



Effect of microalgae hydrolysate foliar application (*Arthrospira platensis* and *Scenedesmus* sp.) on *Petunia x hybrida* growth

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Received: 12 November 2017 / Revised and accepted: 19 February 2018
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Abstract

In horticultural practice accelerated plant development and particularly earlier flowering, has been reported with microalgae applications. Therefore, the objective of this work was to study the effects of foliar spraying with *Scenedesmus* sp. and *Arthrospira platensis* hydrolysates on *Petunia x hybrida* plant development and leaf nutrient status. Three treatments were tested: T1 (foliar application with water, the control), T2 (foliar application with *Arthrospira*), and T3 (foliar application with *Scenedesmus*). Foliar spraying was applied five times (0, 14, 28, 35, and 42 days after transplanting). The concentration of both microalgae was 10 g L⁻¹. At the end of the trial biometric parameters and nutrient concentration in photosynthetic organs (the leaves) were measured. The results of this assay show that foliar application of *Scenedesmus* accelerated plant development in terms of higher rates of root growth, leaf and shoot development, and earliness of flowering. *Arthrospira* enhanced the root dry matter, the number of flowers per plant, and the water content. Nevertheless, a reduction was found in the conductive tissue (stem + petiole) dry weight with *Arthrospira* compared with *Scenedesmus* and the control. The results also show that microalgae hydrolysate supply can improve the plant nutrient status. Based on these results, it is advisable to use *Scenedesmus* hydrolysates in foliar applications to increase the blooming of *Petunia x hybrida*.

Keywords Hormones · Flowering · Number of leaves · Number of shoots · Leaf nutrient concentration

Introduction

There is a growing need to develop environmentally friendly strategies for sustainable culturing of horticultural crops with high yields involving less environmental damage (Alves-Dias et al. 2016). This consists of reducing or substituting the use of chemical inputs, especially fertilizers and pesticides, with natural or biological substances (Elarroussi et al. 2016). Biofertilizers, organic manuring, and biocontrol have emerged as promising components for integrating the nutrient supply system in agriculture (Faheed and Fattah 2008). In this regard the development of natural substances able to promote plant

growth, named “plant biostimulants”, is receiving increased attention (Elarroussi et al. 2016). Microalgae represent a potential sustainable alternative for the enhancement and protection of agricultural crops (Garcia-Gonzalez and Sommerfeld 2016). Plants have shown different responses when microalgae products have been applied. These include vigorous growth, higher yield, increased nutrient uptake, and more resistance to biotic and abiotic stresses (fungal diseases, insect attack, and frost), improved quality, and longer fruit shelf life (Tarraf et al. 2015).

Given that microalgae contain high levels of micronutrients and macronutrients essential for plant growth (Shaaban 2001), they have a potential application as biofertilizers (Garcia-Gonzalez and Sommerfeld 2016). Biofertilizers are products that contain living microorganisms or natural compounds derived from organisms such as bacteria, fungi, and algae that improve the soil’s chemical and biological properties, stimulate plant growth, and restore soil fertility (Garcia-Gonzalez and Sommerfeld 2016). Biofertilizers from algae are currently used in agriculture; most of these products come from macroalgae (Suganya et al. 2016) and they are mainly sold as a source of amino acids. However, microalgae contain a higher protein

