Selcuk Journal of Agriculture and Food Sciences

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Research Article

SELCUK UNIVERSITY PRESS

(2024) 38(2), 356-367 DOI:10.15316/SJAFS.2024.032 e-ISSN: 2458-8377 Selcuk J Agr Food Sci

Use of An Animal-Derived Biostimulant for Alleviating the Effects of Drought Stress on Sugar Beet

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HIGHLIGHTS

- Recent studies have focused on the ability of biostimulants to alleviate plant stress.
- Sugar beet is extremely sensitive to the lack of water during the early growth stages.
- Foliar biostimulant applications may reduce the adverse effects of drought stress to the growth of sugar beet seedlings.
- The beneficial effects of animal-derived biostimulant were determined to mitigate drought stress.

Abstract

This study focused on mitigating effects of an animal-derived biostimulant on sugar beet plantlets subjected to drought stress. The experiment was performed at the Seed Science and Technology Laboratory of the Eskişehir Osmangazi University, Faculty of Agriculture, Department of Field Crops, in 2024. It was established by the randomized plot 2×5 factorial experimental design (ANOVA) with four replications. The sugar beet cultivar Mohican was sprayed by an animal-derived biostimulant (Andolamin®) containing 11% amino acids. Different levels of the biostimulant (control, 12.5, 25, 50, and 75 mL/L) were treated twice at 2-day intervals. Morphological and physiological measurements were made at 7 days after the first application on sugar beet plants grown under two irrigation regimes (water deficit (WD) 50% of field capacity and well-watered (WW) 80% of field capacity). The findings showed that drought had a hazardous impact on sugar beet's number of leaves (NL), fresh (LFW) and leaf dry weight (LDW), relative water content (RWC), and leaf area (LA). Leaf surface temperature (LST), chlorophyll content (Chl), and electrolyte leakage (EL) were higher in plants under water deficit. Foliar biostimulant application mitigated the effect of drought stress on seedlings through improving LFW, LDW, Chl, EL, and LA. On the other hand, biostimulant treatment had no significant effects on NL, and RWC in seedlings exposed to drought stress. It was concluded that animal-derived biostimulant application may be used for alleviating the harmful effects of drought stress and may stimulate the growth of sugar beet seedlings.

Keywords: *Beta vulgaris* L.; drought stress; tolerance; biostimulant

Citation: Kulan EG, Kaya MD (2024). Use of an animal-derived bio stimulant for alleviating the effects of drought stress on sugar beet. *Selcuk Journal of Agriculture and Food Sciences*, 38(2), 356-367. https://doi.org/10. 15316/SJAFS.2024.032
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Received date: 05/12/2023
Accepted date: 01/08/2024

1. Introduction

The sugar beet *(Beta vulgaris* L.), a member of the *Chenopodiaceae* family, is a biennial plant adapted to arid and semi-arid climates (Khan et al. 2018). Its optimum development occurs at moderate or cool temperatures, typically between 10 and 20 °C (Lombardi et al. 2022). The crop has multiple uses in manufacturing industries, including sugar production (Mall et al. 2021).

Around the world, a wide range of environmental stresses such as heat, salt, flooding, and drought have an adverse impact on every stage of crop development during the life cycle (Ghadirnezhad Shiade et al. 2023). Drought affects approximately fifty percent of the earth's arid and semi-arid areas (Nadeem et al. 2019) and is a prolonged period of drastically less rainfall in a certain region (Saeidnejad et al. 2016). A shortage of water may occur from many factors, including insufficient rainfall, unequal amounts of precipitation, the intensity and duration of droughts, and the rate of stress accumulation (Pessarakli and Marcum 2013). In response to drought conditions, plants reduce leaf pigments and close stomata reducing photosynthesis, growth, and other important physiological and biochemical processes (Yan et al. 2016; Saudy et al. 2021; McDowell et al. 2022; Pepe et al. 2022) and have a considerable detrimental impact on plant growth, development, and production (Muhammad Aslam et al. 2022; Yahaya and Shimelis 2022).

