Biostimulant, Seaweed, Physiological Characteristics, Proline

Introduction

The existence of environmental stresses, especially drought stress, has always been considered one of the factors limiting the growth and achieving optimal performance of agricultural and garden plants in arid and semi-arid areas. Stress is caused by the action of one or more living or non-living factors in the environment, which causes the physiological activities of the plant to be disrupted, which is reflected in the reduction of plant growth and performance. In other words, stress indicates the exposure of the plant to one or more environmental factors that cause the loss of growth and production efficiency and also decrease the value of the plant (Fang et al., 2015). The amount of water available to the plant is one of the most important climatic factors that affect the growth of plants all over the globe, and its deficiency causes morphological, physiological, and biochemical changes in agricultural and garden plants. Drought stress occurs when the plant's access to water decreases (Blum, 2017). Drought stress is one of the most crucial environmental

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stresses that hurts plant growth and thus significantly reduces the level of agricultural production. Lack of rainfall and irregular distribution of rain during the growing season are the major causes of drought stress in garden plants. Drought stress is one of the most common and destructive non-living stresses in the agricultural sector, which causes a decrease in the yield of garden plants in different regions of the world, including Iran, most of which is under a semi-arid climate (Golbashy et al., 2010). When plant growth is reduced due to drought stress, a high yield of garden plants becomes challenging to achieve. According to reports, about 40% of the world's agricultural lands are located in semi-arid areas, which shows the importance of drought stress in reducing plant production and requires the measures adopted to protect plants from this stress (Mohammadi et al., 2017).

Lettuce (Lactuca sativa) is a garden plant with high nutritional and economic value, which has always seen high demand in domestic and international markets. On the one hand, this plant is a rich source of vitamins, minerals, and nutrients necessary for human growth and health, and on the other hand, it is highly marketable for various types of food production. Like other plants, lettuce is also sensitive to drought stress, and a vital part of the growth and performance potential is lost when faced with drought stress (Murtic et al., 2018). Plant resistance to stress is a polygenic trait when droughts cause stress, reactions such as the expression of stress-specific genes, the accumulation of metabolites, the expansion of the root network, and the reduction of leaf water level occur (Munné-Bosch et al., 2023). Because the metabolic pathway of stress resistance is very complex and different genes are involved in it; therefore, the use of methods based on genetic engineering to increase the resistance of plants to stress has not been very successful (Shukla et al., 2018). On the other hand, the use of biostimulant compounds is an effective, and at the same time economical approach to increasing the resistance of plants against drought stress. For this reason, in the last two decades, much attention has been paid to biostimulant compounds as a solution to overcome living and other stresses (Lephatsi et al., 2023; Hamedeh et al., 2022).

Seaweed extracts are one of the most important biological stimulants which are widely used in the production of agricultural and horticultural crops (Baltrusch et al., 2023). The use of seaweed extract in the soil or by spraying increases the chlorophyll content improves the efficiency of photosynthesis, and absorption of nutrients by plants, improves the ability to absorb and retain water and generally increases the resistance of plants to living and non-living stresses (Mousavi et al., 2023). The positive effects of the extract obtained from different species of *Sargassum* brown algae have been reported in various studies so far (Shahriari et al., 2021). Although the positive effects of seaweed extract on improving yield and improving the resistance level of the garden and agricultural plants to living and non-living stresses have been reported by many researchers (Alharbi et al., 2022); However, many researchers regarding the molecular mechanisms and how the compounds present in seaweed extract affect the physiological and morphological characteristics of plants have not been done, and still many unanswered questions in this regard have remained. At the same time, knowing how algae extract affects the mechanisms involved in stress resistance will make it possible to adopt better strategies for using natural compounds to increase the resistance of garden plants to various stresses, including drought stress. Considering the above information and considering the potential ability of *Sargassum* seaweed extract in improving the resistance of plants to drought stress, or considering the importance of lettuce as a garden product with high market value, this research aims to evaluate the effect of *Sargassum angustifolium* seaweed extract on the growth and yield indices of lettuce under drought stress conditions. The aim of this research is to determine the mechanism of action of the mentioned algae extract in increasing the resistance level of lettuce plants to drought stress. In fact, this research is an attempt to determine the morphological, biochemical and molecular mechanisms that are involved in creating resistance to drought stress under the treatment of seaweed extract.

