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

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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 *Scenedesmu*s 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 5 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*.

1.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). Bio-fertilizers, organic manuring and bio-control 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 content (up to 70%) and growth rate (doubling in size within 3.5-24 hours) than macroalgae. Microalgae biodiversity is also much greater. It has been estimated that between 20,000–800,000 species exist, of which between 40–50,000 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 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 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). 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 content 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, microalgae polysaccharides showed good capacity for improving plant growth, offering an interesting potential use as biostimulants (Elarroussi et al., 2016). Microalgae contain active compounds, such as free amino and organic acids, phytohormones, enzymes and bioactive secondary metabolites, vitamins and vitamin precursors (Battacharyya et al., 2015). The cellular extracts and growth medium of several microalgae species have been shown to contain phytohormones (auxins, cytokinins, gibberellins, abscisic acid (Tarakhovskaya et al., 2007; Schwarz and Krienitz, 2005) 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, 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 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.

2. Material and methods

2.1. Field trial

The trials were carried out in a 150 m^2 greenhouse with zenith ventilation and relative humidity and temperature control. Average temperature, vapour pressure deficit and photosynthetically active radiation were 20.2 ºC, 1.14 KPa and 19.5 E 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 $H_2PO_4^-$, NO_3^-, NH_4^+, SO_4^{2-}, K^+, Ca^{2+} and Mg^{2+}, respectively. The pH and EC were 6.5 and 1.6 dS m^{-1}, respectively.

2.2. Plant material.

Rooted plants of the *Petunia x hybrida* cultivar Surfinia Purple were used in the experiment. The cuttings were 10 cm high, had 7 leaves per plant, a 2.1 cm^{-2} 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 colour and medium-sized flowers with darker lines (Cantor et al, 2015).

2.3. 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 5 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=40ºC) for four hours 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.

2.4. Nutrient solutions analysis

The parameters determined in the nutrient solutions tested were pH, EC, NO_3^-, SO_4^{2-}, $H_2PO_4^-$, Ca^{2+}, Mg^{2+} 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, SO_4^{2-}, $H_2PO_4^-$, Ca^{2+}, Mg^{2+} and K^+ were determined by HPLC (High Performance Liquid Chromatography; Metrohm 883 Basic IC Plus). NO_3-, SO_4^{2-}, $H_2PO_4^-$ were quantified using a Metrosep A SUPP 4 column (IC conductivity detector range 0–15000 μS cm−1). The mobile phase was prepared by mixing

190.6 mg of CO_3^{2-} and 142.8 mg of HCO_3^- and then diluting this in 1 L of deionized water, acidified with H_2SO_4 (50 mM). The Ca^{2+}, Mg^{2+} and K^+ were quantified using a Metrosep C4 column (IC conductivity detector range 0–15000 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.

2.5. 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 NüveE FN500 oven (range 30 to 300 ºC) at 60ºC for 48 h to determine dry weight (DW). Total FW and total DW were calculated as the sum of roots, stems and petioles, leaves and flowers (FW and DW), respectively. Total FW and DW were used to calculate the water content (WC) as (FW-DW)/FW.

2.6. Plant and algae analysis

A subsample of the dry matter was ground in a Wiley mill and digested (96% H_2SO_4) 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, Essex, England). Total Ca^{2+} and Mg^{2+} 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 0.5mL of cold (–20 °C) extraction mixture, methanol/water (80/20, v/v). Solids were separated by centrifugation (20000g, 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 (SepPakPlus, 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 0.22µm pore size (Millipore, Bedford, MA, USA). Next, 10µL of filtrated extract were injected in a U-HPLC–MS system consisting of an Accela Series U-HPLC (ThermoFisher Scientific, Waltham, MA, USA) coupled to an Exactive mass spectrometer (ThermoFisher Scientific, Waltham, MA, USA) using a heated electrospray ionization (HESI) interface. The mass spectra were determined using the Xcalibur software version 2.2 (ThermoFisher Scientific, Waltham, MA, USA). For quantification of the plant hormones, calibration curves were constructed for each component analyzed (1, 10, 50, and 100µg l-1) and corrected for 10µg l-1 deuterated internal standards.

