Original Research

Research on Crop Ecophysiology
Vol.19/1, Issue 1 (2024), Pages: 30 - 42

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

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Received: 15 September 2023 Accepted: 6 January 2024

ABSTRACT

This research investigates the effects of foliar spraying of biological stimulants on the growth characteristics and antioxidant capacity of lavender in Najafabad City in 2024. The treatments evaluated included seaweed (1, 2, and 3 ml/L), amino acids (1, 3, and 5 ml/L), and Crecer organic fertilizer (5, 10, and 15 ml/L), conducted in three replicates alongside a control treatment. The measured traits included the number, length, fresh weight, and dry weight of flowering stems, as well as the antioxidant capacity of the pure flowers. The results indicate that foliar spraying with biological stimulants significantly improved vegetative traits and antioxidant capacity compared to the control treatment. The highest number of flowering stems (359 stems) was observed with the seaweed treatment at 3 ml/L. The longest flowering stems (27.70 cm) were associated with the Crecer organic fertilizer at 10 ml/L. Additionally, the highest fresh weight (0.067 g) and dry weight (0.027 g) were recorded for the amino acid treatment at 1 ml/L. The control treatment exhibited the highest antioxidant capacity at 71.92%, closely followed by the amino acid treatment at 3 ml/L, which showed an antioxidant capacity of 71.81%. In contrast, the control treatment recorded the lowest values for vegetative traits, while the seaweed treatment at 1 ml/L exhibited the lowest antioxidant capacity at 42.14%. In conclusion, the application of biological stimulants positively influenced vegetative traits, particularly highlighting the amino acid treatment at 3 ml/L in enhancing antioxidant capacity

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

INTRODUCTION

Plants play a central role in human diets and medicinal compounds, garnering increased attention and study in recent years. The World Health Organization reports that over 80 percent of the global population relies on traditional and herbal medicine for disease treatment (Wang *et al.,* 2016). Medicinal plants comprise a diverse range of species containing effective substances that aid in the prevention and treatment of diseases. The utilization of medicinal plants is one of humanity's earliest achievements in healthcare. Throughout the development of human societies and civilizations, a close relationship has persisted between plants and humans (Rodríguez-García *et al*., 2007).

The global trend towards herbal medicine has heightened interest in identifying medicinal plants, encouraging a return to nature. Iran, rich in diverse flora, hosts many unique plants known for their therapeutic properties. Given Iran's potential and climatic diversity, research and development in this sector can significantly contribute to economic growth and entrepreneurship.

Due to the detrimental effects of environmental degradation resulting from the indiscriminate use of chemical inputs, there is a growing necessity to focus on alternative cultivation methods. One key aspect of sustainable agriculture is the application of biological fertilizers in agricultural ecosystems, aiming to replace and reduce chemical fertilizers (Zand et al., 2017).

*Lavandula angustif*olia L. is a perennial woody plant from the Lamiaceae family, native to southern Europe. It thrives in light sandy soils at altitudes up to 1700 meters above sea level, particularly in southern and central Italy, Greece, southern France, and Spain (Bernath, 1993; Currie, 1980; Prusinowska and Smigielski, 2014).

Biological fertilizers consist of various free-living microorganisms that convert primary nutrients from unavailable forms to accessible ones (Rajendran & Devaraj, 2004). Some of these microorganisms are beneficial to plant growth and are classified as Plant Growth-Promoting Rhizobacteria (PGPR) (Abdul Jaleel *et al.,* 2007). These bacteria, found in the rhizosphere, stimulate plant growth and development (Girish & Umesha, 2005).

Amino acids and other nutrients, such as humic acid, hydrolyzed proteins, and extracts from algae and marine plants, form the basis of biological growth stimulants (Gawronaka, 2008; Thomas *et al.,* 2009; Niyogi & Fink, 1992). Biostimulants can be categorized into eight groups: organic compounds, humic substances, beneficial chemical elements, inorganic salts (e.g., phosphites), chitin and chitosan derivatives, seaweed extracts, antiperspirants, and nitrogen compounds (Hakimi *et al.,* 2019). Amino acids, a prominent group of biostimulants, positively affect plant growth, enhance yield, and reduce damage from abiotic stresses (Kahlel & Soltan, 2019). They are crucial for metabolic processes, structural functions, and the synthesis of plant hormones, ultimately improving nutrient absorption and crop quality (Calvo *et al*., 2014). Additionally, amino acids enhance respiratory processes, chlorophyll concentration, and overall plant growth (Sanikhan *et al.,* 2019).