Many field crops have a higher vulnerability to low temperatures and drought, particularly during germination and seedling growth. Drought stress hinders seed imbibition, thereby inhibiting germination (Islam et al. 2018). Also, it limits the beet yield (Monreal et al. 2007) and quality (Nause et al. 2020) of sugar beet production. Lack of water availability leads to reduced growth and yield (Salem et al. 2021). This occurs due to the generation of reactive oxygen substances (ROS), which begin lipid peroxidation of membranes and interaction with other macromolecules (Bistgani et al. 2017). Sugar beet is extremely affected by water limitations, mostly during its early growth stages, and there is a positive correlation between consumption of water and root yield. Also, pathogenic and natural factors can cause seedling damage, hence delaying seedling emergence prolongs the critical stage of growth and increases the risk. Genetic and environmental factors, as well as the effects of seed pre-treatment and seeding applications, strongly influence the stages of germination and seedling development in sugar beet (Helaly et al. 2009).

Different groups classify plant biostimulants, including seaweed extracts, humic and fulvic acids, beneficial chemical elements, chitin and chitosan, microorganisms, inorganic salts, peptides, and amino acids (Du Jardin 2015; Rouphael and Colla 2020). These biostimulants promote plant growth by stimulating natural processes like nutrient uptake performance and stress resistance (Abdel Latef et al. 2021; Attia et al. 2021). Also, they have practical applications in agriculture (Bulgari et al. 2019). Applying biostimulating substances in small quantities, either to the soil or to the leaves of plants, can have a beneficial impact on plant growth (Barone et al. 2019). These substances encourage the development of both roots and shoots, enhancing the rate of photosynthesis, and improving the quality of crops such as beans (Abrantes et al. 2011; Latif and Mohamed 2016), pepper (Palangana et al. 2012), wheat (Hammad and Ali 2014; Farooq et al. 2017), fenugreek (Abdel Latef et al. 2017), tomato (Colla et al. 2017), soybean (Kocira et al. 2018), and sorghum (Ahmad et al. 2016). Biostimulants improve the capacity of plants to tolerate drought stress (Ertani et al. 2013; Colla et al. 2015). Paul et al. (2019) documented enhanced tomato growth in response to biostimulant treatment during periods of drought induced. Hence, products containing biostimulant properties appear as an effective choice for combining seedling production. This leads to the growth of strong seedlings with optimal nutrient levels and well-established root systems. Additionally, these products promote an increase in leaf area and number, thereby enhancing photosynthetic capacity. Consequently, these seedlings demonstrate optimum growth, ultimately resulting in high-quality crop yields. (Bettoni et al. 2022).

2. Materials and Methods

An experiment was conducted at the Seed Science and Technology Laboratory of the Eskişehir Osmangazi University, Faculty of Agriculture, Department of Field Crops, in 2023. Seeds of cultivar Mohican were sown in vials filled with a mix of soil:peat:perlit:vermiculite (4:5:1:1). When the seedlings reached 2 true leaves, they were transplanted to plastic cups (75×95 mm) and irrigated at field capacity. The plants were grown in a climate chamber at 24/18 °C with 16 hours of light / 8 hours of darkness. Andolamin® was used as an animal-derived biostimulant. The product is composed of 55% organic matter; 25% organic carbon; 8.5% organic nitrogen; and 11% free amino acids, with a pH of 5.8-7.8. The biostimulant application was carried out at intervals of 2 days, the first being at 27 days after sowing (DAS), via a foliar spray. At 34 DAS, all seedling samples of each replication were selected, and the following characters were evaluated as the number of leaves (NL), leaf fresh weight (LFW), leaf dry weight (LD), leaf surface temperature (LST), chlorophyll content (Chl), relative water content (RWC), electrolyte leakage (EL), and leaf area (LA). For LFW, samples were washed and weighed on an analytical balance. For the determination of LDW, samples were placed in an electric oven at 70°C ± 5°C for 48 h, then they were calculated. LST was determined by an infrared thermometer. Chl was read using a Konica Minolta SPAD-502 meter on the 3rd or 4th leaf of the seedlings. RWC was determined from two plants in each replicate, specifically from the mature leaves. Two leaves were taken from all replicates and promptly measured to determine the fresh weight (FW). Samples were soaked in distilled water in a falcon tube for 24 hours to regain turgidity and then the turgor weight (TW) was measured. The leaf samples were put in an air oven at 70°C for 48 h in order to determine the dry weight (DW). RWC of the leaves was detected in the formula by described Ghoulam et al. (2002), RWC (%) = (FW – DW)/(TW – DW) × 100 (Eq. 1). EL was analyzed by using the four discs of young leaf from each treatment. The leaf samples were washed with deionized water to get rid of any electrolytes remaining on the leaf surface. Four leaf disks with a 10 mm diameter were excised, weighed, and placed into glass tubes filled with 20 mL of deionized water. After the incubation period of 24 h at 25°C, the solution's EC (Lt) was directly read by the EC meter. Then, they were taken into the water bath for 45 minutes at 90°C, and the EC (Lo) was recorded again at 25°C after equilibration (Yadav et al. 2012). The electrolyte leakage was calculated by the formula (Ghoulam et al. 2002): Electrolyte leakage (%) = (Lt / Lo) × 100 (Eq. 2). After all leaves were cut and scanned in the scanner, leaf area (LA) measurements were calculated with the use of the Image J® software program.

