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The Effects of Biostimulant Application on Growth Parameters of Lettuce Plants Grown under Deficit Irrigation Conditions †

Christina Chaski and Spyridon A. Petropoulos *

Department of Agriculture, Crop Production and Rural Environment, University of Thessaly, Fytokou Street, 38446 Volos, Greece

* Correspondence: spetropoulos@uth.gr

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Abstract: The aim of the present study was to examine the potential ameliorative effects of biostimulant application on lettuce plants grown under deficit irrigation conditions. For this purpose, we evaluated the effect of five biostimulant products with a varied composition (e.g., seaweed extracts + macronutrients + amino acids (SW); humic + fulvic acids (HF); Si + Ca (SiC); Si (Si); vegetable proteins + amino acids (VP)) and the control treatment (no biostimulant added (NB)) on field-grown lettuce plants (*Lactuca sativa* L.: Romaine type cv. Doris) under deficit irrigation conditions (Control treatment: rain-fed plants; I1: 50% of field capacity; I2: 100% of field capacity). The growth parameters tested were plant weight (aerial part), number of leaves, fresh and dry weight of leaves, plant height, leaf area index (LAI) and specific leaf area (SLA), and SPAD index. Our results indicate that the biostimulant with seaweed extracts + macronutrients + amino acids (SW) combined with deficient irrigation (I1) presented the highest values in terms of plant weight, leaf weight, LAI, as well as the chlorophyll content in lettuce plants. According to the SPAD values, the biostimulant treatments performed higher values of chlorophyll in the case of the rain-fed plants compared to those that were fully irrigated (I2). In addition, the Si treatment presented the higher plant height under deficit irrigation (I1) as well as the greatest number of leaves. In general, all of the biostimulants showed a better response to deficit irrigation and to rain-fed plants compared to those with full irrigation in almost all of the measurements.

Keywords: *Lactuca sativa*; seaweed extracts; humic and fulvic acids; silicon; amino acids; deficit irrigation

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1. Introduction

Considering global warming, one of the main abiotic factors that threatens agricultural productivity is the progressive expansion of the water deficit in different areas of the world. Water stress constitutes one of the most important factors limiting plant growth and development [1]. A new, innovative, environmentally friendly approach is the application of natural plant biostimulants (PBs) in various crops. These products are capable of enhancing flowering, plant growth, fruit set, crop productivity, and nutrient use efficiency, especially under biotic and abiotic stressors [2,3]. There are several products available on the market that can be used as biostimulants in various crops [4]. According to du Jardin [5] the main categories of biostimulants are products based on humic substances, seaweed extracts, chitin and chitosan derivatives, antitranspirants, free amino-acids, N-containing substances, etc. In this context, the application of biostimulants could be considered as a good production strategy for obtaining a high yield of nutritionally valuable vegetables [6].

Lettuce is an important horticultural crop which is widely consumed in various salad mixes. Therefore, its demand is constantly increasing since it contributes to the nutritional requirements of the human diet on a daily basis [7]. This is mainly due to the fact that lettuce is consumed fresh, meaning it retains most of its nutrients compared to other vegetables that are cooked or processed prior to consumption. In addition, the consumption of salads consisting of young leaves (cotyledons or microgreens) or seedlings (baby leaf) has been gaining popularity as a culinary trend [8]. In the present study, we evaluated the effect of five biostimulant products with varied composition on plant growth and crop performance of field grown lettuce plants (*Lactuca sativa* L.: Romaine type cv. Doris) under deficit irrigation conditions.