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content (up to 70%) and growth rate (doubling in size within 3.5–24 h) than macroalgae. Microalgae biodiversity is also much greater. It has been estimated that between 20,000 and 800,000 species exist, of which between 40 and 50,000 species are described (Suganya et al. 2016), providing a wide range of possibilities that allows us to select strains with different compositions or adapted to living in different environments. Many can be produced easily with wastewater, reducing the production cost (Acien et al. 2016). In order to get a more effective product it is necessary to break the cell wall to release all the intracellular components; this is easily carried out by enzymatic hydrolysis—a low cost and easily scalable process (Romero García et al. 2012). In this regard a number of studies were conducted to evaluate the effect of microalgae fertilizers on different crops. Some of these studies found a positive effect of microalgae fertilizers, especially when applied to leaves (Alves-Dias et al. 2016). Foliar application of seaweed extract appeared to be most effective if applied in the morning when the leaf stomata are open (Battacharyya et al. 2015). Abo Sadera et al. (2010) revealed that spraying fenugreek plants with algae extract at 0.5 and 1 g L⁻¹ significantly increased total nitrogen, phosphorus, and potassium in plant foliage. They allow the efficient recovery of the nitrogen (N) and phosphorus (P) present in wastewaters by concentrating these nutrients in algal biomass (Coppens et al. 2016; Schreiber et al. 2018). Moreover, as an organic fertilizer, microalgae have the potential to prevent nutrient losses through a gradual release of N, P, and K, which is attuned to plant requirements (Coppens et al. 2016). However, extensive studies on the chemical composition of various extracts made from a diversity of seaweed revealed that the nutrient contents of the extracts were insufficient to elicit physiological responses at the typical concentrations when the seaweed extracts were applied in the field (Battacharyya et al. 2015).

On the other hand, microalgal polysaccharides showed good capacity for improving plant growth, offering an interesting potential use as biostimulants (Elarroussi et al. 2016). Algae contain active compounds such as free amino and organic acids, phytohormones, enzymes and bioactive secondary metabolites, vitamins, and vitamin precursors (Battacharyya et al. 2015; Mógor et al. 2017). The cellular extracts and growth medium of several microalgae species have been shown to contain phytohormones (auxins, cytokinins, gibberellins, abscisic acid (Schwarz and Krienitz 2005; Tarakhovskaya et al. 2007), and salicylic acid) which are known to play crucial roles in plant development. Plant hormones can be essential in various aspects of plant growth and development. Cytokinins stimulate cell division, regulate shoot and root development, and promote leaf growth as well as flower, fruit, and seed formation; they stabilize photosynthetic machinery, suppress senescence, and enhance sink strength and nitrogen acquisition (Vankova 2014). Gibberellins constitute a group of plant hormones that control developmental processes such as germination, shoot

elongation, tuber formation, flowering, and fruit set, along with growth in diverse species (Serrani et al. 2007). Some auxins strongly promote root elongation (Zhao and Hasenstein 2009). Abscisic acid is a plant hormone, whose biosynthesis increases with water stress to induce adaptive stress responses (Agehara and Leskovar 2017). However, the overall effect of abscisic acid is shoot growth suppression and an increase in the root-to-shoot ratio (Agehara and Leskovar 2017). Salicylic acid, known as important signaling molecules in plants, could be promising compounds for reducing stress sensitivity (Dong et al. 2016). Salicylic acid has a major involvement as a signaling molecule in plants, specifically during the defense reaction against pathogens (Rangel Sánchez et al. 2010).

The aim of this work was to study the effect of foliar spraying with *Scenedesmus* sp. and *Arthrospira* sp. microalgae hydrolysates on *Petunia x hybrida* growth and leaf nutrient status.

Material and methods

Field trial

The trials were carried out in a 150 m² greenhouse with zenith ventilation and relative humidity and temperature control. Average temperature, vapor pressure deficit, and photosynthetically active radiation were 20.2 °C, 1.14 KPa, and 19.5 photons m⁻² day⁻¹, respectively.

Single *Petunia x hybrida* plants were transplanted into 1.5 L pots and the substrate used was a mixture of peat and perlite 80:20 (v/v). Fertigation was applied manually and the dose was 100 mL per plant per day. The concentrations in the standard nutrient solution were 0.52, 7.2, 0.7, 1.7, 3.0, 2.5, and 1.0 mmol L⁻¹ of H₂PO₄⁻, NO₃⁻, NH₄⁺, SO₄²⁻, K⁺, Ca²⁺, and Mg²⁺, respectively. The pH and EC were 6.5 and 1.6 dS m⁻¹, respectively.