The plots included two irrigation regimes of water deficit (WD) (50% of field capacity) and well-watered (WW) (80% of field capacity), and five doses of an animal-derived biostimulant (control, 12.5, 25, 50, and 75 mL/L). The Levene test was used to test for homogeneity. Data given in percentages were subjected to an arcsine transformation before statistical analysis. The data were analyzed by the randomized plot 2×5 factorial experimental design (ANOVA) with four replications. The MSTAT-C (Freed et al. 1991) statistical program was used for the analysis of variance, and a comparison of the means was performed using the Least Significant Difference (LSD) test ($p<0.05$).

3. Results

For all characteristics except LST, there was a significant difference between the levels of drought, but biostimulant doses were significant for LFW, LDW, LST, Chl, EL, and LA (Table 1). Under drought stress, the NL, LFW, LDW, RWC, and LA were significantly suppressed; however, increasing biostimulant doses enhanced the LFW, LDW, RWC, EL, and LA. There was a significant interaction between drought stress and biostimulant doses for the morphological traits, except for NL. The highest (6.30 number per plant) and lowest (5.80 number per plant) NL were measured at a biostimulant dose of 25 mL/L, without and with drought stress, respectively. Sugar beet seedlings in well-watered conditions had a larger leaf size than in plants under drought (Fig. 1). LFW and LDW increased with gradually increasing biostimulant doses, and significant differences were determined (Fig. 2). Drought stress influenced all the physiological parameters of sugar beet seedlings. LST, Chl, and EL were higher in drought-stressed plants. The minimum Chl (53.2 SPAD) was recorded in seedlings applied with the lowest dose of biostimulant and it was enhanced by increasing doses, reaching the maximum value with 55.3 SPAD at a biostimulant level of 50 mL/L. Drought reduced RWC of leaves; however, increased biostimulant doses led to linearly induced RWC. For the EL, a significant interaction was observed (Fig. 3). The EL, with the minimum values (19.3% in drought and 13.9% in well-water) recorded at 25 mL/L of biostimulant, showed that higher ELs were observed under drought stress. Sugar beet seedlings produced a larger LA per plant as the biostimulant dose increased it considerably (Fig. 4). The LA per plant increased up to a dose of 50 mL/L, but it dropped at 75 mL/L, which was considered as overdose.

