

# Effects Of Some Biostimulant Foliar Application On Antioxidant Capacity and Some Morphological Characteristics Of *Lavendula angustifolia L.*

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## ABSTRACT

In this research, the effect of foliar spraying of some biological stimulants on the growth characteristics and antioxidant capacity of lavender was investigated. The treatments include seaweed (1, 2, 3 ml/L), amino acid (1, 3, 5 ml/L) and Curser organic fertilizer (5, 10, 15 ml per liter) was evaluated in three replicates along with the control treatment. The measured traits included number, length, fresh and dry weight of flowering stems and antioxidant capacity of pure flowers. The results of this research showed that foliar spraying with biological stimulants had a significant difference in vegetative traits and antioxidant capacity with the control treatment. The highest number of flowering stems related to the seaweed (3 ml/liter) is 359 stems, the highest length of the flowering stem related to Cercer organic fertilizer (10 ml/liter) is 27.70 cm. The highest fresh and dry weight related to Amino acid (1 ml/liter) at the rate of 0.067 g and 0.027 g, respectively, the highest antioxidant capacity related to the control treatment at the rate of 71.92 percent, and amino acid (3 ml/liter) was 71.81%. Also, the lowest amount in the vegetative traits related to the control treatment and the lowest amount of antioxidant capacity in the seaweed treatment (1 ml/liter) was estimated to be 42.14%. The general conclusion indicates that the biological stimuli had a positive effect on the vegetative traits of lavender and a negative effect on the antioxidant capacity.

**Keywords:** Seaweed, Amino acid, Organic fertilizer, antioxidant.

## INTRODUCTION

Plants are the main part of the diet and medicinal compounds in such a way that in recent years they have received more attention and study by humans. The report of the World Health Organization indicates that more than 80 percent of the world's population relies on traditional and herbal medicine to treat their diseases (Wang *et al.*, 2016). Medicinal plants are a large and diverse range of plants that have certain effective substances and are effective in preventing and treating diseases. Undoubtedly, the use of medicinal plants by humans is the oldest achievement of humans in the treatment of diseases, and during the development of human societies and civilizations, there has always been a close relationship between plants and humans (Rodríguez-García *et al.*, 2007). The world's approach to the use of herbal medicines has increased the attention of most countries in the world to identify medicinal plants and return to nature. Iran is the habitat of various and diverse plants, most of which are unique in terms of therapeutic properties. Considering the

existence of potential talents in Iran and having a great climate diversity, research and activity in this sector can play a very important role in growth and entrepreneurship to perform.

Due to the destructive effects of the common environment caused by the indiscriminate use of chemical inputs, the importance and necessity of paying attention to alternative cultivation is increasing day by day. In this regard, one of the main pillars of sustainable cultivation is the use of biological fertilizers in agricultural ecosystems with the aim of replacing and eliminating chemical fertilizers (Zand *et al.*, 2017).

*Lavendula angustifolia L.* is a perennial and woody plant from the Lamiaceae family. Its origin is southern Europe and it grows wild in the south and center of Italy, Greece, southern France and Spain in light sandy soil at an altitude of 1700 meters above sea level. This is a true plant (not a two-vein or hybrid) and it seeds in the lead (Bernath. 1993; Currie, 1980).