Plant material

Rooted plants of the *Petunia x hybrida* cultivar Surfinia Purple were used in the experiment. The cuttings were 10 cm high, had seven leaves per plant, a 2.1-cm² leaf area (average value), and 0.04 g total dry weight. *Petunia* is considered the leading cultivated bedding plant. The leaves of *Petunia* are sessile and usually oval-shaped. There are four distinct categories of petunias, based on flower size and growth habits (multiflora, grandiflora, milliflora, groundcover, or spreading) (Cantor et al. 2015). Surfinia belongs to the groundcover category. Groundcover petunias are vigorous growing plants that spread like groundcover. Spreading petunias possess excellent heat and drought tolerance and require little maintenance (Jauron 2013). Surfinia Purple has a pink-purple to light cyclamen

color and medium-sized flowers with darker lines (Cantor et al. 2015).

Treatments

Three treatments were performed: T1 (foliar application with water, the control), T2 (foliar application with *Arthrospira*), and T3 (foliar application with *Scenedesmus*). The concentration of both microalgae (*Scenedesmus almeriensis* (CCAP 276/24) and *Arthrospira* (Strain BioVeg-F025 donated by biotechnology group of the University of Antioquia (Colombia)) was 10 g L⁻¹. Foliar spraying was applied five times (0, 14, 28, 35, and 42 days after transplanting). The hydrolysates of both microalgae were produced starting from a sludge containing up to 100 g L⁻¹ of biomass, and performing enzymatic hydrolysis under controlled conditions (pH = 8, temperature = 40 °C) for 4 h providing 0.2% *p* biomass/*p* of proteases (Alcalase and Flavourzyme from Novozyme). The cell walls were previously broken by high pressure homogenization. The hormonal components and nutrient content of the microalgae are shown in Tables 1 and 2.

Nutrient solutions analysis

The parameters determined in the nutrient solutions tested were pH, EC, NO₃⁻, SO₄²⁻, H₂PO₄⁻, Ca²⁺, Mg²⁺, and K⁺. The pH was measured with a Crison MicropH 2001 pH-meter, and the EC with a Crison Micro CM 2200 conductivity-meter. Nitrate, SO₄²⁻, H₂PO₄⁻, Ca²⁺, Mg²⁺, and K⁺ were determined by HPLC (high-performance liquid chromatography; Metrohm 883 Basic IC Plus). NO₃⁻, SO₄²⁻, and H₂PO₄⁻ were quantified using a Metrosep A SUPP 4 column (IC conductivity detector range 0–15,000 μS cm⁻¹). The mobile phase was prepared by mixing 190.6 mg of CO₃²⁻ and 142.8 mg of HCO₃⁻ and then diluting this in 1 L of deionized water, acidified with H₂SO₄ (50 mM). The Ca²⁺, Mg²⁺, and K⁺ were quantified using a Metrosep C4 column (IC conductivity

Table 1 Hormone content of the microalgae hydrolysate (ng g⁻¹)

		<i>Arthrospira</i>	<i>Scenedesmus</i>
Ethylene	ACC	545.55	341.32
Cytokinins	Trans-Zeatin	191.37	0.84
	Zeatin riboside	0	6.82
	Isopentenyl adenine	2560.73	45,561.97
Gibberellins	GA1	24.97	208.81
	GA3	–	–
	GA4	31.27	9.09
Auxins	Indoleacetic acid	10.3	5965.0
Other hormones	ABA	1.03	3718.25
	Salicylic acid	0.61	156,713.72
	Jasmonic acid	0.84	75.13

Table 2 Nutrient content of the microalgae hydrolysate (%)

	<i>Arthrospira</i>	<i>Scenedesmus</i>
N	1.198 ± 0.005	1.009 ± 0.020
P	0.187 ± 0.004	0.297 ± 0.027
K	0.157 ± 0.005	0.0952 ± 0.000
Ca	0.157 ± 0.004	1.1605 ± 0.068
Mg	0.048 ± 0.001	0.0548 ± 0.002

detector range 0–15,000 μS cm⁻¹) and the mobile phase was prepared by mixing 117 mg of 2,6-pyridinedicarboxylic acid and 1.7 mL of nitric acid (1 M) diluted in 1 L of deionized water.