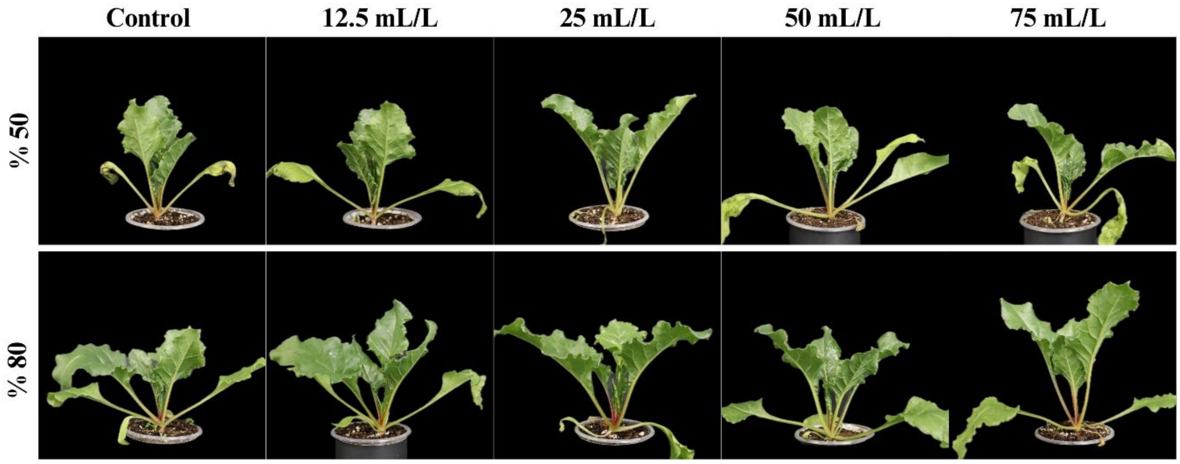

Figure 1. Front views of seedlings sprayed with different biostimulant doses under drought (50% of field capacity) and well-watered (80% of field capacity) conditions.

Table 1. Means and analysis of variance for the number of leaves, leaf fresh and dry weights, leaf surface temperature, chlorophyll content, relative water content, electrolyte leakage, and leaf area of sugar beet plants subjected to different biostimulant doses under well-watered and drought stress.

	Number of Leaves (number plant^{-1})	Leaf Fresh Weight (mg plant^{-1})	Leaf Dry Weight (mg plant^{-1})	Leaf Surface Temparature (°C)	Chlorophyll Content (SPAD)	Relative Water Content (%)	Electrolyte Leakage (%)	Leaf Area (cm^2)
Drought Stress (A)								
50%	5.40^{b} ± 0.13	4357^{b} ± 99.5	392^{b} ± 10.0	25.2 ± 0.15	60.9^{a} ± 0.55	63.4^{b} ± 0.55	22.9^{a} ± 0.63	93^{b} ± 2.30
80%	6.15^{a} ± 0.08	6658^{a} ± 64.1	518^{a} ± 10.0	25.0 ± 0.09	48.4^{b} ± 0.66	74.6^{a} ± 0.34	15.7^{b} ± 0.72	143^{a} ± 1.49
Biostimulant Dose (B)								
Control	5.88 ± 0.22	5375^{c} ± 509	457^{a} ± 36.4	25.1^{bc} ± 0.10	55.9^{a} ± 1.94	68.7 ± 2.27	18.6^{b} ± 2.18	115^{b} ± 8.71
12.5 mL/L	5.63 ± 0.26	5512^{bc} ± 537	464^{ab} ± 34.8	24.9^{cd} ± 0.15	53.2^{c} ± 2.58	68.8 ± 2.14	20.1^{a} ± 2.05	119^{b} ± 10.9
25 mL/L	6.00 ± 0.19	5650^{b} ± 377	489^{a} ± 15.3	25.3^{b} ± 0.15	54.8^{ab} ± 3.42	69.8 ± 2.29	16.6^{c} ± 1.04	120^{b} ± 10.4
50 mL/L	5.88 ± 0.23	5882^{a} ± 354	465^{ab} ± 18.4	24.5^{d} ± 0.10	55.3^{ab} ± 1.17	69.5 ± 1.57	21.1^{a} ± 0.46	126^{a} ± 6.55
75 mL/L	5.50 ± 0.19	5118^{d} ± 422	402^{c} ± 22.4	25.7^{a} ± 0.18	54.1^{bc} ± 3.03	68.4 ± 2.78	20.5^{a} ± 1.91	109^{c} ± 10.7
Analysis of Variance								
A	<0.01**	<0.01**	<0.01**	0.11ns	<0.01**	<0.01**	<0.01**	<0.01**
B	>0.23ns	<0.01**	<0.01**	<0.01**	<0.01**	>0.50ns	<0.01**	<0.01**
A × B	>0.26ns	<0.01**	<0.01**	0.15ns	<0.01**	<0.04*	<0.01**	<0.01**

Means ± SD of replicates connected with the same letter(s) in the same column are not significantly different by the LSD test at p≤0.05. **: significant at 1%; *: significant at 5%; ns: non-significant.