Seaweed extracts, which promote growth, are environmentally friendly alternatives to chemical fertilizers, being non-toxic and preventing environmental degradation (Del Poso *et al.,* 2007). Rich in growth hormones such as auxins and cytokinins, vitamins, and micronutrients, seaweed extracts stimulate root growth, delay aging, and enhance tolerance to environmental stress (Zodape *et al*., 2011).

Reactive oxygen species (ROS) are naturally produced during various metabolic pathways and play crucial roles in cellular signaling (Aruoma & Cuppette, 1997). However, excessive

ROS can damage cellular components, including proteins, nucleic acids, phospholipids, and membranes (Valko *et al*., 2007). Antioxidants, essential compounds that protect cells from oxidative damage, can be categorized as enzymatic (e.g., superoxide dismutase, catalase, glutathione peroxidase) or non-enzymatic. Significant antioxidant properties have been documented in plant extracts, attributed to various secondary metabolites, including tocopherols, folic acid, ascorbic acid, carotenoids, and polyphenols (Zhang *et al*., 2006; Yu *et al*., 2002).

MATERIALS AND METHODS

This research was conducted in 2024 at a private farm in Najafabad City, Isfahan Province, located at 32°38'N latitude and 51°21'E longitude, with an altitude of 1600 meters above sea level. A randomized complete block design was utilized, consisting of three treatments at three concentration levels and three replicates. A control treatment (distilled water) was included, resulting in a total of nine treatment combinations, with each treatment replicated three times, yielding 27 plots of 15 plants each.

The studied plants were four-year-old lavender, with 15 plants allocated for each replicate. The spacing was 40 cm between plants within a row and 80 cm between rows, utilizing a drip irrigation system with weekly irrigation cycles. The treatments included: 1) amino acids at three concentrations (1, 3, and 5 ml/L), 2) seaweed at three concentrations (1, 2, and 3 ml/L), 3) Crecer organic fertilizer at three concentrations (5, 10, and 15 ml/L), and 4) the control treatment (distilled water), all applied via foliar spraying. Treatments were administered in the spring of 2024, following winter dormancy, with two applications spaced 14 days apart. Data collection occurred on June 9, 2024, one month post-second spraying.

For growth assessment, the number of flowering stems (including ready-to-harvest flowers and buds) was recorded, and the branches were harvested. The lengths of randomly selected branches were measured with a caliper, and the weight of 100 branches per treatment was recorded using a digital scale (precision to 0.001 g). The harvested flowers were dried in an oven at 37°C for 48 hours, after which their dry weight was recorded.

Antioxidant measurement method

To measure antioxidant capacity, 5 grams of pure flowers from each replicate were mixed with 10 ml of 80% ethanol, followed by centrifugation at 4°C and 5000 rpm for 10 minutes. A 1:1 solution of the supernatant and DPPH (0.1 M) was prepared, and absorbance was measured at 517 nm using a spectrophotometer. The antioxidant capacity was calculated as the percentage of DPPH inhibition (Hosseini *et al*., 2019).

RESULTS

Sampling of dried flowers and leaves of the lavender medicinal plant was conducted to measure growth characteristics, including the number of flowering branches, branch length, fresh weight, dry weight, and antioxidant levels.

Vegetative traits

Results from statistical analyses, 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 different treatments is illustrated in Figure 1. Variance analysis indicated that the effect of treatments on the number of flowering stems was significant at the 5% probability level (Table 1). The comparison of means revealed that the highest number of flowering stems (359) was associated with the S3 treatment (seaweed at 3 ml/L), while the lowest number (213) was found in the control treatment (distilled water). Other treatments did not show significant differences (Figure 1).