2. Materials and Methods

2.1. Description of the Treatments and Experimental Design

The experiment took place during the spring–summer growing period of 2021, at the experimental field of the University of Thessaly, in Velestino, Greece. The lettuce plants (*Lactuca sativa* L.: Romaine type cv. Doris) were transplanted on April 1 (7 weeks after the sowing stage of 3–5 true leaves), while the harvest took place on May 27. The area of each experimental plot was 2.5 m^2 and in each plot, 34 plants were grown. The experimental layout was designed according to the split-plot design (n = 3), considering irrigation as the main plot and the biostimulant application as the sub-plot. The biostimulants studied included five products with a varied composition (e.g., a mixture of plants and seaweed extracts, amino acids and trace elements (SW), humic and fulvic acids balanced solution (HF), 35% *w/v* CaO and 35% *w/v* SiO_2 + calcium utilization, mobilization and translocation factor (SiC), 0,3% stabilized orthosilicic acid (Si), vegetable proteins and amino acids: 11% free L-amino acids, 24% short chain peptides, 20% proteins (VP)), and the control treatment (without addition of biostimulants (NB)). All of the biostimulant products are experimental formulations provided by Agrology S.A. (Thessaloniki, Greece). The biostimulants were applied as follows: SiC: 15 L/ha Si and 1 L/ha Ca; HF: 20 L/ha, SW: 100 mL/100 L; Si: 100 mL/10 L, VP: 300 mL/100 L. The irrigation regime included three treatments, namely the control treatment: rain-fed plants (the control); I1: 50% of field capacity; I2: 100% of field capacity. Prior to transplanting, the roots of the plants were immersed in the corresponding biostimulants (the control plants were immersed in water). During the growing period, three applications of the biostimulants were carried out at 5, 15, and 25 days after transplanting, except for the treatment of seaweed extracts + macronutrients + amino acids (SW) which, according to the application guide, was not applied at 5 days. In addition, the biostimulants HF and SiC were applied directly to the roots via fertigation, while the rest of the formulations were applied by foliar application.

2.2. Plant Sampling and Analyzed Parameters

The height of the lettuce plants was recorded one day after each biostimulant application. The harvest took place when the plants reached the marketable size (about >300 g/head). On the day of harvest, the tested growth parameters were plant weight (aerial part), number of leaves, fresh and dry weight of leaves, leaf area index (LAI) and specific leaf area (SLA). Dry weight was determined after drying at 72 °C until constant weight (approximately after 72 h). Moreover, the measurements of the plant height took place after the application of biostimulants at 5, 15, and 25 days after the transplantation and the results are presented in Table 1. The chlorophyll's content (SPAD index) was recorded before harvesting with the use of a portable SPAD-502 chlorophyll meter (Konica Minolta Inc., Osaka, Japan). For the SPAD determination, a measurement was made on one fully developed leaf (in the middle of the lettuce head) and the measurement was repeated on ten plants from each treatment and replication. The LAI values were determined in five of the lettuce plants with the LI-3100C Area Meter (LI-COR Biosciences; Hellamco S.A., Athens, Greece) and then these values were used to determine the SLA value using the formula: SLA = LAI/dry weight expressed in m^2/g.

Table 1. Plant height (cm) and SPAD index values of lettuce palnts at harvest.

Biostimulants	Irrigation	Plant Height (cm)	SPAD Index
NB	Control	28.7 ± 3.1 Aab	26.7 ± 1.5 Ab
	IR.1	28.3 ± 3.7 Aab	28.1 ± 2.1 Aab
	IR.2	26.9 ± 3.1 Bbc	19.5 ± 1.6 Bc
SiC	Control	29.3 ± 1.3 Aab	31.3 ± 1.2 Aab
	IR.1	24.0 ± 2.2 Bc	28.9 ± 1.5 Bab
	IR.2	24.4 ± 2.5 Bc	20.5 ± 1.5 Cc
HF	Control	28.8 ± 2.1 Aab	31.2 ± 1.8 Aab
	IR.1	28.1 ± 2.6 Aab	25.4 ± 1.0 Bb
	IR.2	26.0 ± 2.8 Bbc	24.6 ± 1.0 Bb
SW	Control	27.7 ± 3.0 Abc	27.3 ± 1.2 Bab
	IR.1	26.8 ± 2.4 ABbc	33.3 ± 1.2 Aa
	IR.2	25.2 ± 2.7 Bc	19.0 ± 1.0 Cc
Si	Control	24.7 ± 1.4 Bc	29.5 ± 1.5 Aab
	IR.1	30.1 ± 3.1 Aa	29.9 ± 1.0 Aab
	IR.2	24.9 ± 2.5 Bc	19.7 ± 1.3 Bc
VP	Control	27.6 ± 2.9 Abc	31.9 ± 1.8 Aab
	IR.1	28.1 ± 2.2 Aab	26.5 ± 1.3 Bb
	IR.2	25.7 ± 1.9 Bbc	14.9 ± 2.0 Cd