Biological fertilizer is a substance that contains different types of free-living microorganisms that can convert the main nutrients from an unavailable form to an accessible form (Rajendran & Devaraj, 2004). Some of these microorganisms have beneficial effects on the growth and development of plants and are known as Plant Growth Promoting Rhizobacteria (PGPR) (Abdul Jaleel *et al.*, 2007). In other words, growth-promoting rhizobacteria include a group with a high diversity of root environment cloning bacteria and Diazotrophic microorganisms, and when they grow associatively with a plant, they will stimulate the growth and development of the plant (Girish & Umesha, 2005). Amino acids or amino acids mixed with nutrients, humic acid, hydrolyzed proteins, extracts of algae and marine plants and other metabolites are the basis of the formulation of biological and growth stimulants in new inputs (Gawronaka, 2008; Thomas *et al.*, 2009; Niyogi & Fink, 1992). Biostimulants are classified into eight groups, which include: organic compounds, humic substances, useful chemical elements, inorganic salts such as phosphite, chitin and chitosan derivatives, seaweed extracts, antiperspirants and amino acids or compounds Nitrogen (Hakimi *et al.*, 2019). One of the famous biological stimulants are amino acids, which have many effects on plants, and the most important of these effects can be the positive effect on growth, increasing the quantitative and qualitative performance of plants, significantly reducing damage caused by abiotic stresses in plants. Direct or indirect effect on the physiological activities of plants (Kahlel & Soltan, 2019). Carrying out metabolic processes, structural and exchange activities in plants (Aminifard *et al.*, 2020), Precursor for the synthesis of plant hormones and other growth-stimulating compounds, improving the efficiency of plant metabolism, which increases the quantitative and qualitative yield of crops and the plant's tolerance to abiotic stresses, facilitating the absorption of nutrients in the plant and finally improving the quality characteristics (Calvo *et al.*, 2014). Also, strengthening the respiratory processes and helping to improve its process, increasing the concentration of chlorophyll, which is effective on photosynthesis, as well as protein synthesis and plant growth are among its other effects (Sanikhan *et al.*, 2019). Seaweed extract is one of the compounds that stimulate growth, which, unlike chemical fertilizers, prevents environmental degradation, is non-toxic and does not cause dangerous pollution for humans, animals and birds (Del Poso *et al.*, 2007). Seaweeds are non-polluting and toxic and are environmentally friendly and due to the presence of growth hormones such as auxins and cytokinins, vitamins, micronutrients, and amino acids, they increase plant growth, stimulate root growth, and postpone Aging and increasing the threshold of tolerance to environmental stress (Zodape *et al.*, 2011). ROS are produced naturally in different metabolic pathways in cells and have an important role in the signaling process in the cell (Aruoma & Cuppette, 1997). High

amounts of these reactive oxygen species pose a danger to cells and destroy the components of cells, including proteins, nucleic acids, phospholipids, and cell membranes (Valko *et al.*, 2007). On the opposite side of free radicals, there are antioxidants, which are very important compounds that can protect cells from oxidation and prevent and treat oxidative damage (Zhang *et al.*, 2006). Antioxidants are divided into two groups, enzymatic and non-enzymatic. The most important enzyme antioxidants include superoxide dismutase, catalase and glutathione peroxidase, which directly react with reactive oxygen species and convert them into non-radical compounds. Significant antioxidant properties have been reported in plant extracts due to the presence of various compounds of secondary metabolites. The most important antioxidants in plants are tocopherols, folic acid, ascorbic acid, carotenoid pigments, phenyl acrylic acids and polyphenols (flavonoids and anthocyanin's), which are the most important and broadest group of natural phenols. These antioxidants are generally able to act in different ways, such as cleaning reactive oxygen species, chelating, stimulating or activating antioxidant enzymes, and by reducing or eliminating the damage caused by free radicals to help biological systems (Yu *et al.*, 2002).

## MATERIALS AND METHODS

This research was carried out in 2024 in a private farm in Najaf Abad city (Isfahan province) with geographical characteristics of 32 degrees and 38 degrees north longitude and 51 degrees and 21 minutes east with an altitude of 1600 meters above sea level. The design format was randomized complete blocks with 3 treatments at 3 levels (concentration) and with 3 replications. Also, a treatment with 3 repetitions that was sprayed with distilled water was considered as a control treatment (a total of 9 treatment combinations, each treatment combination including 3 repetitions and a total of 27 plots of 15 plants). The tested plants were four-year-old lavender plants and 15 plants were considered for each repetition. The distance between the plants in the row was 40 cm and between the two rows was 80 cm, and the drip irrigation system and irrigation cycle was once a week. The studied treatments include 1- Amino acid in three concentrations (1-3 and 5 ml/liter) 2- Seaweed in three concentrations (1-2 and 3 ml/liter) 3- Curser organic fertilizer in three concentrations (5 -10 and 15 ml/liter) and 4- the control treatment (distilled water) were all in the form of foliar spraying. Spraying for the treatments of this research was done in the spring of 2024, after wintering and starting to grow, in two occasions with an interval of 14 days. The results were collected on June 9, 2024 (one month after the second spraying). For this purpose, first the studied plants were counted and recorded in terms of the number of flowering stems (including flowers ready for harvest and buds) and then the branches were covered. After that, the size of the harvested branches was measured randomly with a caliper, and 100 branches were randomly separated from each treatment and their weight was recorded by a digital scale of one thousandth of a gram. Then the harvested flowers were placed in an oven with a temperature of 37 degrees for 48 hours to dry. After drying, the weight was again measured by a digital scale of 0.001/g and recorded as the dry weight of 100 branches. In order to measure the amount of antioxidants, pure flower samples from each replicate were packed separately and transported to the horticulture laboratory of Islamic Azad University, Isfahan branch (Khorasgan).