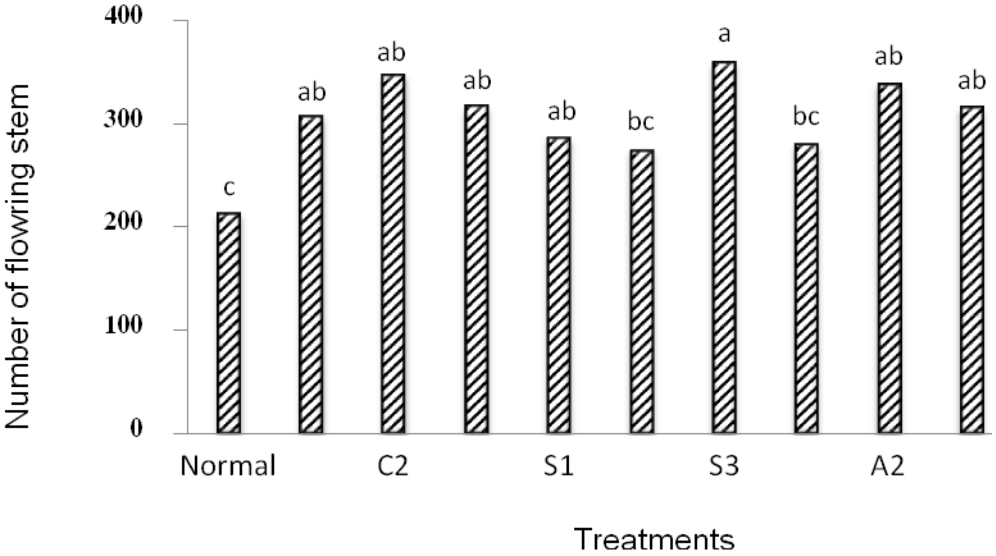

Figure 1. Number of flowering stem with different treatments (N:Normal, C:Cercer, S:Seaweed, A:Amino acid)

The average length of flowering stems under the various treatments is illustrated in Figure 2. Variance analysis indicated that the effect of different treatments on stem length was significant at the 1% probability level (Table 1). Furthermore, comparison of the average data revealed that the highest stem length (27.70 cm) was associated with the C2 treatment (Crecer organic fertilizer at a concentration of 10 ml/L), while the lowest stem length (18.71 cm) was observed in the control treatment (distilled water). No significant differences were noted among the other treatments, as shown in Figure 2.

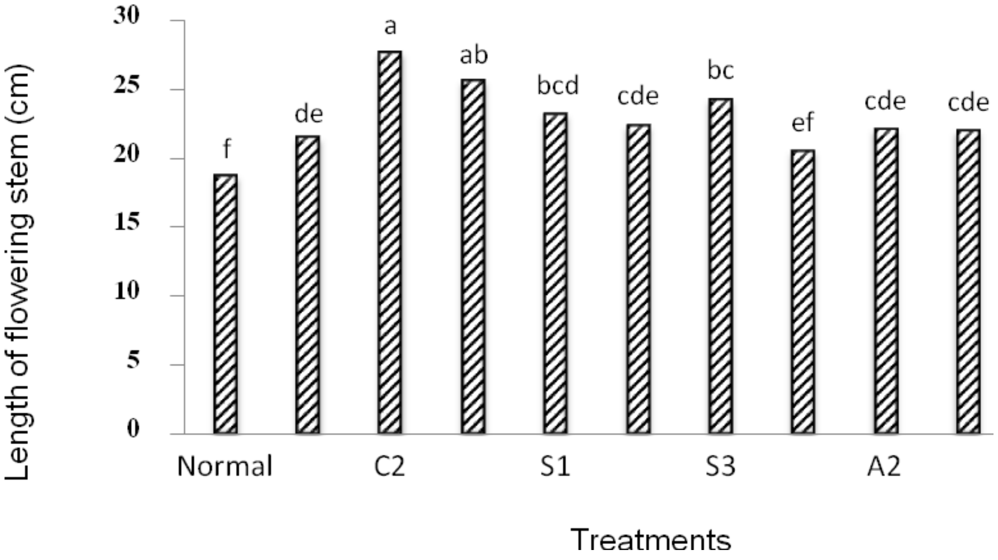

Figure 2. Length of flowering stem(cm) with different treatments (N:Normal, C:Cercer, S:Seaweed, A:Amino acid)