Biometric parameters

The number of leaves, shoots, and flowers per plant was counted every week during the crop cycle. Fresh and dry weights, internode length, leaf length, and width were registered at the end of the cultivation (49 days after the beginning of the trial). Internode length, leaf length, and width were measured using a ruler. After removing the substrate the plant material of 12 plants per treatment was separated into different vegetal fractions: absorption organs (roots), conductive organs (stems and petioles), photosynthetic organs (leaves), and flowers, which were weighted separately on a COBOS G M5-1000 scale (precision 0.005 g) to determine fresh weight (FW). All of the samples were washed and dried in a Nüve FN500 oven (range 30 to 300 °C) at 60 °C for 48 h to determine dry weight (DW). Total FW and total DW were calculated as the sum of roots, stems and petioles, and leaves and flowers (FW and DW), respectively. Total FW and DW were used to calculate the water content (WC) as (FW-DW)/FW.

Plant and algae analysis

A subsample of the dry matter was ground in a Wiley mill and digested (96% H₂SO₄) for the analysis of organic N, P, K, Ca, and Mg. Total K⁺ was directly measured by flame spectrophotometry (Lachica et al. 1973), using an Evans Electro Selenium LTB Flame Photometer (Halstead, England). Total Ca²⁺ and Mg²⁺ were analyzed by atomic-absorption spectrophotometry (Hocking and Pate 1977), using a Perkin Elmer Atomic Absorption Spectrometer 3300. Phosphorus was analyzed using the method proposed by Hogue et al. (1970) and nitrogen using the method proposed by Krom (1980).

Regarding hormone content, AA, GA1, GA3, GA4, CKs (tZ and iP), riboside, ethylene precursor ACC, ABA, SA, and JA concentrations were analyzed as in Ghanem et al. (2008) with some modifications. Briefly, 30 mg of homogenized dry biomass were dropped in 0.5 mL of cold (-20 °C) extraction mixture, methanol/water (80/20, v/v). Solids were separated

by centrifugation (20,000×g, 15 min) and re-extracted for 30 min at 4 °C in an additional 0.5 mL of the same extraction solution. Pooled supernatants were passed through a Sep-PakPlus C18 cartridge (Waters, USA) to remove interfering lipids and part of the plant pigments and evaporated at 40 °C in a vacuum, either to near dryness or until the organic solvent was removed. The residue was dissolved in 1 mL methanol/water (20/80, v/v) solution using an ultrasonic bath. The dissolved samples were filtered through Millex nylon membrane filters, 13 mm diameter of 0.22 µm pore size (Millipore, USA). Next, 10 µL of filtered extract was injected in a U-HPLC–MS system consisting of an Accela Series U-HPLC (Thermo Fisher Scientific, USA) coupled to an Exactive mass spectrometer (Thermo Fisher Scientific) using a heated electrospray ionization (HESI) interface. The mass spectra were determined using the Xcalibur software version 2.2 (Thermo Fisher Scientific). For quantification of the plant hormones, calibration curves were constructed for each component analyzed (1, 10, 50, and 100 µg L⁻¹) and corrected for 10 µg L⁻¹ deuterated internal standards.

Experimental design and statistical analysis

The experimental design was a completely randomized block with three treatments, four replicates per treatment, and three plants (pots) per replicate. Twelve plants per replicate and treatment were sampled 49 days after transplanting. The significance of the effect of the treatments was examined by the standard analysis of variance (one-way ANOVA) and Fisher's Least Significant Difference (LSD) test, carried out using Statgraphics Centurion XVI.II (Statpoint Technologies, Inc., USA).