### Antioxidant measurement method:

First, 5 grams of pure flower from each replicate was mixed with 10 ml of 80% ethanol, and then the supernatant was centrifuged at 4 degrees and 5000 rpm for 10 minutes. Then, one to one solution of supernatant and DPPH with a concentration of 0.1 M was added and its absorbance was read at a wavelength of 517 nm in a spect device. Then the antioxidant capacity of the extracts was calculated as DPPH inhibition percentage (Hosseini *et al.*, 2019).

## RESULTS

Sampling of dried flowers and leaves of lavender medicinal plant was done in order to measure and investigate some growth characteristics such as the number of flowering branches, the length of flowering branches, fresh weight and dry weight of flowering branches, as well as the amount of antioxidants.

### Vegetative traits:

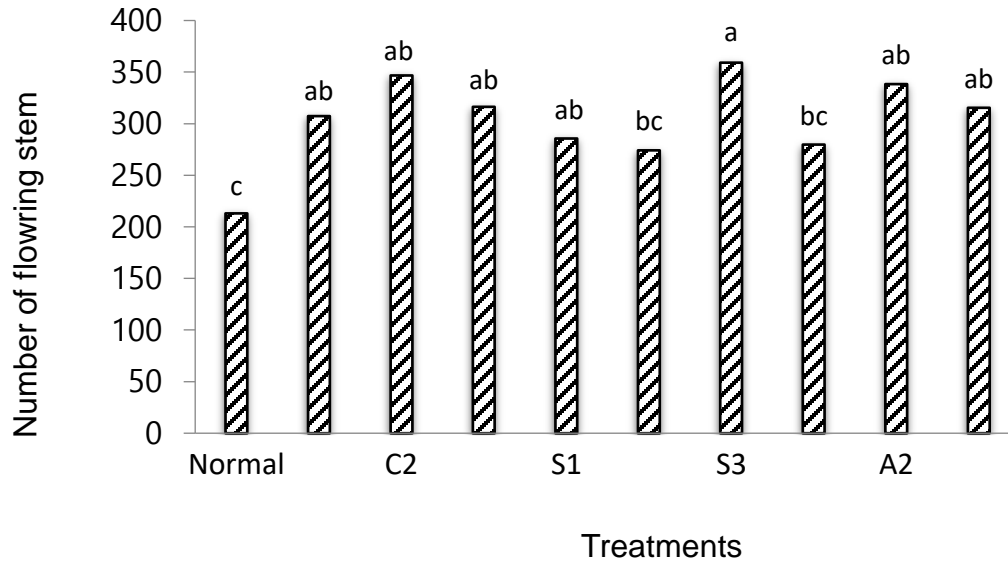
The results obtained from statistical analyzes according to Duncan's test are presented in table (1) and figures (1) to (4).