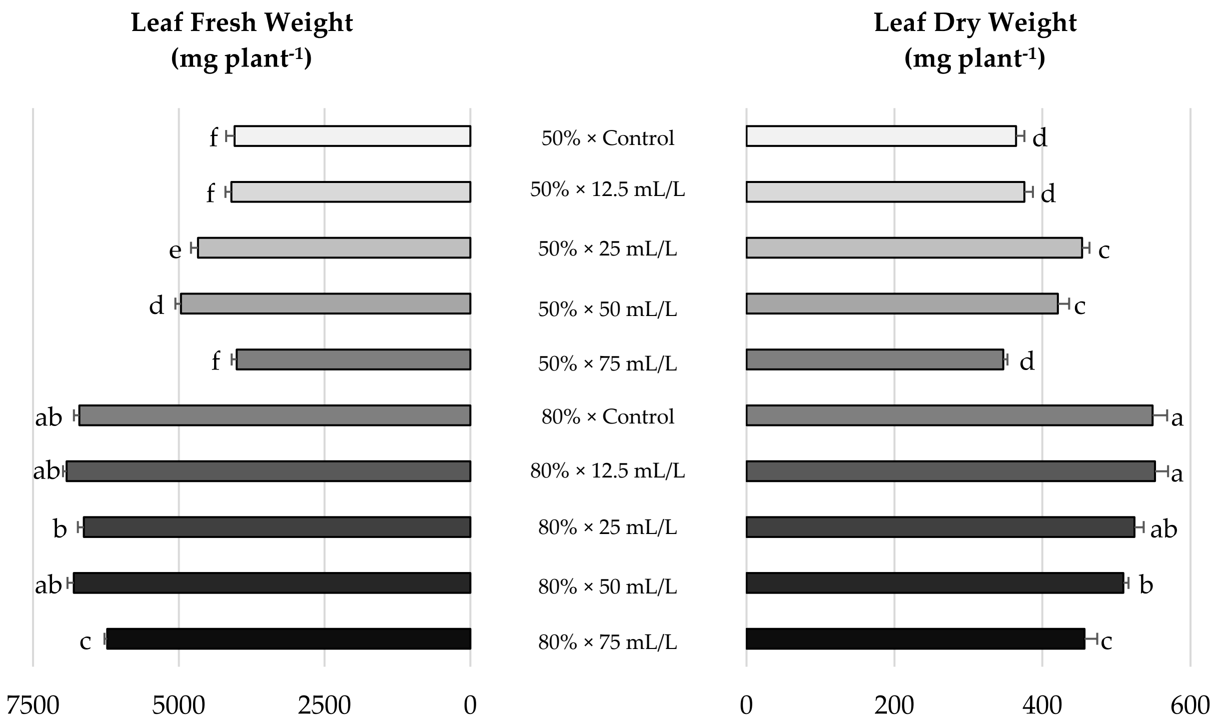

Figure 2. The interaction of drought stress and biostimulant doses on leaf fresh weight and leaf dry weight. Letters on each bar denote the significance level at $p<0.05$.