Materials and methods

Preparation of algae extract (AE)

The brown algae S. *angustifolium* was collected from the coast of Chabahar, and transferred to the laboratory, and its waste materials were removed through washing. Algae samples were dried under shade conditions and then powdered using a mill. 15 grams of algae powder was mixed with 300 ml of ethanol (70%) and stirred on a shaker for 24 hours at room temperature. The resulting extract was passed through filter paper and placed in a rotary evaporator to remove ethanol. The supernatant containing the algal extract was dried at 40 degrees Celsius and the algal pellet was re-suspended in distilled water and the dilution process was carried out to reach a concentration of 2 g/l.

Plant cultivation and applying drought stress

Lettuce seedlings var. Ferdos were grown in pots containing soil with pH=5.5-6.5 and electrical conductivity (EC) equal to 1.2-1.8mS/cm. To apply the treatments, algae extract solution (including three concentrations of zero (control), 1 g/l, and 1.5 g/l) was sprayed in the amount of 100 ml per seedling. Drought stress was applied at three levels: Field Capacity= 100%, Field Capacity=70%, and Field Capacity=50%. The weight method is used to determine FC. For this purpose, the pot was first filled with one kilogram of dry soil and then watered until the soil became saturated. After 24 hours, the pot was weighed, and the weight difference between before and after irrigation was considered as the maximum agricultural capacity (FC=100%). Other FC values were also determined according to the weight of the pots. The experimental design is factorial.

Measurement of growth parameters

Plant height and dry weight

The height of two-week-old plants was measured in centimeters. At the end of the experiment, the lettuce seedlings were separated from the soil and transferred to the laboratory. First, the fresh weight of the seedlings was measured with a precise digital scale. Then the aerial parts of the seedlings were separated and placed in an oven at 80° C. Finally, the dry weight of the seedlings was measured.

The photosynthetic pigments content including chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid content were measured using the method of Lichthen Thaler (1987). For this purpose, 0.2 grams of leaves of seedlings were treated with liquid nitrogen and mixed with 15 ml of 80% acetone. The amount of absorption (A) was measured using a spectrometer at three wavelengths of 646, 663, and 740 nm, and the number of photosynthetic pigments was calculated in micrograms per gram of fresh weight using the following formulas:

$$Chl\ a = 12.25\ A663 - 2.79\ A646$$

$$Chl\ b = 21.21\ A646 - 5.1\ A663$$

$$Chl\ T = Chl\ a + Chl\ b$$

$$Car = (1000\ A470 - 1.8\ Chl\ a - 85.02\ Chl\ b)/198$$

Where, Chl a, Chl b, Chl T, and Car represent chlorophyll a, chlorophyll b, total chlorophyll content, and carotenoid content, respectively.

Antioxidant activity

The power to eliminate free radicals was measured according to the method proposed by Shukla et al. (2018). For this purpose, 200 mg of leaf samples were mixed with 15 ml of 80% ethanol and centrifuged at 5000 rpm for 3 minutes. 250 µl of this extract was added to 250 µl of methanol and 500 µl of 1-1-diphenyl-2-picrylhydrazyl (DPPH). This mixture was kept in the dark for 30 minutes, and the absorbance was measured at 515 nm. Trolox reagent was used as a positive control, and the results were reported in Trolox equivalents per gram of dry weight.

Proline measurement

To measure the proline, the method of Batts et al. (1970) was used, in which proline concentration was determined in mg/g of fresh leaf tissue using a standard curve. The unit is expressed as milligrams per gram of body weight.

Measurement of superoxide dismutase activity

Superoxide dismutase (SOD) activity was measured using the method of Mansouri et al. (2014). The reaction mixture was added to 200 µL of an enzyme mixture. Then the reaction mixture was irradiated at 20° C, and the absorbance was measured at 560 nm. Inhibition percentage (%I) was measured using the following formula:

$$\%I = Ab - As/As$$

In this formula, Ab represents the absorbance of the standard (blank), and As represents the absorbance of the sample. SOD activity is expressed in units per mg of protein (U/mg protein), where each unit represents a

50% change in inhibition percentage.

Statistical analysis

This research was done as a factorial in a completely randomized design with three replications. The experimental factors included the first factor, three different levels of drought stress, and the second factor three different concentrations of algae extract in the form of foliar spraying. The statistical analysis of the data was done using SPSS 20 and comparing the average data with Duncan's multi-range test at the 5% level.