**Table(1)** -The results of analysis of variance of the effect of treatments on some vegetative characteristics

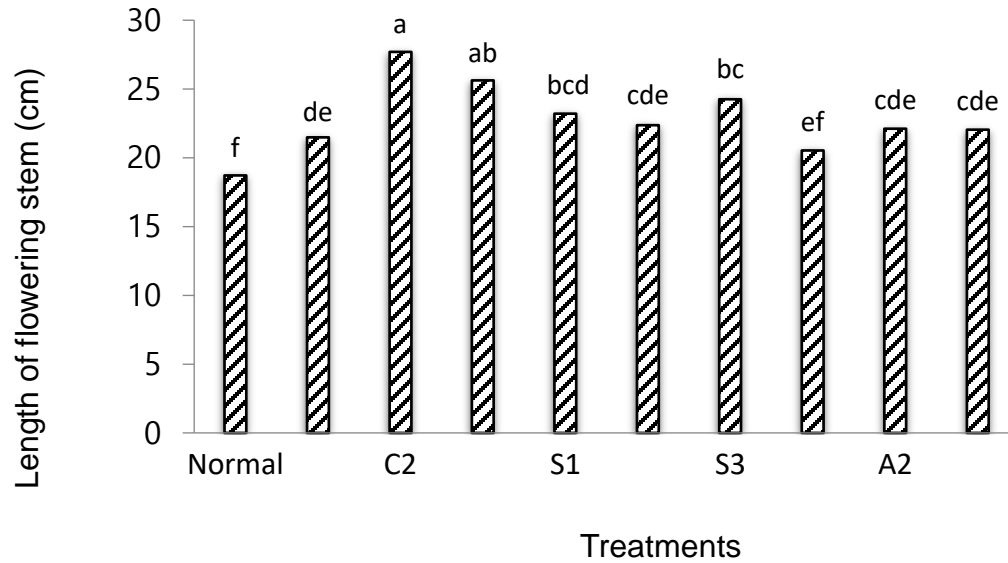
Source of variation	Degree of freedom	Mean square			
		Number of flowering stems	Length of flowering stem	Fresh weight	Dry weight
Block	2	5138	0.12	0.000005	0.000008
Treatment	9	5474*	19.70**	0.0002**	0.000017**
Error	18	1520	1.82	0.000028	0.000005
Coefficient of variance (CV)	%	12.85	5.94	10.71	9.83

\* and \*\*: respectively, becoming significant at the statistical level of 5 and 1 percent.

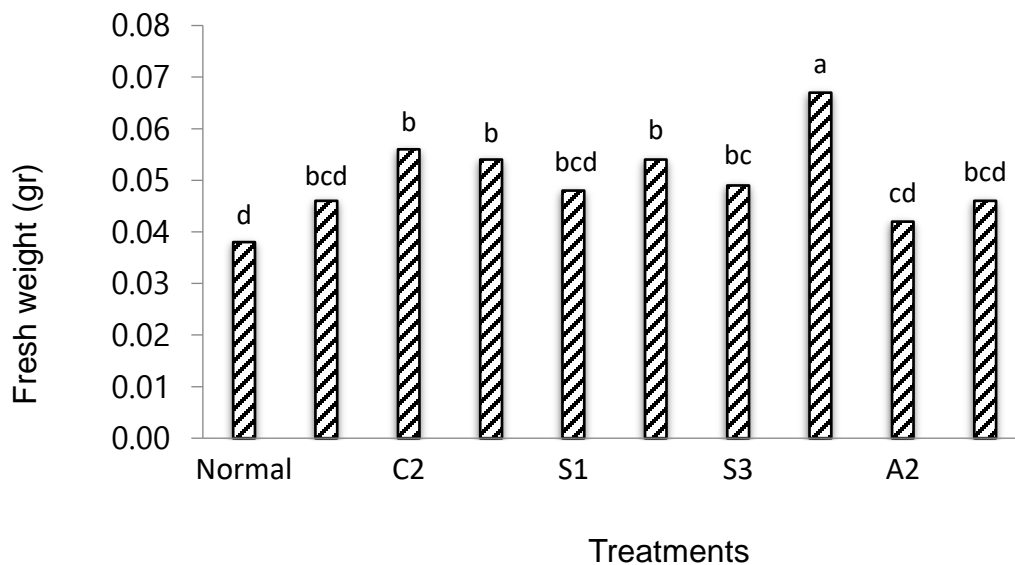
The average number of flowering stems under the spraying of different treatments is shown in Figure 1. Data variance analysis showed that the effect of different treatments on the number of flowering stems was significant at the 5% probability level (Table 1). Also, the comparison of the averages indicates that the highest number of flowering stems is related to the S3 treatment (seaweed with a concentration of 3 ml/liter) as 359 stems and the lowest number of flowering stems is related to the control treatment (sprinkling distilled water) as 213. It was a stem. Other treatments did not have significant differences. (Figure 1).