2.7. Experimental design and statistical analysis

The experimental design was a completely randomized block with 3 treatments, 4 replicates per treatment and 3 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. Warrenton, Virginia, USA).

3. Results

3.1. Biometric parameters

The foliar application of *Arthrospira* and *Scenedesmus* increased root dry weight (Figure 1) and flower dry and fresh weight (Figure 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 *Scenedesmu*s 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 ratio for the control, *Arthrospira and Scenedesmus* was 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 (Figure 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 (Figure 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 (Figures 5 and 6). However, the foliar application of *Scenedesmus* stimulated shoots numbers and leaf numbers (Figures 7 and 8). *Scenedesmus* application increased shoot and leaf numbers by 20% and 24%, respectively, compared with the control.

3.2. Nutritional status

Nitrogen, P, Mg and Ca concentrations in the leaves was inside the range described by Marschner, 2012. In contrast, potassium concentration in the leaves was lower than the 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 (Figure 9).

4. 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 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 response 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 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 (Battacharyya et al., 2015, Tarakhovskaya et al., 2007, Priyadarshani and Rath, 2012 and Shaaban et al 2010). The following phytohormones were detected in algae: auxins, cytokinins, gibberellins, abscisic acid, lunularic acid, jasmonic acid, polyamines and brassinisteroids (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* microalgae, which can accelerate plant development. Cytokinins promote bud development (Tarakhovskaya et al., 2007) and gibberils 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 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).

4. 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 interesting potential use as biofertizers and biostimulants.

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References

Abo Sedera FA, Abd El-Latif AA , Bader LAA, Rezk SM (2010) Effect of NPK mineral fertilizer levels and foliar application with humic and amino acids on yield and quality of strawberry. Egypt J Appl Sci 25:154-169.

Acien FG, Gómez-Serrano C, Morales-Amaral MM, Fernández-Sevilla, JM, Molina-Grima E (2016) Wastewater treatment using microalgae: how realistic a contribution might it be to significant urban wastewater treatment? Appl Microbiol Biotechnol 100 (21):9013-9022.

Alves-Dias G, Rocha RHC, Lopes-Araújo J, Franciraldo de Lima J, Alves Guedes S W(2016). Growth, yield and postharvest quality in eggplant produced under different foliar fertilizers (*Spirulina platensis*) treatments. Ciencias Agrarias 37: 3893-3902.

Agehara S, Leskovar DI (2017) Growth suppression by exogenous abscisic acid and uniconazole for prolonged marketability of tomato transplants in commercial conditions. Hortsci 52 (4): 606-611.

Battacharyya D, Zamani Babgohari M, Rathor P, Prithiviraj B (2015) Seaweed extracts as biostimulants in horticulture. Sci Hort 196: 39-48.

Bose Sk, Kumar Yadav R, Mishra S, Sangwan RS, Singh AK, Mishra B, Srivastava AK, Sangwan NS (2013). Effect of gibberellic acid and calliterponone on plant growth attributes trichomes, essential oil biosyntheiss and pathway gene expression in differential manner in Mentha arvensis L. Plant Physiol Biochem 66: 150-158.

Cantor M, Krizbai E, Buta E (2015) The behavior of some Petunia varieties for improvement the Romanian Assortment. Bull UASVM Hortic 72 (1): 39-44.

Coppens J, Grunert O, Van den Hende S, Vanhoutte I, Boom N, Haesaert G, Gelder LD (2016) The use of microalgae as a high-value organic slow-release fertilizer results in tomatoes with increased carotenoid and sugar level. J Appl Phycol 28:2367-2377.

Dong Y, Chen W, Liu F, Wan Y(2016) Effects of exogenous salicylic acid and nitric oxide on peanut seedlings growth under iron deficiency. Comm Soil Sci Plant Anal 47: 2490-2505.

Elarroussi H, Elmernissi N, Benhima R, Meftah EL Kadmiri I, Bendaou N, Smouni A, Wahby I (2016) Microalgae polysaccharides a promising plant growth biostimulant. J Algal Biomass Utln 7(4):55-63.

Faheed F, Fattah ZAE (2008) Effect of *Chlorella vulgaris* as bio-fertilizer on growth parameters and metabolic aspects of lettuce plant. J Agi Soc Sci 4 (4): 166-16.

