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Insight into the Biostimulant Effect of an Aqueous Duckweed Extract on Tomato Plants

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Abstract: Agricultural systems must improve their sustainability and productivity to meet the growing global demand for food. A cost-effective and sustainable way is the development of biostimulants from plants rich in bioactive compounds. This study aimed to test an aqueous extract from *Lemna minor* L. (duckweed) on tomato plants at different concentrations (LE—0.1, 0.5 and 1.0%—*weight/volume*, *w/v*). Photosystem I and II activity, linear electron flow (LEF), electrochemical gradient across the thylakoid membrane (ECSt), shoot biomass production, root phenotyping, pigment and metabolite content were studied. LE improved many of these traits, with LE 0.5% being the most effective dosage. Compared to the untreated samples, LE significantly stimulated photosystems to use light energy while reducing the amount lost as heat (PhiNPQ and NPQt) or potentially toxic to chloroplasts (PhiNO). These results were supported by the improved shoot biomass production (number of leaves and fresh and dry weight) and root traits (number of tips, surface, volume and fresh and dry weight) found for LE-treated samples compared to untreated controls. Finally, the study highlighted that LE increased pigment and flavonoid contents. In conclusion, the research indicates that this species can be an effective and eco-friendly tool to stimulate beneficial responses in tomato.

Keywords: plant extract; horticultural crop; *Lycopersicon esculentum*; photosynthesis; biomass production; pigment content; antioxidants

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1. Introduction

One of the biggest challenges facing agriculture in the coming years is the growing demand for food, as the world population could reach 9.7 billion by 2050 [1]. In addition, human activities are compromising the quality of natural resources by reducing, for instance, the area dedicated to crop cultivation, and the situation is further exacerbated by climate change [2]. In this context, it should also be considered that agricultural systems based on the extensive use of synthetic chemicals administered to crops to increase yield result in environmental pollution and the degradation of primary resources such as soil and freshwater [3]. Therefore, in a circular economy logic, there is a cogent need for innovative and sustainable biobased solutions to mitigate the impact of cropping systems. Indeed, this vision aims to exploit more efficiently biological resources, even those derived from agroindustrial waste, for application in agriculture, with the scope of increasing crop productivity and quality, reducing pressure on the environment and safeguarding the health of ecosystems [4].

For these reasons, eco-friendly solutions should be searched for in unexplored natural resources and applied in agriculture to improve crop performance. As is well known, biostimulants are natural substances that can stimulate seed germination, affect plant nutrition, improve water uptake and use, influence plant growth and biomass production

and improve primary and secondary metabolism [3]. Moreover, these materials can make crops more tolerant to environmental biotic and abiotic stressors [3]. Based on their origin, biostimulants have been classified as microbial and non-microbial [5,6]. Non-microbial biostimulants can be obtained from plant extracts (plants and algae), protein hydrolysates (both of plant and animal origin), fulvic and humic substances and inorganic (salts) and organic compounds (chitosan) [7].

Currently, there is growing attention being paid to finding new plant extracts rich in bioactive molecules to be exploited as biostimulants and used in agriculture. In particular, plant extracts can show noticeable contents of bioactive compounds such as phenols, amino acids, small peptides, micro- and macro-elements and numerous other components that can stimulate crop metabolism and biomass production and improve the end product's quality [8]. These beneficial effects can be due to the ability of biostimulants to prompt some crucial physiological, morphological and biochemical processes, such as photosynthesis and metabolism [8]. Furthermore, specific molecules with signaling or hormonal activity have been identified in plant extracts, and they are responsible for increased plant biomass production, interactions with proteins to regulate genes and amino acid and metabolite synthesis [9,10]. For instance, it is well known that applying protein hydrolysates to plants can determine many plant-stimulatory effects, as they contain peptides and amino acids that can act as signal molecules [9,11].