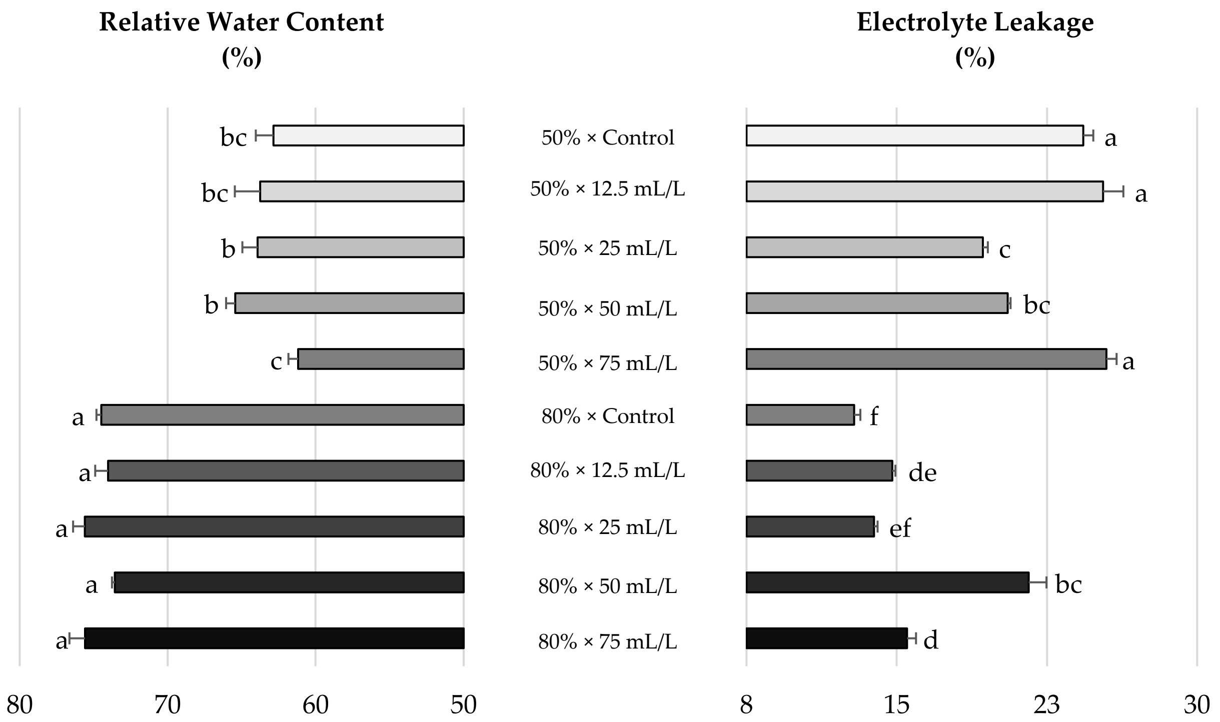

Figure 3. The interaction of drought stress and biostimulant doses on relative water content and electrolyte leakage. Letters on each bar denote the significance level at $p<0.05$.

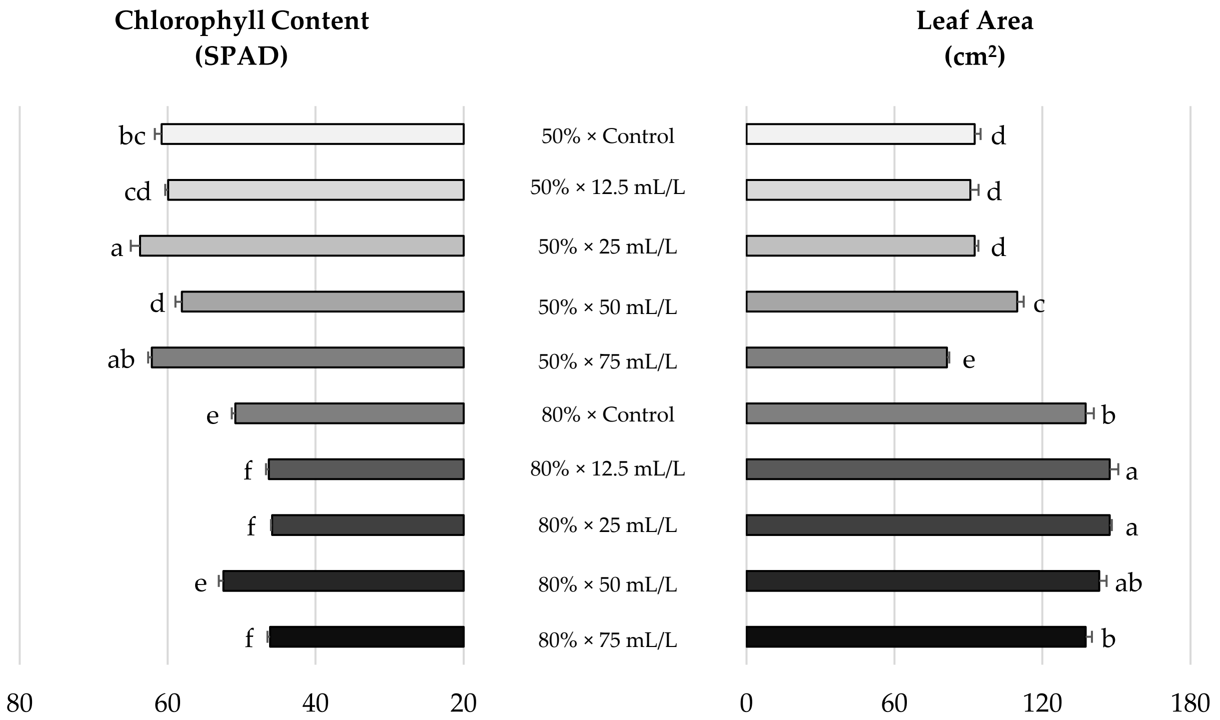

Figure 4. The interaction of drought stress and biostimulant doses on chlorophyll content and leaf area. Letters on each bar denote the significance level at $p<0.05$.