Garcia-Gonzalez J, Sommerfeld M (2016) Biofertilizer and biostimulant properties of the microalga *Acutodesmus dimorphus.* J Appl Phycol 28: 1051-1061.

Ghanem ME, Albacete A, Martínez-Andújar C, Acosta M, Romero-Aranda R, Dodd IC, Lutts S, Pérez-Alfocea F(2008) Hormonal changes during salinity-induced leaf senescence in tomato (*Solanum lycopersicum* L.). J Exp Bot 59: 3039-3050.

Hocking PJ, Pate JS (1977) Mobilization of minerals to developing seeds of legumes, Ann Bot 41 1259–1278.

Hogue E, Wilcox GE, Cantliffe DJ (1970) Effect of soil P levels on phosphate fraction in tomato leaves. J Am Soc Hortic Sci 95, 174-176.

Jauron R (2013) Growing Petunias. Extension and Outreach. Iowa state University. Press. Iowa.

Krom MD (1980) Spectrophotometric determination of NH_4^+: a study of a modified berthelot reaction using salicylate and dichloroisocyanurate. Analyst 105, 305-316.

Lachica M, Aguilar A, Yañez J (1973) Análisis foliar. Métodos utilizados en la Estación Experimental del Zaidín, Anales de Edafología y Agrobiología 32: 1033–1047.

Lambers H, Stuart F, Pons TL (1998) Plant physiological ecology. Springer Verland. New York Berlin. Heidelberg.

Marschner H (2012) Mineral Nutrition of Higher Plants, 3rd ed, Academic Press; London.

McAdam SA, Brodribb TJ, Ross JJ (2016) Shoot-derived abscisic acid promotes root growth. Plant Cell Environ 39, 652-659.

Priyadarshani I, Rath B (2012) Commercial and industrial applications of micro algae- A review. J Algal Biomass Utln 3(4): 89-100.

Rangel Sánchez G, Castro Mercado E, Beltran Peña E, Reyes de la Cruz H, García Pineda E (2010) El acido salicílico y su participación en la resistencia a patógeno en plantas. Biológicas 12 (2): 90-95.

Romero García JM, Acién Fernández FG, Fernández Sevilla JM (2012) Development of a process for the production of l-amino-acids concentrates from microalgae by enzymatic hydrolysis. Bioresource Technol 112:164-170.

Schwarz D, Krienitz L (2005) Do algae cause growth-promoting effects on vegetables grown hydroponically? In: Price MR (ed) Fertigation: optimizing the utilization of water and nutrients. International Potash Institute, Beijing, pp 161–170.

Shaaban M (2001) Green microalgae water extract as foliar feeding to wheat plants. Pak J Biol Sci. 4 (6): 628-632.

Shaaban M, El-Saady AKM, El-Sayed AEB (2010) Green microalgae wáter extract and micronutrients foliar application as promoters to nutrient balance and growth of wheat plants. J Am Sci 6: 631-636.

Serrani JC, Sanjuán R, Omar Ruiz-Rivero MF , Garcia-Martinez JL (2007) Gibberellin regulation of fruit set and growth in Tomato. Plant Physiol 145:246-257.

Suganya T, Varman M, Renganathan S (2016) Macroalgae and microalgae as a potential source for commercial applications along with biofuels production: A biorefinery approach. Renewable and Sustainable Energy Reviews 55: 909-941.

Tarakhovskaya ER, Maslov YuI, Shishova MF (2007) Phytohormones in Algae. Russian J Plant Physiol 54: 163-170.

Tarraf S, Talaat IM, El-Sayed AEK, Balbaa LK (2015) Influence of foliar application of algae extract and amino acids mixture on fenugreek plants in sandy and clay soils. NusantBiosci 7: 33-37.

Vankova R (2014) Cytokinin regulation of plant growth and stress responses. Phytohorm: a window to metabolism, signaling and Biotechnol Appl 55-79.

Zhao Y, Hasenstein KH (2009) Primary root growth regulation: the role of auxin and ethylene antagonists. J Plant Growth Regul 28: 309-320.