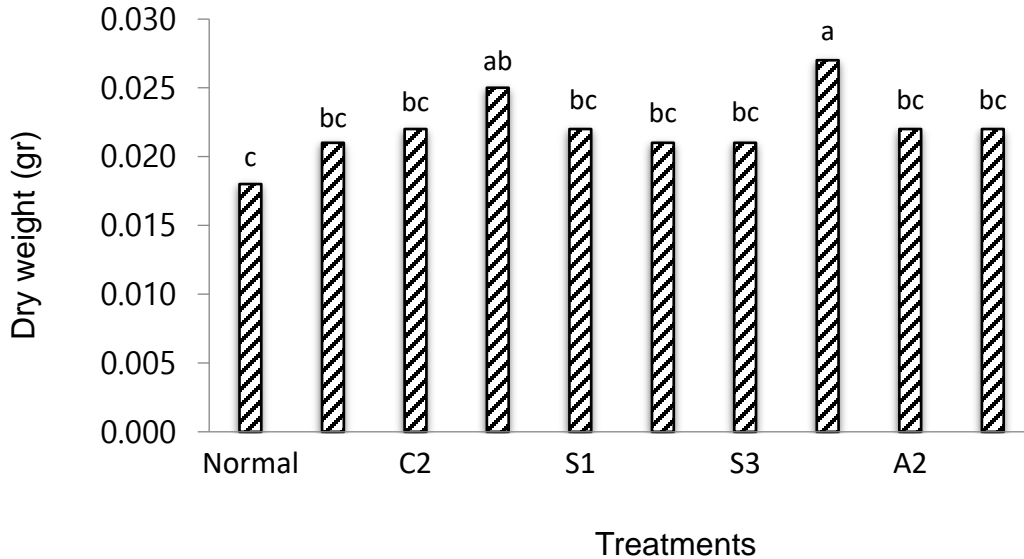
The average length of the flowering stems under the spraying of different treatments is shown in Figure 2. Data variance analysis showed that the effect of different treatments on the number of flowering stems was significant at the 1% probability level (Table 1). Also, the comparison of the average data showed that the highest stem length corresponds to C2 treatments (Cercer organic fertilizer with a concentration of 10 ml/liter) in the amount of 27.70 cm and the lowest stem length corresponds to the control treatment (spraying distilled water) in the amount It was 18.71 cm. No significant difference was observed in other treatments, Figure (2).



The average wet weight of the flowering stems under the spraying of different treatments is shown in Figure 3. The variance analysis of the data showed that the effect of different treatments on the fresh weight of the flowering stem was significant at the 1% probability level (Table 1). Comparison of the average data indicates that the highest fresh weight of the flowering stem in treatments A1 (amino acid with a concentration of 1 ml/liter) is 0.067 grams and the lowest fresh weight of the flowering stem in the control treatment (sprinkling distilled water) is 0.038 g was obtained. Also, in treatments C2 (Cercer organic fertilizer with a concentration of 10 ml/liter) in the amount of 0.056 grams and C3 (Cercer organic fertilizer with a concentration of 15 ml/liter) in the amount of 0.054 grams and S2 (seaweed with a concentration of 2 milliliters per liter) at the rate of 0.054 grams, a significant difference was observed. The weight gain of the C3 and S2 treatments was observed to the same extent (0.054 g) Figure (3).



The average dry weight of flowering stems sprayed with different treatments is shown in Figure 4. The variance analysis of the data showed that the effect of different treatments on the fresh weight of the flowering stem was significant at the 1% probability level (Table 1). Comparison of average data shows that the highest dry weight of the flowering stem corresponds to treatments A1 (amino acid with a concentration of 1 ml/liter) at the rate of 0.027 grams and the lowest amount of dry weight of the flowering stem in the control treatment (sprinkling distilled water) 0.018 g was obtained. No significant difference was observed in other treatments. Figure (4).



**Antioxidant:**

The results obtained from statistical analyzes according to Duncan's test are presented in Table (2) and Figure (5).