Morphological features

The results of the application of seaweed extract on the morphological characteristics of lettuce plants, including plant height and dry weight, are shown in Table 1. As can be seen, algae extract treatment significantly increased the height of lettuce plants under drought-stress conditions ($p< 0.05$). A similar trend was observed regarding the effect of seaweed extract on the dry weight of lettuce plants ($p< 0.05$).

Indeed, the effect of 1.5 g/l treatment had a greater effect on plant height and dry weight compared to the 1 g/l treatment ($p< 0.05$).

Photosynthetic pigments

Figures 1 to 3 show the effect of seaweed extract on the photosynthetic pigment content of lettuce. According to the results, the highest amount of chlorophyll a was observed in treated plants with a concentration of 1.5 g/l. The amount of chlorophyll in plants treated with both concentrations of algae extract was significantly higher than in the control group ($p< 0.05$). This means that AE treatment significantly increased chlorophyll in lettuce seedlings. The contents of chlorophyll b, total chlorophyll, and carotenoid were significantly higher in seedlings treated with AE compared to the control group ($p< 0.05$). In general, AE treatment significantly increased the content of pigments in all stages of drought stress.

The proline concentration

Figure 4 shows the changes made in the amount of proline of lettuce plants under stress in two cases of control and application of seaweed extract. Based on the obtained results, it was found that the use of seaweed extract at both levels of 1 g/l (treatment I) and 1.5 g/l (treatment II) significantly increased the amount of proline in lettuce ($p< 0.05$).

Antioxidant activity

The ability to eliminate free radicals in the treated plants as well as the plants of the control group was measured using the DPPH test. Based on the results shown in this graph, it is clear that seaweed extract treatment significantly increased the ability to remove reactive oxygen species (ROS) in treated plants compared to the control ($p< 0.05$). The highest amount of free radical elimination was observed in treatment II.

Table 1. Morphological traits of lettuce seedlings after 7 days drought

Indices	Height			Dry weight		
	T 2	T1	Control	T 2	T 1	Control
	43.01 ± 1.32^{c}	39.56 ± 2.22^{b}	34.33 ± 1.07^{a}	12.98 ± 1.76^{b}	12.45 ± 0.34^{ab}	11.21 ± 1.16^{a}

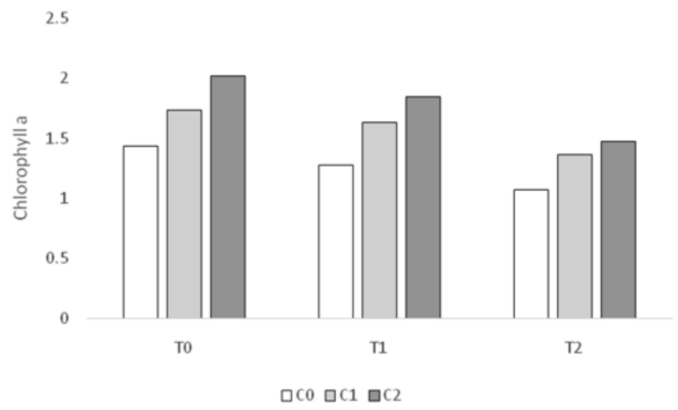

Fig. 1. Effect of stress and algae extract treatment on chlorophyll a content in lettuce seedlings. The values presented in this graph are the average of three replicates, C0, C1 and C2 stand for three levels of drought stress

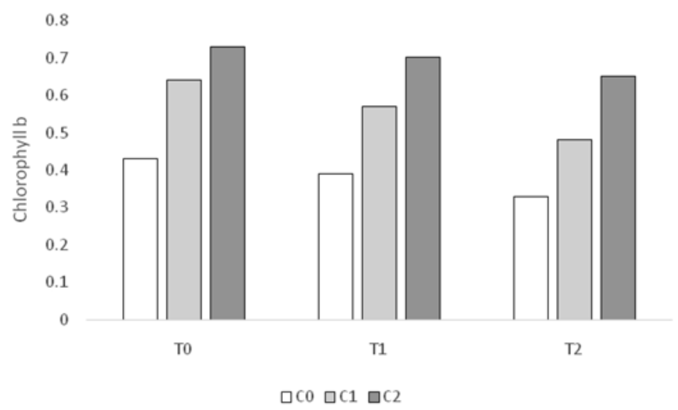

Fig. 2. Effect of stress and algae extract treatment on chlorophyll b content in lettuce seedlings. The values presented in this graph are the average of three replicates, C0, C1 and C2 stand for three levels of drought stress