Figures

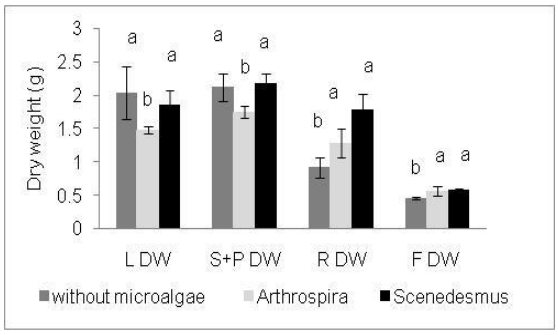

Figure 1. Dry weight (g) of L (leaves), S+P (stems and petioles, conductive organs), R (roots) and F (flowers) at the end of the trial. Different letters indicate significant differences between treatments at the $P<0.05$ level in the LSD test. Values represent the average (n=12); bars represent the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

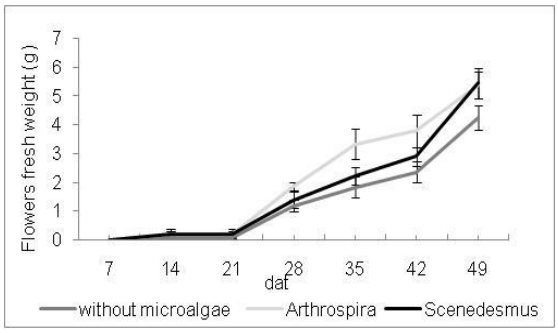

Figure 2. Flower fresh weight during the crop. Values represent the average (n=12); bars represent the standard error.

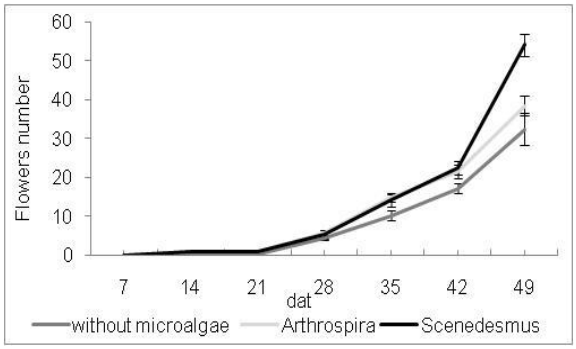

Figure 3. Number of flowers during the crop. Values represent the average (n=12); bars represent the standard error.

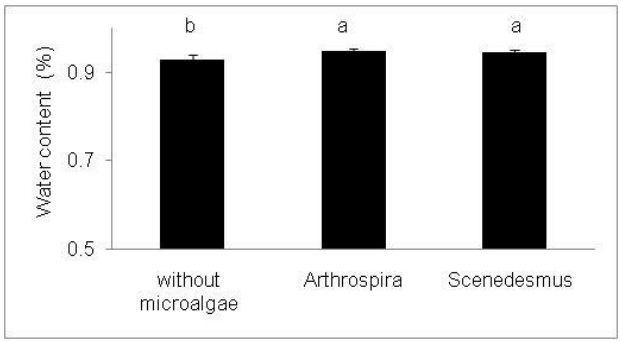

Figure 4. Water content (%). Different letters indicate significant differences between treatments at the P<0.05 level in the LSD test. Values represent the average (n=12); bars represent the standard error.

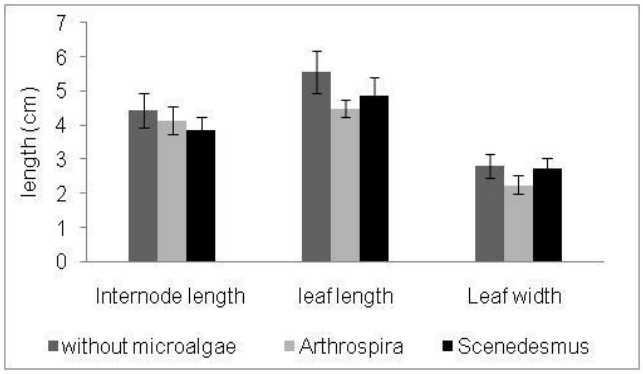

Figure 5. Internode length (cm), leaf length (cm) and leaf width (cm) at the end of the trial. Different letters indicate significant differences between treatments at the P<0.05 level in the LSD test. Values represent the average (n=12); bars represent the standard error.