The average fresh weight of flowering stems under the various treatments is presented in Figure 3. Variance analysis revealed that the effect of different treatments on the fresh weight of flowering stems was significant at the 1% probability level (Table 1). Comparison of the average data indicates that the highest fresh weight (0.067 grams) was associated with treatment A1 (amino acid at a concentration of 1 ml/L), while the lowest fresh weight (0.038 grams) was observed in the control treatment (distilled water). Additionally, treatments C2 (Crecer organic fertilizer at 10 ml/L) and C3 (Crecer organic fertilizer at 15 ml/L) showed fresh weights of 0.056 grams and 0.054 grams, respectively, along with treatment S2 (seaweed at 2 ml/L), which also recorded a fresh weight of 0.054 grams. A significant difference was noted among these treatments. The fresh weight gain for treatments C3 and S2 was equivalent at 0.054 grams, as depicted in Figure 3.

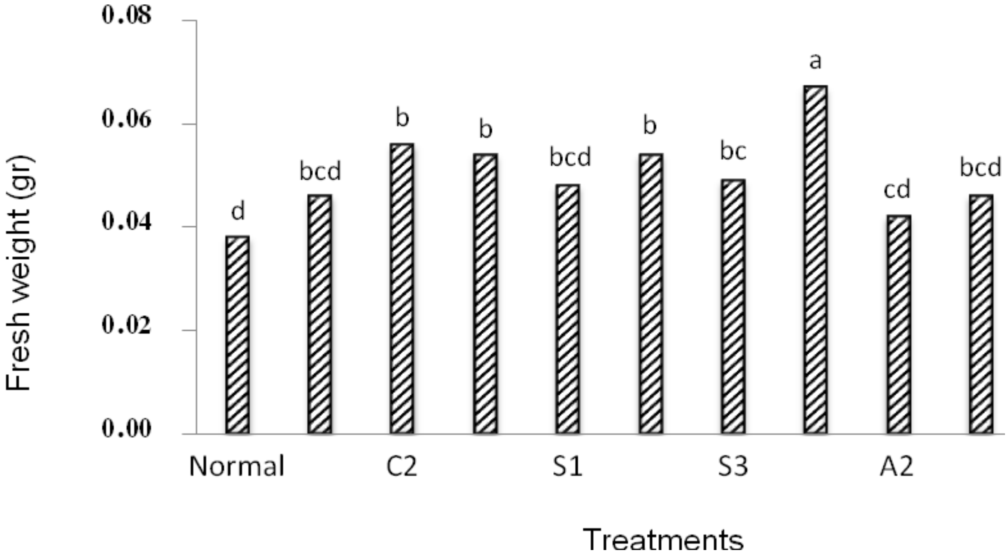

Figure 3. Fresh weight(g) with different treatments (N:Normal, C:Cercer, S:Seaweed, A:Amino acid)

The average dry weight of flowering stems treated with different sprays is illustrated in Figure 4. Variance analysis indicated that the effect of different treatments on the dry weight of flowering stems was significant at the 1% probability level (Table 1). Comparison of the average data shows that the highest dry weight (0.027 grams) was associated with treatment A1 (amino acid at a concentration of 1 ml/L), while the lowest dry weight (0.018 grams) was recorded in the control treatment (distilled water). No significant differences were observed among the other treatments, as depicted in Figure 4.