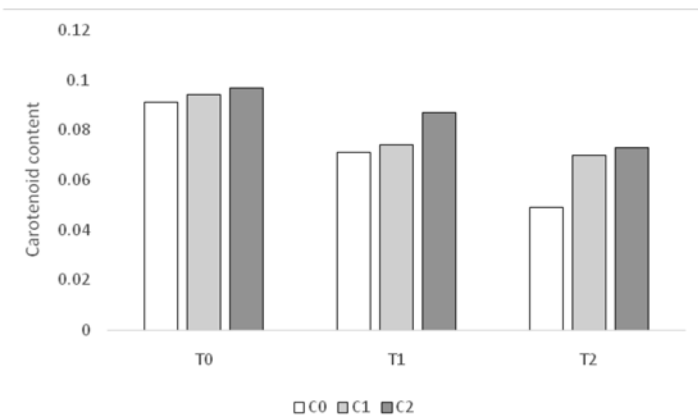

Fig. 3. Effect of stress and algae extract treatment on carotenoid content in lettuce seedlings. The values presented in this graph are the average of three replicates, C0, C1 and C2 stand for three levels of drought stress

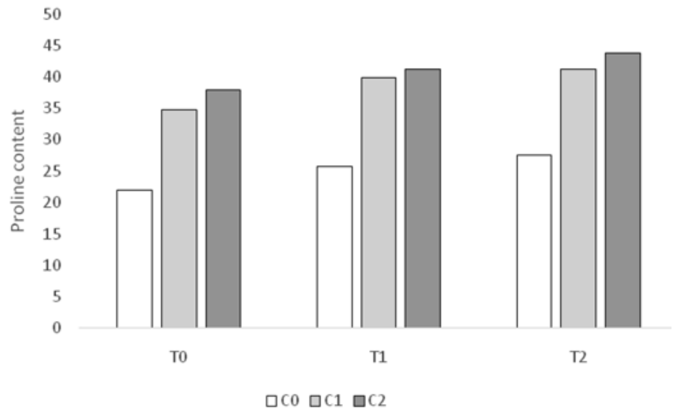

Fig. 4. Comparison of the amount of proline in lettuce plants under stress with and without the application of seaweed extract. The values presented in this graph are the average of three replicates, C0, C1 and C2 stand for three levels of drought stress

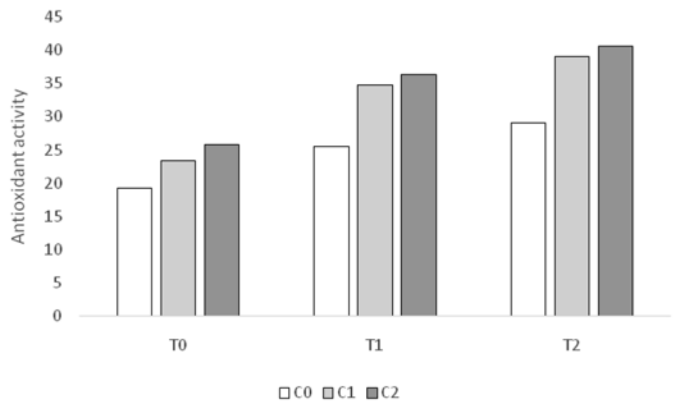

Fig. 5. Increase in the power of killing free radicals due to the application of seaweed extract in lettuce seedlings. The values presented in this graph are the average of three replicates, C0, C1 and C2 stand for three levels of drought stress

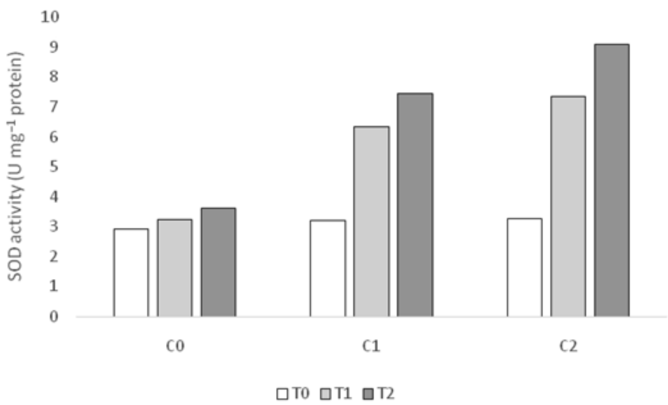

Fig. 6. Changes in superoxide dismutase activity. The values presented in this graph are the average of three replicates, C0, C1 and C2 stand for three levels of drought stress

Superoxide dismutase activity

The activity of the SOD enzyme as an antioxidant mechanism which is activated during plant stress was measured and its results are shown in Figure 8. According to antioxidant activity in SOD, the highest activity was observed in treatment II (concentration of 1.5 g/L of seaweed extract). The difference between treated and control plants in terms of superoxide dismutase (SOD) activity was significant ($p < 0.05$).