Results

Biometric parameters

The foliar application of *Arthrospira* and *Scenedesmus* increased root dry weight (Fig. 1) and flower dry and fresh weight (Fig. 2). *Arthrospira* enhanced root dry weight (35%), flower dry weight (19%), and the fresh weight of the flower (21%) compared with the control. Nevertheless, a reduction in the conductive tissue (stem + petiole) and the photosynthetic organ (leaf) dry weight was found with *Arthrospira* compared with *Scenedesmus* and the control. The foliar application of *Scenedesmus* increased root dry weight (49%), flower dry weight (20%), and fresh weight of the flower (22%) compared with the control. No differences were found between *Scenedesmus* and the control with respect to the stem + petiole and leaf dry matter. Regarding the root/shoot dry weight, there were significant differences between treatments. The root/shoot ratios for the control, *Arthrospira*

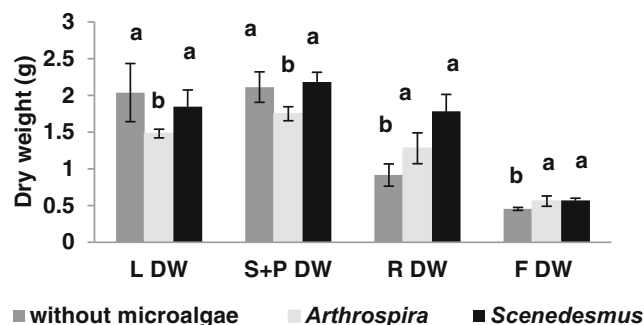


Fig. 1 Dry weight (g) of L (leaves), S + P (stems and petioles, conductive organs), R (roots), and F (flowers) at the end of the trial. Different letters indicate significant differences between treatments at the $P < 0.05$ level in the LSD test. Values represent the average ($n = 12$); bars represent the standard error

and *Scenedesmus*, were 0.27, 0.4, and 0.4 g g⁻¹, respectively. These were higher (32%) when the microalgae were applied.

On the other hand, microalgae accelerated plant development in terms of earliness of flowering and higher flower numbers (Fig. 3). The foliar application of *Arthrospira* and *Scenedesmus* increased flowers numbers (66 and 18%), respectively, compared with the control. Water content in the whole plant was also affected by treatments (Fig. 4). Microalgae application increased water content by 2.3 and 2% when *Arthrospira* and *Scenedesmus* were applied, respectively. In contrast, no difference in internode length, leaf length and width, and leaf area was observed between the treatments (Figs. 5 and 6). However, the foliar application of *Scenedesmus* stimulated shoot numbers and leaf numbers (Figs. 7 and 8). *Scenedesmus* application increased shoot and leaf numbers by 20 and 24%, respectively, compared with the control.

Nutritional status

Nitrogen, P, Mg, and Ca concentrations in the leaves were inside the range described by Marschner 2012. In contrast, potassium concentration in the leaves was lower than the

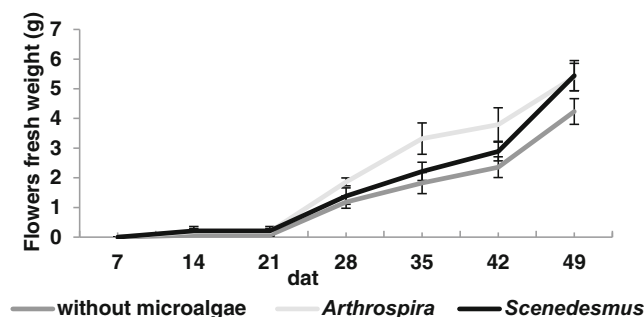


Fig. 2 Flower fresh weight during the crop. Values represent the average ($n = 12$); bars represent the standard error