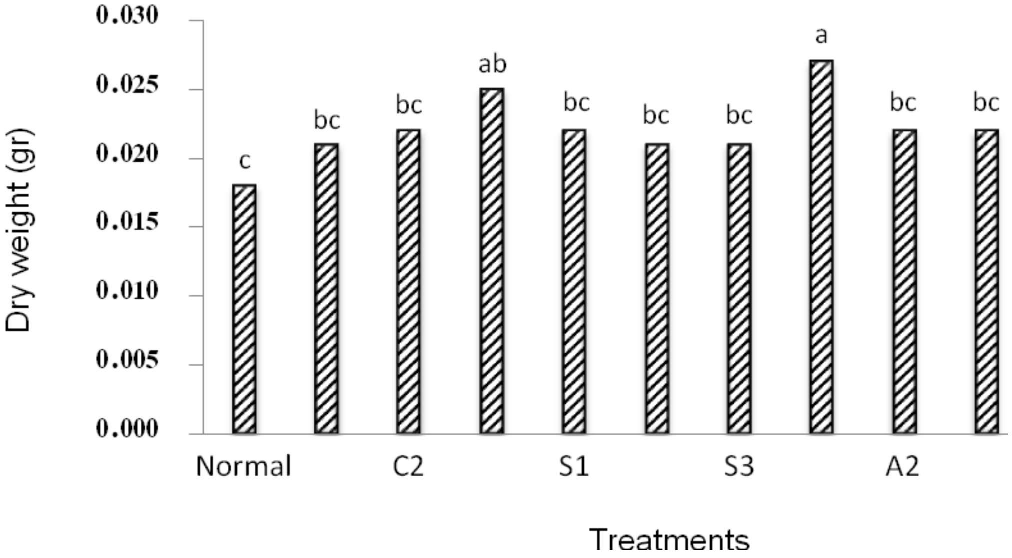

Figure 4. Dry weight(g) with different treatments (N:Normal, C:Cercer, S:Seaweed, A:Amino acid)

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

		Mean square
Source of variation	Degree of freedom	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 antioxidant capacity of pure flowers under the different treatments is illustrated in Figure 5. Variance analysis revealed that the effect of the various treatments on the antioxidant levels was significant at the 1% probability level (Table 2). Comparison of the average data indicates that the highest antioxidant capacity was found in the control treatment (distilled water) at 71.92%, closely followed by treatment A2 (amino acid at a concentration of 3 ml/L) at 71.81%. Conversely, the lowest antioxidant content was observed in treatment S1 (seaweed at a concentration of 1 ml/L) at 42.14%. No significant differences were detected among the other treatments, as shown in Figure 5.

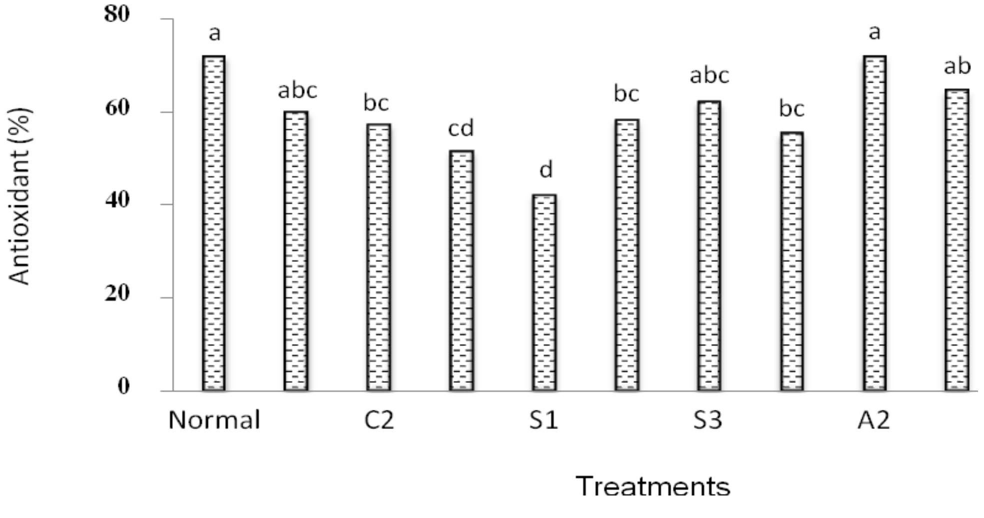

Figure 5. Antioxidant value(%) with different treatments (N:Normal, C:Cercer, S:Seaweed, A:Amino acid)

DISCUSSION

The number of flowering stems per plant was assessed, revealing that the highest count was achieved with the application of seaweed treatment at a concentration of 3 ml/L (S3), resulting in 395 stems. This was followed by the application of Crecer organic fertilizer at 10 ml/L (C2), which produced 346.7 flowering stems, ranking second. The amino acid treatment at 3 ml/L (A2) also demonstrated a positive effect, yielding 338.3 flowering stems per plant. All treatments showed significant differences compared to the control (distilled water), which had the lowest production at 213 stems. Thus, the application of seaweed notably enhanced the number of flowering stems compared to the control treatment.