Discussion

Drought stress is one of the most important factors that reduce the performance of garden plants, which negatively affects garden production (Kuromori et al., 2022). In particular, this stress hurts the growth and functional characteristics of the lettuce plant and the climatic conditions of Iran. It is considered one of the main factors reducing the yield of the lettuce plant (Shahriari et al., 2021). On the other hand, considering the major benefits of seaweed as a source of micronutrients and reducing stress, the use of seaweed as biological fertilizers and growth stimulants has experienced a growing trend in recent decades. The present study was conducted to investigate the use of brown seaweed S. *angustifolium* as a biological agent to reduce the negative effect of water stress on the morphology and physiology of lettuce plants. The results obtained from this experiment showed that AE leaf spray caused a significant increase in shoot dry weight and seedling height compared to the control group. This positive effect is consistent with results reported for seaweed usage in other agricultural and horticultural crops. For example, in the research conducted by Haghparast et al. (2012), it was also observed that spraying with natural substances, especially seaweed extract, could increase the growth of chickpea plants under drought stress. Hernández-Herrera et al. (2018) also reported that the use of seaweed extract has a positive effect on the growth of plants under drought stress. The positive effect of seaweed on growth parameters may be attributed to the presence of auxin (Martynenko et al., 2016), cytokinin (Zhang et al., 2010), and other growth-promoting factors as well as micronutrients and micronutrients (Kumari et al., 2011).

he results obtained in this research show the positive effect of AE on photosynthetic pigments. The improvement of the performance of the photosynthesis apparatus may justify the increase in the dry weight of the branch and plant growth due to the application of seaweed extract. It is believed that the enhancement of the effect of seaweed extract on photosynthetic pigments is primarily due to the reduction of chlorophyll degradation during water deficit (Wahab et al., 2022). Betaine is a significant biological agent in seaweed extract that increases the chlorophyll content of plants, so the improvement in chlorophyll content of lettuce plants treated with AE may be due to the content of betaines in seaweed extract (Tinte et al., 2022). Considering that the loss of photosynthetic pigments is one of the main factors of growth reduction and crop yield reduction during drought stress, it is suggested that the protective effect of AE on chlorophyll and other photosynthetic pigments affects the application of AE extrapolation in plant promotion.

Drought stress is one of the main factors that cause oxidative stress in plants, which disrupts the balance between ROS production and antioxidant and defense activities of the plant; this is why oxidative stress always occurs during drought stress (Bitarafan et al., 2019). Water stress leads to the accumulation of antioxidant compounds in the vacuoles of epidermal tissues of plants; however, sometimes the increase in the number of natural antioxidants is not high enough to prevent destructive oxidative reactions (Shukla et al., 2016). Therefore, it is necessary to increase the production of antioxidant compounds in plants under stress by using different treatments, to mitigate the destructive oxidative effects caused by drought stress. Seaweed extracts have attracted great attention in recent years due to their high potential to remove free radicals (Mansori et al., 2015). The results obtained in this research showed that the use of *Sargassum* seaweed extract has a positive effect on increasing the absorption power of free radicals and significantly increases the activity of the superoxide dismutase enzyme as a natural antioxidant agent. This finding is consistent with the results reported by other researchers regarding the effect of seaweed extract on improving the power of removing free radicals (Tinte et al., 2022; Zhang et al., 2010; Martynenko et al., 2016; Murtic et al., 2018).

Another result obtained in this research was an increase in the amount of proline with treatment by seaweed extract. Proline is an amino acid that plays a role in creating resistance against almost all non-living stresses. Based on the results obtained in this study, it was found that both levels of *Sargassum* algae extract have a significant effect on increasing proline concentration in lettuce. This finding is consistent with the results reported by other researchers (Elferjani et al., 2018; Erulan et al., 2009). In total, the results obtained in Sayer's research, by other published articles in this field, once again prove that seaweed extract can be a natural stimulant that significantly improves the resistance of plants against drought stress. This finding indicates that due to the prevalence of drought stress in many regions of the country, *Sargassum* algae extract can be used as a cheap and effective source to increase the resistance of garden plants to drought stress.

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