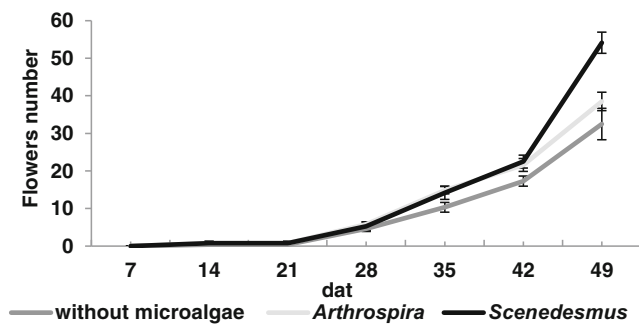


Fig. 3 Number of flowers during the crop. Values represent the average ($n = 12$); bars represent the standard error

levels described by Marschner (2012). However, no symptoms of potassium deficiency were found. In the leaves, P, K, Ca, and Mg were significantly higher in the microalgae treatments. However, nitrogen was only affected by *Arthrospira* treatment. *Arthrospira* application increased the leaf nitrogen content by about 6%. Moreover, the foliar application of *Arthrospira* and *Scenedesmus* increased P (34 and 31%), K (12 and 10%), Ca (29 and 28%), and Mg (22 and 29%), respectively, compared with the control (Fig. 9).

Discussion

Previous microalgae works have been reported generally using the whole biomass for fertilization and plant growth stimulation (Elarroussi et al. 2016). Extract foliar application at 3.75 g L^{-1} concentration resulted in increased plant height and a greater numbers of flowers and branches per plant in Roma tomato plants (Garcia-Gonzalez and Sommerfeld 2016). Foliar application of algae extract to fenugreek plants significantly increased plant height, the number of leaves, the number of branches, and the fresh and dry weight of the plants at the vegetative growth stage and flowering stage, especially in plants treated with 5 g L^{-1} algae extract (Tarraf et al. 2015). However, some researchers observed no effect on cucumber yield and a reduction of fresh weight on lettuce heads and cucumber shoots when in the presence of algae (Schwarz and Krienitz 2005). Nonetheless, fewer studies have been

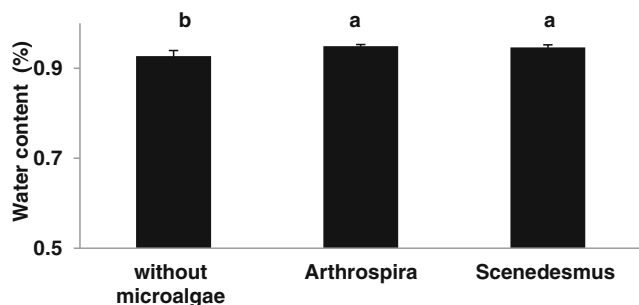


Fig. 4 Water content (%). Different letters indicate significant differences between treatments at the $P < 0.05$ level in the LSD test. Values represent the average ($n = 12$); bars represent the standard error

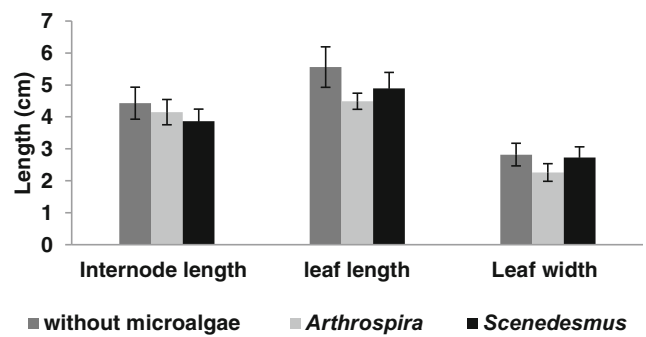


Fig. 5 Internode length (cm), leaf length (cm), and leaf width (cm) at the end of the trial. Different letters indicate significant differences between treatments at the $P < 0.05$ level in the LSD test. Values represent the average ($n = 12$); bars represent the standard error