Regarding stem length, the maximum value was recorded for the Crecer organic fertilizer treatment at 10 ml/L (C2), measuring 27.70 cm, while the 15 ml/L concentration (C3) followed with 25.63 cm. The control treatment yielded the shortest stem length at 18.71 cm. These findings indicate that all treatments—Crecer organic fertilizer, seaweed, and amino acids—significantly increased the flowering stem length in lavender plants.

In terms of fresh weight, the highest value was associated with the amino acid treatment at 1 ml/L (A1), which registered 0.067 grams. Following this, the Crecer organic fertilizer at 10 ml/L (C2) had a fresh weight of 0.056 grams, and both the 15 ml/L (C3) and 2 ml/L seaweed treatment (S2) recorded 0.054 grams. The control treatment yielded the lowest fresh weight at 0.038 grams. Similarly, for dry weight, the amino acid treatment at 1 ml/L (A1) also had the highest value at 0.027 grams, followed by Crecer organic fertilizer at 15 ml/L (C3) at 0.025 grams, and 10 ml/L (C2) at 0.022 grams. The control group again had the lowest dry weight at 0.018 grams. These results confirm that the various treatments significantly enhanced both fresh and dry weights of the flowering stems.

Previous research supports these findings, indicating that biological fertilizers can improve growth and increase fresh and dry weight in plants, such as hyssop (Koocheki *et al*., 2008). The use of organic compounds has been shown to significantly enhance the yield, growth, and chemical composition of other medicinal and aromatic plants, including Ocimum (El-Naggar *et al*., 2015) and Matricaria (Salehi *et al*., 2016). Furthermore, studies indicate that amino acids positively affect vegetative growth and biochemical traits in medicinal plants. For instance, foliar spraying with L-tryptophan at concentrations of 50 and 100 ppm resulted in significant growth and yield increases in lavender (Elshorbagy *et al*., 2020). The application of amino acid fertilizers has also been effective in enhancing morphological traits and performance in peppermint (Asadi *et al*., 2018).

Additionally, the external application of the amino acid glycine in hyssop has been shown to mitigate stress from irrigation deficits and enhance overall growth (Khajahhosseini *et al*., 2020). Research indicates that various amino acids can significantly improve plant height, branching, dry and wet weights, as well as the yield of bioactive compounds (Wahba *et al*., 2015). In the context of seaweed applications, studies have demonstrated its role in improving growth, photosynthetic efficiency, and biochemical traits, particularly under salinity stress (Korkmaz & Cicek, 2024). Specifically, the highest values for plant height, leaf area, dry weight, and essential oil yield in hyssop were obtained with 10 ml/L of seaweed fertilizer (Pirani *et al.*, 2020). These findings are consistent with the results of this research, highlighting the beneficial effects of biological stimulants on lavender growth.

Antioxidant

The antioxidant activity in lavender flowers varied significantly among treatments. The control treatment (water spray) recorded the highest antioxidant level at 71.92%, closely followed by the amino acid treatment at 3 ml/L (A2) with 71.81%. Notably, no significant difference was found between these two treatments. Conversely, the lowest antioxidant level was observed in the seaweed treatment at 1 ml/L (S1), which measured 42.14%. These findings suggest that the application of various treatments, including Crecer organic fertilizer, amino acids, and seaweed, may reduce the antioxidant content in lavender flowers.

Prior studies have reported positive effects of amino acids on the antioxidant activity of medicinal plants. For instance, foliar spraying with a concentration of 1 mg/L in Salvia resulted in enhanced antioxidant activity (Bahadour *et al.*, 2024). In another study, Achillea plants treated with 3 ml/L of amino acids exhibited a 17% increase in antioxidant activity compared to controls (Shafie *et al.*, 2021). Similarly, seaweed applications have been shown to enhance antioxidant capacity in plants such as Achillea (Shafie *et al.*, 2021) and Foeniculum vulgare (Rasouli *et al.*, 2023). Furthermore, organic fertilizers have positively influenced antioxidant activity in species like Mesembryanthemum edule L. (Fallah *et al.*, 2018), Marrubium vulgare L. (Zahedifar & Najafian, 2023), and Ocimum basilicum (Nguyen *et al.*, 2010).