Among the species that can be used to obtain plant extracts with bioactive properties and promote benefits in crops, duckweed is attracting increasing interest. This species is a small free-floating aquatic plant belonging to the Lemnaceae family that naturally occurs worldwide in wetland ecosystems, such as lagoons, swamps and ponds, as well as in irrigation ditches. Duckweed is considered invasive due to its fast growth rate and high capacity to adapt to different climatic conditions (temperatures in the range of 5–35 °C) and unfavorable aquatic environments (pH levels between 3.5 and 10.5) [11]. In addition, duckweed can tolerate and survive high concentrations of toxic compounds, and this resistance makes it a suitable species for phytoremediation and ecotoxicity studies [12,13]. Indeed, it was successfully used for phytoremediation purposes, such as wastewater treatment, as it can remove and bioaccumulate pollutants, ranging from organic compounds to metal trace elements [12,13].

Moreover, recent studies demonstrated that duckweed produces a plethora of secondary metabolites with bioactive properties, especially glucosinolates and phenols [11]. Indeed, metabolomics studies conducted by the authors of this research have revealed that this aquatic plant has a broad spectrum of bioactive substances [11,14–17]. Among them, compounds including phenols, glucosinolates, flavonoids and substances with antioxidant properties and protective action should be mentioned because they correlate with biostimulant properties [11,14–16]. For instance, glucosinolates, extensively studied for other species such as Brassicaceae, can exert protective action in plants against physical damage, such as wounds and those caused by pest attacks and even abiotic stressors (high temperatures, salinity and UV) [11]. In addition, it has been proposed that these compounds may act as signaling molecules capable of activating plant defense systems [18]. Phenols and their exogenous applications have shown a wide range of benefits in treated plants, due to their involvement in regulating and stimulating various physiological processes. These include growth regulation, photosynthesis, pigment synthesis induction and oxidative stress mitigation [19]. These bioactives can play a crucial role in the adaptation to challenging environmental conditions, and it has been demonstrated that their application to crops can help plants cope with abiotic stresses [20].

Despite the interesting traits of duckweed and its richness in bioactive compounds, few studies have explored its biostimulatory potential on crops, except for some recent research on olive and maize crops [11,14–16]. For horticultural species, on the other hand, there are no traces in the literature that address the biostimulant effect of duckweed on these crops. To fill this gap, the present work aimed to evaluate the effects of an aqueous duckweed extract (LE) on a horticultural species. In particular, LE was applied by foliar

spraying on tomato, which was chosen because this crop is one of the most important and widespread worldwide. The photosynthetic machinery and specific aspects, such as the photosystem functionality, aerial and root biomass development, pigment content and some major classes of antioxidants, were then investigated in LE-treated tomato plants. All this was undertaken to ascertain any beneficial effects of the extract on the horticultural species in question.

2. Materials and Methods

2.1. Preparation of the Aqueous Extract of Duckweed

All chemicals used in this research were purchased from Merck Life Science S.r.l. (Milan, Italy) and used as received without further purification.

Duckweed was grown in polyethylene trays (35 × 28 × 14 cm) according to a previously published procedure, renewing the culture medium every two weeks [21]. Briefly, the nutrient solution (pH 6.5) contained 3.46 mmol L^{-1} KNO_3, 1.25 mmol L^{-1}, $Ca(NO_3)_2 \cdot 4H_20$, 0.66 mmol L^{-1} KH_2PO_4, 0.071 mmol L^{-1} K_2HPO_4, 0.41 mmol L^{-1} $MgSO_4 \cdot 7H_2O$, 0.28 mmol L^{-1} K_2SO_4, 1.94 µmol L^{-1}, H_3BO_3, 0.63 µmol L^{-1} $ZnSO \cdot 7H_2O$, 0.18 µmol L^{-1} $Na_2MoO_4 \cdot 2H_2O$, 1 µmol L^{-1} $MnSO_4 \cdot H_2O$, 21.80 µmol L^{-1} Fe-EDTA and 1 µmol L^{-1} $CuSO_4$. Trays were maintained in a growth chamber at 24 ± 2 °C, 80 µmol m^{-2} s^{-1} of light intensity, and a photoperiod of 8 h light and 16 h dark.