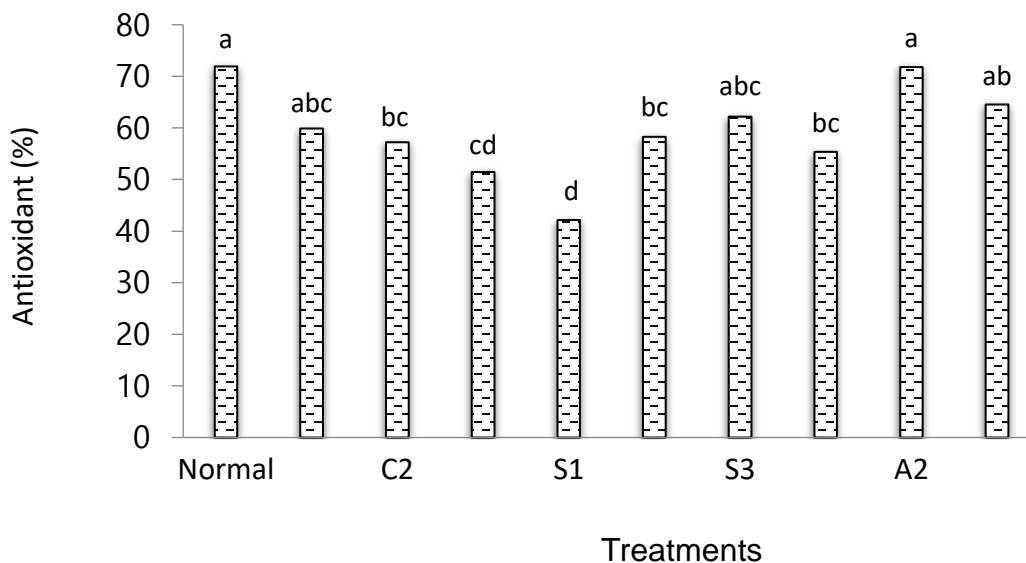
Table (2) -The results of analysis of variance of the effect of treatments on Antioxidant

Source of variation	Degree of freedom	Mean square
		Antioxidant
Block	2	3.93
Treatment	9	242.8**
Error	18	44.88
Coefficient of variance (CV)	%	11.26

\* and \*\*: respectively, becoming significant at the statistical level of 5 and 1 percent.

The average of pure flower antioxidant under the spraying of different treatments is shown in Figure 5. Variance analysis of the data showed that the effect of different treatments on the amount

of pure flower antioxidants was significant at the 1% probability level (Table 2). Comparison of average data indicates that the highest amount of pure flower antioxidant is in the control treatment (sprinkling distilled water) at the rate of 71.92% and the A2 treatment (amino acid with a concentration of 3 ml/liter) at the rate of 71.81%. And the lowest antioxidant content of pure flower was observed in treatment S1 (seaweed with a concentration of 1 ml/liter) at the rate of 42.14% and no significant difference was observed in other treatments (Figure 5).



## DISCUSSION

Flowering stems were counted and reported based on the number per plant. According to the average comparison chart, it was found that the most flowering stems were obtained after applying seaweed treatment with a concentration of 3 ml/liter (S3) with a number of 395 stems. Also, the application of Curser organic fertilizer treatment with a concentration of 10 ml/liter (C2) with the production of 346.7 flowering stems was estimated to be the second highest number of this trait in lavender. Also, amino acid treatment with a concentration of 3 ml/liter (A2) with the production of 338.3 flowering stems per plant shows the positive effect of this compound on increasing the production of flowering stems in lavender plants. Since all the treatments were significantly different from the control treatment (sprinkling distilled water), it was found that the control treatment was ranked last with the production of 213 stems (the lowest production of flowering stems). Therefore, the application of seaweed had a significant effect on the number of flowering stems compared to the control treatment.

The maximum stem length obtained in this study related to the treatment of Curser organic fertilizer with a concentration of 10 ml/liter (C2) was estimated to be 27.70 cm. Also, Curser organic fertilizer with a concentration of 15 ml/liter (C3) was also ranked second with the longest flowering stem length with 25.63 cm. Since the lowest stem length related to the control treatment (sprinkling distilled water) was 18.71 cm, so it can be concluded that all treatments (cursor organic fertilizer, seaweed and amino acid) significantly increase the stem length. Flowering in the lavender plant.

The highest fresh weight of the flowering stem was related to the amino acid treatment with a concentration of 1 ml/liter (A1) at the rate of 0.067 grams. After that, respectively, the treatment of Curser organic fertilizer with a concentration of 10 ml/liter (C2) in the amount of 0.056 grams



and Curser organic fertilizer with a concentration of 15 ml/liter (C3) in the amount of 0.054 grams and seaweed with a concentration of 0.054 grams. 2 ml/liter (S2) in the amount of 0.054 grams are in the next positions. The lowest fresh weight of the flowering stem was obtained in the control treatment (spraying with distilled water) at the rate of 0.038 grams. Also, the obtained results indicate that the highest dry weight of the flowering stem was related to the amino acid treatment with a concentration of 1 ml/liter (A1) at the rate of 0.027 grams. After that, the treatment of Curser organic fertilizer with a concentration of 15 ml/liter (C3) in the amount of 0.025 grams and Curser organic fertilizer with a concentration of 10 ml/liter (C2) in the amount of 0.022 grams in the next place with the highest weight Dry flowering stems were placed. The lowest amount of dry weight of the flowering stem related to the control treatment (sprinkling distilled water) was 0.018 grams. In all the results related to the fresh and dry weight of the flowering stem, it was proved that the different treatments in the study (cursor organic fertilizer, seaweed and amino acid) caused a significant increase in the fresh and dry weight in all concentrations.