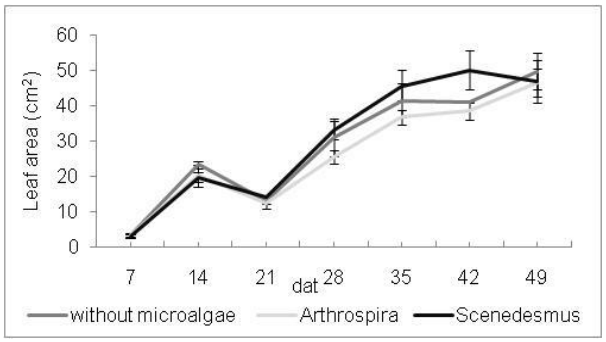

Figure 6. Leaf area during the crop (cm^2). Values represent the average (n=12); bars represent the standard error.

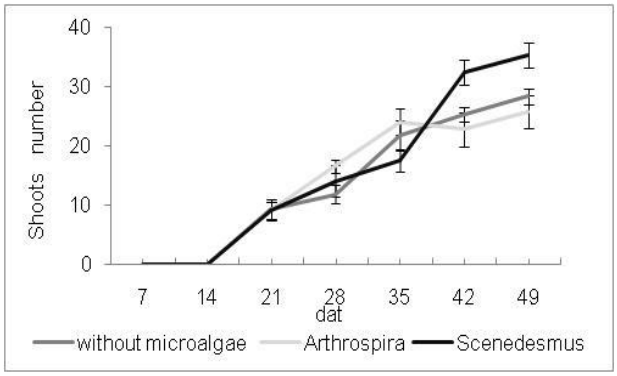

Figure 7. Number of shoots during the crop. Values represent the average (n=12); bars represent the standard error.

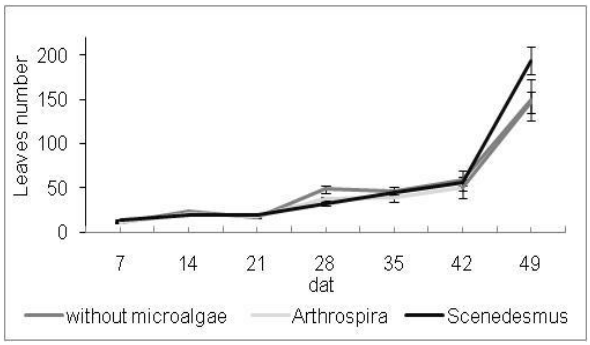

Figure 8. Number of leaves during the crop. Values represent the average (n=12); bars represent the standard error.

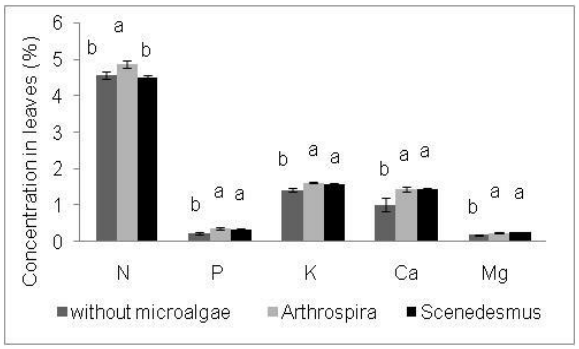

Figure 9. Concentration in leaves (%). Different letters indicate significant differences between treatments at the P<0.05 level in the LSD test. Values represent the average (n=12); bars represent the standard error.

Tables.

Table 1. Hormone content of the microalgae hydrolysate (ng g^{-1})

		Arthrospira	*Scenedesmus*
Ethylene	ACC	545.55	341.32
Cytokinins	Trans-Zeatin	191.37	676.3
	Zeatin riboside	0	6.82
	Isopentenyl adenine	2560.73	45561.97
Gibberellins	GA1	24.97	208.81
	GA3	-	-
	GA4	31.27	9.09
Auxins	Indoleacetic acid	10.3	5965.01
Other hormones	ABA	1.03	3718.25
	Salicylic acid	0.61	156713.72
	Jasmonic acid	0.84	75.13

Table 2. Nutrient content of the microalgae hydrolysate (%).

	Arthrospira	*Scenedesmus*
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