The enhancement of antioxidant activity due to organic fertilizers is attributed to their positive impact on soil physical and chemical properties, as well as increased organic matter content, which improves nutrient availability and absorption (Nguyen *et al.*, 2010). This contrasts with our findings, where all treatments, despite providing essential nutrients, did not enhance antioxidant activity.

Additionally, previous research indicates that the application of amino acids, seaweed, and fulvic acid organic fertilizer did not affect the antioxidant activity in sage, with the control treatment yielding the highest values (Farruggia *et al.*, 2024), consistent with our observations. The synthesis of secondary metabolites, which includes antioxidants, is influenced by various internal and external factors (Rajabi *et al.*, 2014; Ben Akacha *et al.*, 2023). Stress conditions are known to stimulate the production of these metabolites (Figueiredo *et al.*, 2008; Kulak *et al.*, 2020). Therefore, it is plausible that the treatments provided optimal growth conditions, thereby reducing the synthesis of certain secondary metabolites and resulting in lower antioxidant activity.

Research focusing on nitrogen and phosphorus levels in lavender highlighted that nitrogen concentrations below 200 mg/L and phosphorus below 60 mg/L positively influenced antioxidant activity (Chrysargyris *et al.*, 2016). Moreover, an increase in antioxidant activity was noted at a nitrogen dose of 50 kg/ha, while higher doses (100 and 200 kg/ha) led to a decrease in antioxidant levels (Beisiada *et al.*, 2008). Furthermore, a study in Brazil identified a specific sequence for nutrient accumulation in lavender, indicating that optimal nutrient levels are critical for maximizing biochemical traits, particularly antioxidants

(K>N>Ca>Mg>P>S for macronutrients and Fe>Mn>Zn>B>Cu>Ni>Mo for micronutrients) (Pecanha *et al*., 2021).

Given the complex interactions of nutrients, it is possible that increased absorption of certain nutrients from organic fertilizers, seaweeds, and amino acids may have interfered with the absorption of others, thereby impacting antioxidant production. The observed decrease in antioxidant levels, coupled with discrepancies between our findings and other studies that report increased antioxidant production, may stem from differences in plant species, genetic characteristics, environmental conditions, and experimental timelines that influence secondary metabolite synthesis.

CONCLUSION

This research demonstrated that the application of biological stimulants significantly impacts various growth traits in lavender plants. The treatments resulted in increased vegetative and reproductive growth compared to the control group, likely due to enhanced availability and absorption of essential nutrients. However, the control treatment exhibited a higher antioxidant capacity than the treated groups, indicating that the application of these stimulants may have reduced antioxidant levels.

It is possible that the nutrient balance was disrupted by the addition of high nutrient concentrations or growth hormones, leading to increased absorption of some elements at the expense of others. This imbalance may have contributed to the observed decrease in antioxidant capacity. Nonetheless, other biochemical traits, including phenolic compounds, chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids, generally exhibited an increasing trend with the application of biological stimulants compared to the control.

Overall, the use of biostimulants—specifically amino acids, seaweed, and Crecer organic fertilizer—effectively enhanced growth characteristics in lavender. Notable results included the highest number of flowering stems (359) from the seaweed treatment at 3 ml/L, the maximum flowering stem length (27.70 cm) associated with Crecer organic fertilizer at 10 ml/L, and the highest fresh (0.067 g) and dry weights (0.027 g) linked to amino acid treatment at 1 ml/L. Conversely, the lowest antioxidant capacity was found in the seaweed treatment at 1 ml/L (42.14%). The most favorable outcomes across all traits were observed with the amino acid treatment at a concentration of 3 ml/L. Ultimately, while the biostimulants enhanced growth characteristics, they did not significantly improve antioxidant levels compared to the control.

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