conducted on *Scenedesmus*. The results of this assay show that the foliar application of *Scenedesmus* and *Arthrospira* increased the number of flowers and flower fresh and dry matter per plant and root dry weight. The application of dry microalgae improves plant nutrition, which in turn enhances all the physiological reactions that lead to good growth (Faheed and Fattah 2008). In the present study, the foliar application of *Arthrospira* and *Scenedesmus* hydrolysates enhanced the P, K, Ca, and Mg foliar concentration, which can accelerate plant development in terms of higher rates of root growth and earliness of flowering. The nutrients present in the seaweed extracts are readily absorbed by leaves through stomata and cuticle hydrophilic pores (Battacharyya et al. 2015). Plant responses to elements supplied via foliar sprays are normally more rapid than to soil treatment (Marschner 2012). Such elements are present in a reasonable percentage in algae extract, especially phosphorus (Shaaban 2001), potassium, iron, magnesium, and calcium (Priyadarshani and Rath 2012). Another reason for increasing nutrient uptake is the significant increase in root volume caused by adding microalgae (Shaaban 2001). On the other hand, algae extract as a natural plant cell sap contains certain amounts of enzymes and vitamins that may improve nutrient assimilation and solute translocation, leading to significant increases in yield (Shaaban 2001). In this regard, Tarraf et al. (2015) found that treatment of fenugreek plants with algae extract markedly

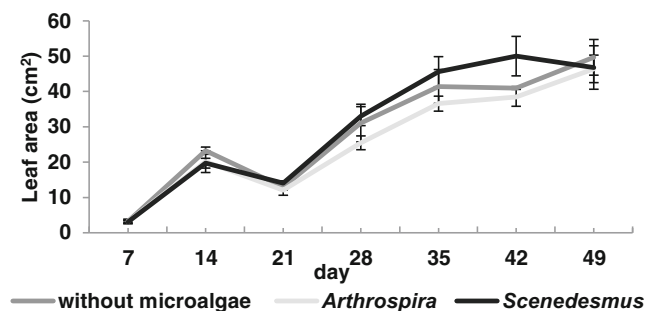


Fig. 6 Leaf area during the crop (cm^2). Values represent the average ($n = 12$); bars represent the standard error

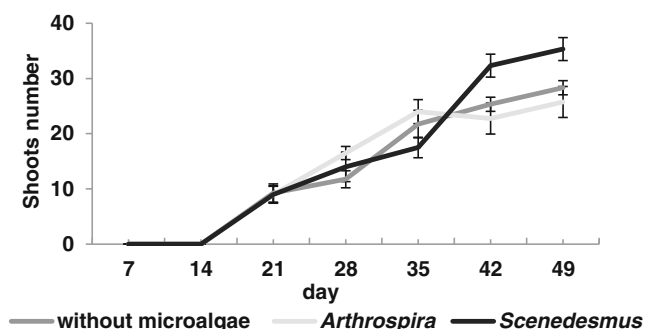


Fig. 7 Number of shoots during the crop. Values represent the average ($n = 12$); bars represent the standard error

increased nitrogen, phosphorus, and potassium contents, especially at 5 g L^{-1} . Foliar application of algae cell extract was found to increase the uptake of most nutrients by wheat plant shoots (Shaaban 2001). However, Alves-Dias et al. (2016) found that foliar application of *Arthrospira* at low concentrations did not affect the leaf content of N, P, K, and Na. Additionally, the growth-promoting effects of seaweed extracts are related to the direct or indirect effect of phytohormones present in the extracts (Tarakhovskaya et al. 2007, Shaaban et al. 2010; Priyadarshani and Rath 2012; Battacharyya et al. 2015). The following phytohormones have been detected in algae: auxins, cytokinins, gibberellins, abscisic acid, lunularic acid, jasmonic acid, polyamines, and brassinosteroids (Tarakhovskaya et al. 2007). In this assay, *Scenedesmus* had a higher concentration of cytokinins (isopentenyl adenine), gibberellins (GA1), auxins (indoleacetic acid), salicylic acid, and abscisic acid compared with *Arthrospira*, which can accelerate plant development. Cytokinins promote bud development (Tarakhovskaya et al. 2007) and gibberellins are associated with various growth and development processes such as floral organ development, reduced time of flowering, and increased flower number (Bose et al. 2013). Therefore, *Scenedesmus* treatment resulted in a great number of flowers, shoots, and leaves. Moreover, foliage-derived abscisic acid was found to promote root growth relative to shoot growth (McAdam et al. 2016). High abscisic acid levels suppress ethylene synthesis, which in turn reduces auxin transport and biosynthesis in the root tip, removing this primary inhibitor of root growth, and thereby