4. Discussion

Drought stress harms plant growth and development at all stages, beginning at sowing and continuing until harvest. Sugar beet is vulnerable to drought stress during every stage of its life cycle. To produce high quality sugar and root yields, sugar beet requires much more water compared to other crops. In this study, sugar beet seedlings were treated with different levels of foliar animal-derived biostimulant to alleviate the hazardous effects of drought stress. In this study, there was a decline in NL, LFW, LDW, RWC, and LA under drought stress. According to Ahmad et al. (2019) and Paponov et al. (2020), the existence of water deficit conditions significantly decreased the NL in maize. Our results showed that LFW and LDW increased with increasing biostimulant doses, and the differences were significant. Similarly, Borcioni et al. (2016) observed that fulvic acid application increased LFW and LDW in lettuce. Santos (2014) observed a significant increase up to 85% in the LDW of plants, while investigating the effects of applying humic substances to the leaves of sweet pepper. Widuri et al. (2018) reported a significant decline in shoot dry weight in common bean under drought conditions. RWC plays an essential role in controlling many physiological processes in plants. The primary indication of the drought stress response is the decrease in RWC (Hussain et al., 2018). A decrease in RWC and LA in sugar beet was demonstrated by Khodadadi et al. (2020). Drought stress generally causes a decline in leaf RWC in barley, according to Istanbuli et al. (2020). The incidence of cell membrane damage and subsequent release of electrolytes from plant cells are significant indicators in the plants exposed to drought stress (ElBasyoni et al. 2017). Similarly, a considerable increase in EL was observed in sugar beet seedlings under drought. Drought stress reduced LA and whole plant biomass, as observed by Kumar et al. (2022). This decline was due to the limitations of leaf growth and the interruption of the photosynthetic process. For many crops, LA considerably reduced under drought stress, such as wheat (Naz and Perveen 2021) and maize (Cai et al. 2020). In this study, foliar animal-derived biostimulant positively induced RWC, EL, and LA. Amino acids may serve as osmoprotectant agents in plants suffering drought stress (Zulfiqar et al. 2020), hence contributing to the improvement of plant tissue's RWC. In our study, animal-derived biostimulant treatments increased the RWC of leaves. Similar results demonstrated an increase in RWC on soybean (Teixeira et al. 2020), tomato (Alfosea-Simon et al. 2020), and broccoli (Kaya 2023) leaves when a biostimulant containing amino acids was applied. In general, drought stress can reduce Chl by harming the membrane and structure of chloroplasts (Shin et al. 2021). In our study, the beneficial effect of animal-derived biostimulant on Chl can be explained by the stimulatory effects of amino acids on chlorophyll biosynthesis (Noroozlo et al. 2019). Many studies showed a significant increase in Chl following treatment with biostimulants in bean (Sadak et al. 2015), soybean (El-Aal 2018), timothy (Radkowski and Radkowska 2018), and cotton (Ergin et al. 2024); however, insignificant differences in Chl were found in potato (Farhad et al. 2011), chamomile (Pirzad et al. 2011), and apple (Ping et al. 2015).

5. Conclusions

The use of biostimulants via foliar spray is a remarkable and new technology that is friendly to the environment and enhances agricultural sustainability. Furthermore, it has the potential for yield and quality improvement in crops under a wide range of conditions. Drought stress induced an important adverse effect on the evaluated morpho-physiological parameters in our experiment. Overall, the application of animal-derived biostimulant by spraying showed a promising effect on the development of sugar beet plantlets under drought stress. Especially at 25 and 50 mL/L doses of the biostimulant, improvements in RWC, EL, and LA characteristic were observed. In conclusion, foliar application of the animal-derived biostimulant may be beneficial to alleviate the negative impacts of drought and may stimulate the growth of sugar beet seedlings.

Author Contributions: Engin Gökhan KULAN planned, established, and conducted the study. Mehmet Demir KAYA analyzed the data obtained in the study, and Engin Gökhan KULAN and Mehmet Demir KAYA wrote the article. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Data Availability Statement: The data that supports the findings of this study are available within the manuscript.

Acknowledgments: The authors are thankful to the staff of the Seed Science and Technology Laboratory, Department of Field Crops, Eskişehir Osmangazi University.

Conflicts of Interest: The authors declare no conflict of interest.

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