Means in the same column of the same biostimulant treatment followed by different capital letters are significantly different according to Tukey's HSD test at p = 0.05. Means in the same column followed by different letters are significantly different according to Tukey's HSD test at p = 0.05. SW: algae extracts + macronutrients + amino acids; HF: humic + fulvic acids; SiC: Si + Ca, Si: Si, VP: plant proteins + amino acids; NB: without addition of biostimulants. Control: rain-fed plants, I1: 50% of field capacity; I2: 100% of field capacity.

2.3. Statistical Analysis

The statistical analysis was carried out with JMP v. 16.1 (SAS Institute Inc., Cary, NC, USA). Before the conduction of the statistical analysis, all of the data were examined for normal distribution according to the Shaphiro–Wilk test. The results of the study are expressed as mean values and standard deviations (SD). Data were analyzed using the two-way analysis of variance (two-way ANOVA), while means were compared using the Tukey HSD-test at p = 0.05.

3. Results

Plant Biomass and Growth Parameters

The results regarding the plant height are presented in Table 1. According to these results, slight differences in plant heights were recorded at first sampling date, while the effect of biostimulant and irrigation treatments was more profound at the last sampling date. In particular, a varied response was recorded with the highest values being recorded for the Si at deficit irrigation conditions (Si × I1) and SiC × Control treatment. Table 1 presents the chlorophyll content (SPAD index) of leaves at harvest. SPAD values increased when plants treated with vegetable proteins + amino acids (VP) at rain-fed conditions or seaweed extracts + macronutrients + amino acids (SW) at deficit irrigation (I1: 50% of field capacity). Moreover, a noteworthy observation is that all biostimulants showed higher content of chlorophyll under the rain-fed conditions (Control treatment) than full irrigation (I2), while no significant differences from deficit irrigation where recorded. Regarding the combination of irrigation and biostimulant treatments, a varied response was observed with the application of SW under rain-fed conditions presenting the highest overall values and the treatments of VP × IR2 the lowest ones.

Plant growth parameters are presented in Table 2. Total plant weight, weight of leaves and LAI were the highest in the half irrigation treatment (I1) for plants treated with the SW treatment, whereas the number of leaves increased for the plants that received half irrigation (I1) and Si. The highest dry matter content and SLA values were recorded for plants that did not receive biostimulants under rain-fed or full irrigation (I2), respectively. Comparing the weight of leaves and total plant weight for each biostimulant and irrigation level, the results of HF, SW, Si as well as the NB treatment showed that deficit irrigation resulted in higher weight of leaves and plants compared to the control and the full irrigation treatment. Similar trends were recorded for the number of leaves and LAI values for the treatments of SW, Si and NB under rain-fed conditions, indicating that plant weight was higher due to the larger number of leaves. In contrast, the rain-fed plants (control treatment) were the highest when treated with SiC or VP biostimulants. Dry matter content was the highest for rain-fed plants, regardless of the biostimulant treatment, except for the case of VP treatment, where deficit irrigation resulted in the highest dry matter content. Finally, SLA values where the highest for fully irrigated plants, regardless of the biostimulant treatment, except for the case of VP treatment where rain-fed conditions increased SLA.

Table 2. Growth parameters of lettuce plants in relation to irrigation regime and biostimulant application (means ± SD).