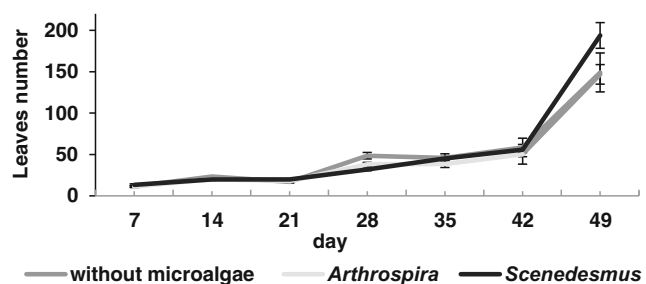


Fig. 8 Number of leaves during the crop. Values represent the average ($n = 12$); bars represent the standard error

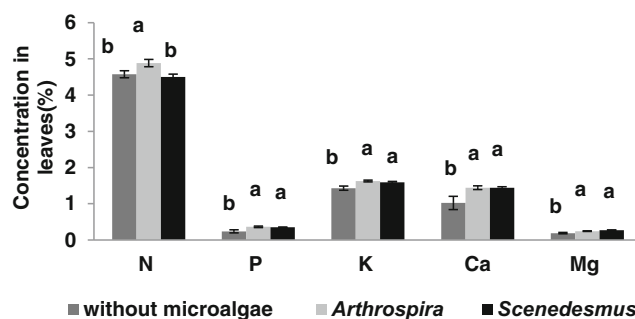


Fig. 9 Concentration in leaves (%). Different letters indicate significant differences between treatments at the $P < 0.05$ level in the LSD test. Values represent the average ($n = 12$); bars represent the standard error

promoting root growth (McAdam et al. 2016). Also, a high concentration of indole acetic acid induces the process of root formation (Tarakhovskaya et al. 2007). This study demonstrated that the application of microalgae enhanced root dry weight. However, this increase was higher in the *Scenedesmus* treatment compared with the *Arthrospira*, microalgae, with a higher concentration of indole acetic acid and abscisic acid. Despite cytokinins promoting the rate of leaf expansion (Lambers et al. 1998) in this assay no difference was found in leaf area, length, and width between treatments. On the other hand, in the present study, an increase in water content was found when microalgae were applied, as was reported by Faheed and Fattah (2008).

Conclusions

From the present work it can be concluded that the foliar application of microalgae hydrolysates increased the number of flowers, the flower fresh and dry matter per plant, and the root dry weight. Nevertheless, a reduction was found in the conductive tissue (stem + petiole) dry weight with *Arthrospira* compared with *Scenedesmus* and the control. The foliar application of *Scenedesmus* hydrolysates also increased the number of leaves and shoots, and enhanced P, K, Ca, and Mg foliar concentration. Therefore, *Scenedesmus* hydrolysates demonstrated good capacity for improving plant growth and this indicates their potential use as biofertilizers and biostimulants.

Funding information This research has received funding from the European Union's Horizon 2020 Research and Innovation program under the Grant Agreement No. 727874 SABANA, and ERANET LAC GREENBIOREFINERY ELAC 2014/BEE-0357.

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