Based on previous researches, the use of different types of biological fertilizers in the hyssop medicinal plant improved the growth of the plant and increased the amount of fresh and dry weight in its aerial parts (Koocheki *et al.*, 2008). The report of the researchers showed that the use of different organic compounds in the soil significantly increases the yield, growth and chemical composition of medicinal and aromatic plants such as Ocimum (El-Naggar *et al.*, 2015) and Matricaria (Salehi *et al.*, 2016). Also, the results of most researches indicate the positive effects of amino acids on increasing vegetative growth and biochemical traits in medicinal plants. For example, based on the report of a research in Egypt about foliar spraying with a concentration of 50 and 100 ppm L-tryptophan amino acid on lavender plants, a significant increase in growth and yield was obtained in the plant with 100 ppm amino acid treatment (Elshorbagy *et al.*, 2020). Also, the researchers reported that the use of aminosore fertilizer is effective in increasing the morphological traits and performance of peppermint (Asadi *et al.*, 2018). Also, the research results indicate that the external application of amino acid glycine in the medicinal plant hyssop, in addition to reducing stress damage caused by lack of irrigation, increased the growth power of the plant and improved its efficiency (Khajahosseini *et al.*, 2020). It is stated in a report that consumption of different types of amino acids significantly improves the plant height, number of branches, dry and wet weight of the plant and seed yield as well as chemical compounds (the total content of fatty acids and Caffeic acid derivatives) in the nettle plant. The most effective effect was obtained using a concentration of 100 ppm tryptophan (Wahba *et al.*, 2015). Also, the application of seaweed on lavender plant to investigate growth quality, photosynthetic and biochemical traits in the face of salinity stress and without stress showed that seaweed plays a significant role in reducing the negative effects of salinity stress in all traits. Also, foliar application of seaweed in the absence of salinity stress increased all the above traits in the highest amount compared to the control (Korkmaz & Cicek, 2024). The consumption of seaweed in hyssop showed that the most studied traits had a significant increase compared to the control plant, such that the highest plant height (49.66 cm), the highest leaf area (0.77 cm), the highest dry weight of the plant (22.89 g) and the yield of essential oil (2.12 g/m<sup>2</sup>) was obtained when 10 ml/l of seaweed fertilizer was applied (Pirani *et al.*, 2020). These results are consistent with the findings of this research.

#### **Antioxidant:**

The highest amount of pure flower antioxidant was observed in the control treatment (distilled water spray) at the rate of 71.92% and in the amino acid treatment with a concentration of 3 ml/liter (A2) at the rate of 71.81%. Therefore, no significant difference was observed between the amino

acid treatment with a concentration of 3 ml/liter and the control treatment (spraying distilled water). The lowest amount of pure flower antioxidant in seaweed treatment with a concentration of 1 ml/liter (S1) was reported as 42.14%. The results of this research indicate that the application of different treatments (cursor organic fertilizer, amino acid and seaweed) has reduced the amount of antioxidants in pure flowers of lavender plant.