Biostimulants	Irrigation Treatment	Plant Weight (g)	Number of Leaves	Weight of Leaves (g)	LAI (cm^2)	Dry Weight (%)	SLA
NB	Control	402.7 ± 12.0 Bde	36 ± 1 Bh	298.5 ± 7.1 Be	5905.4 ± 173.6 Bd	8.3 ± 3.9 Aa	26.8 ± 1.2 Cik
	IR.1	437.4 ± 10.6 Aab	42 ± 1.4 Acd	362.4 ± 6.9 Aab	6647.6 ± 108.3 Ab	5.0 ± 0.3 Bg	36.6 ± 1.5 Bc
	IR.2	363.1 ± 18.3 Cf	36.8 ± 1.6 Bgh	284.8 ± 5.9 Bef	5209.1 ± 134.9 Cfg	3.8 ± 0.8 Ck	51.1 ± 1.6 Aa
SiC	Control	429.1 ± 12.8 Abc	43.6 ± 1.3 Bbc	346.6 ± 18.5 Ac	5997.0 ± 129.7 Ad	7.4 ± 0.7 Ab	23.9 ± 2.6 Cl
	IR.1	312.9 ± 11.0 Cik	44 ± 1.8 Aab	257.8 ± 13.9 Chi	4630.9 ± 198.6 Bi	6.9 ± 0.6 Bc	27.8 ± 2.9 Bhi
	IR.2	348.1 ± 8.1 Bgh	36.2 ± 1.3 Ch	280.4 ± 14.7 Bfg	4808.8 ± 109.0 Bh	5.6 ± 0.5 Cf	32.1 ± 1.9 Ade
HF	Control	392.1 ± 10.4 Be	45.4 ± 1.6 Aab	322.5 ± 9.2 Bd	6375.5 ± 120.8 Ac	6.6 ± 0.6 Ad	31.0 ± 1.0 Bef
	IR.1	438.9 ± 14.2 Aab	37.6 ± 1.0 Cfg	355.5 ± 12.4 Abc	6472.7 ± 193.1 Ac	6.2 ± 0.4 Be	30.0 ± 1.6 Bf
	IR.2	311.5 ± 8.4 Cik	42 ± 1.8 Bcd	253.0 ± 8.7 Chi	4813.7 ± 163.3 Bh	5.5 ± 0.5 Cf	35.3 ± 2.0 Ac
SW	Control	323.6 ± 18.8 Chi	41.2 ± 2.2 Ade	260.4 ± 12.9 Bhi	5176.5 ± 198.0 Bg	6.9 ± 1.4 Ac	29.5 ± 1.2 Bfg
	IR.1	460.5 ± 10.4 Aa	42.6 ± 1.9 Ac	379.3 ± 8.0 Aa	6928.8 ± 147.6 Aa	6.4 ± 0.7 Bd	28.8 ± 1.9 Bgh
	IR.2	440.1 ± 14.4 Bab	37.2 ± 1.6 Bfg	362.8 ± 7.5 Aab	6718.7 ± 146.3 Aab	4.2 ± 0.5 Ci	44.5 ± 1.9 Ab
Si	Control	325.4 ± 11.2 Chi	43.2 ± 1.8 Bbc	267.5 ± 6.4 Bgh	5392.1 ± 118.0 Bf	8.1 ± 1.7 Aa	25.8 ± 1.9 Ck
	IR.1	451.2 ± 12.8 Aa	46.8 ± 1.0 Aa	357.3 ± 7.3 Abc	6542.8 ± 109.4 Abc	6.2 ± 0.7 Be	30.3 ± 1.8 Bf
	IR.2	361.3 ± 11.8 Bfg	40.4 ± 1.9 Ce	283.4 ± 5.2 Bef	5167.4 ± 124.9 Bg	5.6 ± 0.7 Cf	33.1 ± 1.7 Ad
VP	Control	417.9 ± 19.1 Acd	41.2 ± 1.6 Ade	324.9 ± 6.7 Ad	5679.3 ± 109.1 Ae	4.5 ± 1.7 Ch	46.8 ± 2.0 Ab
	IR.1	381.3 ± 13.8 Bef	39.6 ± 1.4 Be	297.3 ± 9.9 Be	5125.4 ± 152.7 Bg	6.9 ± 0.4 Ac	25.4 ± 1.5 Ck
	IR.2	302.7 ± 14.2 Ck	37.4 ± 1.10 Cfg	245.6 ± 1.0 Ci	4495.3 ± 105.8 Ck	5.2 ± 0.6 Bg	36.1 ± 1.4 Bc

Means in the same column of the same biostimulant treatment followed by different capital letters are significantly different according to Tukey's HSD test at p = 0.05. Means in the same column followed by different letters are significantly different according to Tukey's HSD test at p = 0.05. SW: algae extracts + macronutrients + amino acids; HF: humic + fulvic acids; SiC: Si + Ca, Si: Si, VP: plant proteins + amino acids; NB: without addition of biostimulants. Control: rain-fed plants, I1: 50% of field capacity; I2: 100% of field capacity.

4. Discussion

Our results indicate that the biostimulant SW in combination with the deficit irrigation (I1) gave the highest average in terms of plant weight, leaf weight, LAI as well as the level of chlorophyll in the plants. It is interesting to highlight that in most biostimulants (e.g., HF, SW, NB and VP) the application of full irrigation resulted in lower plant height compared to the other irrigation treatments, which probably indicates that the applied irrigation exceeded plant requirements resulting in stressful conditions. In addition, the biostimulant with Si on deficit irrigation (I1) presented the higher plant height as also the greatest number of leaves. These results are consistent with other previous research which suggested that Si through modification of plant water relation, stimulates cell division and cell elongation, boosts plant immune system and enhances plant growth [9,10]. Similar results were presented by Goñi et al. [11] who performed a pot experiment with tomato plants and tested three commercial biostimulants that contained *Ascophyllum nodosum* extract under irrigation stress conditions. Their results showed that two of the three formulations under reduced irrigation showed significantly higher chlorophyll content than untreated drought plants. According to Di Mola et al. [12], the application of seaweed extracts and protein hydrolysates significantly improved yield and LAI values of baby leaf lettuce plants grown under greenhouse conditions. Moreover, the use of protein hydrolysates or fertilizers containing peptides and amino acids significantly increased crop yield and chlorophyll content through the stimulating effects on phyllosphere plant growth promoting bacteria that consequently affect plant growth [13].

The biostimulant treatment with humic+fulvic acids (HF) recorded higher values in IR1 in terms of plant weight, leaf weight and LAI values, compared to rain-fed and fully irrigated plants, whereas the number of leaves and dry matter content increased under rain-fed conditions. According to Hernandez et al. [14], the application of humates may improve growth rate resulting in early harvesting of lettuce plants, while it can also increase yields through the formation of more leaves. Similarly to our study, the same authors indicate that humates did not affect chlorophyll content, while the same authors suggested that morphological responses of lettuce plants to biostimulant application should be attributed to physiological responses [14]. Moreover, protein hydrolysates may increase marketable yield of lettuce plants, especially under stress conditions [15]. This report is in agreement with our results, since the highest values of the tested growth parameters were observed for the control irrigation treatment, followed by the IR1 treatment where plants either did not receive irrigation (rain-fed) or were irrigated according to 50% field capacity. Therefore, in both cases where plants were subjected to stress conditions protein-based biostimulants resulted in the highest values. Finally, our results are in accordance with the findings of Asgharipour and Masapour [16] as silicon foliar spray under water deficit condition showed positive interaction of leaf area index.

The positive effects of biostimulants on lettuce plants include several other products containing bacteria, amino acids or minerals [17–19], indicating the complexity of mechanisms of actions that need to be revealed.

5. Conclusions

Our results indicate that the biostimulant with seaweed extracts+macronutrients+amino acids (SW) combined with deficient irrigation (I1) presented the highest values in terms of plant weight, leaf weight, LAI as well as the chlorophyll content in lettuce plants. According to SPAD values, the biostimulants treatments performed higher values of chlorophyll in the case of rain-fed plants compared to those that were fully irrigated (I2). Also, the biostimulant with Si presented the higher plant height under deficit irrigation (I1) as also the greatest number of leaves. In general, all biostimulants showed a better response to deficit irrigation and to rain-fed plants compared to those with full irrigation in almost all measurements. In conclusion, each biostimulant product may act differently depending on the irrigation conditions as well as on the tested species or variety. Therefore, continuous research on biostimulants as well as on deficit irrigation is needed in order to provide useful information regarding the water use efficiency of crops and the alleviation of the effects of water shortages on crop productivity.

Supplementary Materials: The poster presentation can be downloaded at: https://www.mdpi.com/article/10.3390/IECHo2022-12499/s1.

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