Reports have been published about the positive effect of amino acids on the antioxidant activity of medicinal plants. For example, in plants such as *Salvia* after foliar spraying with a concentration of 1 mg per liter (Bahadour *et al.*, 2024), the highest antioxidant activity of *Achillea* from plants treated with 3 ml/l amino acid with a 17% increase compared to the control (Shafie *et al.*, 2021) Positive effects of amino acid application on antioxidant activity have been reported. There are also reports indicating the positive effects of seaweed on increasing antioxidant capacity in plants such as *Achillea* (Shafie *et al.*, 2021), *Foeniculum vulgare* (Rasouli *et al.*, 2023) has been published. Organic fertilizer also has an effect on the antioxidant activity of *Mesembryanthemum edule* L. (Fallah *et al.*, 2018) *Marrubium vulgare* L.( Zahedifar & Najafian, 2023), *Ocimum basilicum* (Nguyen *et al.*, 2010). It has been effective. According to the report of the researchers, the use of organic fertilizers increases the antioxidant activity due to the positive effect on the physical and chemical characteristics of the soil and the increase in the organic matter of the soil on the one hand, as well as the increase in the availability and ability to absorb more nutrients on the other hand (Nguyen *et al.*, 2010). These results are contrary to the findings of this research, and since all the treatments in question contain amounts of nutrients and elements needed by the plant, it was expected that they would have positive effects on the antioxidant activity of the lavender plant.

On the other hand, the researchers' report indicates that the use of amino acids, seaweed and fulvic acid organic fertilizer in sage plant had no effect on the antioxidant activity of this plant and the control treatment had the highest antioxidant activity values (Farruggia *et al.*, 2024) which is consistent with the findings of this research. On the other hand, it has been proven that the synthesis of secondary metabolites changes under the influence and interaction of internal and external factors of the plant (Rajabi *et al.*, 2014; Ben Akacha *et al.*, 2023) And stress conditions cause the production and synthesis of secondary metabolites (Figueiredo *et al.*, 2008; Kulak *et al.*, 2020). It is possible that the investigated treatments, by creating optimal growth conditions and avoiding stressful conditions, have reduced the production of some secondary metabolites and reduced the antioxidant activity of lavender. Also, based on a research on the lavender plant with the aim of determining the appropriate levels of nitrogen and phosphorus for a better effect on vegetative and biochemical traits, it was determined that nitrogen levels less than 200 mg/liter and phosphorus less than 60 mg/liter are effective in increasing antioxidant activity will not be (Chrysargyris *et al.*, 2016). Another report indicates that the antioxidant activity of lavender increased in the treatment fertilized with nitrogen at a dose of 50 kg/ha, and the antioxidant activity decreased with the increase of nitrogen at the rate of 100 and 200 kg/ha (Beisiada *et al.*, 2008). On the other hand, in a research in Brazil on the lavender plant with the aim of investigating the phosphorus levels in the vegetative and biochemical traits of this plant, it was found that the nutritional levels allow the sequence of accumulation of K>N>Ca>Mg>P>S for macronutrients and Fe>Mn>Zn>B>Cu>Ni>Mo for micronutrients (Pecanha *et al.*, 2021). According to this research, it is clear that the amount of access to food elements for the maximum production of biochemical traits and especially antioxidants has a minimum and maximum level, and outside of this range, there is a possibility of reducing the production of these compounds. According to the compounds present in organic fertilizer, sea algae and amino acid treated plants, there is a possibility that the

increase in the absorption level of some nutrients has led to the decrease in the absorption level of some other elements and affects the production of antioxidants. Also, the decrease in antioxidant production and the discrepancy between the results of this research and some studies that have reported an increase in antioxidant production in different plants may be due to the differences in the studied plants and their genetic characteristics, differences in environmental conditions such as the location of the study, time Experiments and weather conditions that affect the production of secondary metabolites.

## **CUNCLUSION**

According to the results of this research, it was determined that the use of biological stimulants has an effect on all the growth traits studied in the lavender plant. So that the use of biological stimulants led to an increase in all vegetative and growth traits compared to the control, which can be due to the increase in the level of access and higher absorption of food elements, which led to better vegetative and reproductive growth. The higher antioxidant capacity of the control compared to the treatments indicates the reduction of this attribute after the application of the treatments in this research. Since all the investigated treatments had high amounts of nutrients and some of them had growth hormones, it is possible that by adding nutrients less or more than the allowed amount, the lack of proper balance in the absorption of elements required by the plant happened. The increase in the absorption of some elements has decreased the absorption of other elements and the reduction of the antioxidant capacity has been effective. Other biochemical traits such as phenol, chlorophyll a, chlorophyll b, total chlorophyll and carotenoid due to the application of biological stimulants often had an increasing trend compared to the control treatment.

In general, it can be concluded that the use of biological stimulants (amino acids-seaweed and Curser organic fertilizer) is effective on all growth characteristics of lavender. Also, the use of studied biostimulants did not cause a significant increase in antioxidants compared to the control.

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