Three different concentrations of LE were chosen for the experiments on tomato plants: LE 0.1, 0.5 and 1.0% (dry weight/water volume—wt/v). To this scope, 20 g of fresh plant material was thoroughly rinsed with water and dried at 40 °C for 72 h. Then, 1 g of dry biomass was mixed with 100 mL of deionized water (pH value = 7.00), extracted using a mortar with a pestle for 5 min in the presence of small amounts of quartz sand, and left in an orbital shaker overnight (100 rpm) at 23 °C, to complete the extraction. Finally, the suspension was filtered using filter paper and brought to the final volume of 100 mL with deionized water. This allowed us to obtain the most concentrated LE extract (LE 1.0%). This extract was appropriately diluted with deionized water to obtain the other two solutions, designated as LE 0.5 and 0.1%. The three concentrations were selected as previous studies showed they were capable of prompting biostimulatory effects in crops [11,14,15]. Differently, LE can be phytotoxic at higher concentrations (2 and 8%) or lose activity at lower ones [11].

A description of the metabolomic and phytochemical profile of LE 1.0%, ascertained in previous studies on plants bred and extracted according to the above procedure, is given below [11,15]. The LE phytochemical profile was determined by using untargeted metabolomics ultra-high-pressure liquid chromatography associated with a quadrupole-time-of-flight mass spectrometer (UHPLC-ESI/QTOF-MS), according to Del Buono et al. [11]. The results indicated a remarkable content of bioactives such as phenols (6714.99 mg kg^{-1}) and glucosinolates (4563.74 mg kg^{-1}). Also, flavonoids and phenolic acids were found in significant amounts and similar concentrations of 1829 and 1733 mg kg^{-1}, respectively [11]. The most abundant flavonoids were kaempferol and quercetin and their glucosides, followed by myricetin. Furthermore, hesperidin was the most abundant flavone, while caffeic acid was the most abundant of the phenolic acids (812 mg kg^{-1}). In addition, the following low molecular weight phenols were detected: mainly 5-nonadecenylresorcinol, hydroxytyrosol and 4-hydroxycoumarin. Phytohormones (auxins, cytokinins, gibberellins, jasmonate-related metabolites and brassinosteroids) were also found in the LE [15]. The metabolomic profile also revealed the presence of amino acids, phenylpropanoids and alkaloids [15]. Isoprenoids, including triterpenoids, sesquiterpenes and terpene hormones (gibberellins and their precursors, abscisic acid derivatives and brassinosteroids) were well represented. Finally, antioxidant and plant-to-stress response-related compounds were identified (ascorbates and glutathione) [15].

2.2. Growth Conditions of Tomato Plants and LE Treatments

The experiments were conducted on tomato plants (*Lycopersicon esculentum* Mill.) cv. Rio Grande, a variety widely cultivated in Italy that produces large and pear-shaped tomatoes, suitable, for instance, for processing to obtain peeled tomatoes and preserves. The seeds were directly sown in plastic pots containing commercial peat and germinated in the dark for 5 days before light exposure. Tomato seedlings were cultivated in a growth chamber, with a photoperiod of 12/12 h (day/night), light intensity at 300 µmol m^{-2} s^{-1}, at a constant temperature of 24 ± 2 °C, and irrigated daily with water up to 75% field moisture capacity.

The leaves of tomato plantlets were sprayed, using a domestic sprinkler, at 4 (third true leaf stage) and 5 weeks after sowing. The temporal sequence of the treatment was chosen based on the development stage of the seedling, as the third true leaf was well formed. In detail, 2.5 mL per plant of water (control) or LE 0.1, 0.5 or 1.0% were applied, depending on the experimental group. For each treatment, 5 replicates were carried out, according to a completely randomized experimental design. Six-week-old plants were harvested for the physiological, morphological and biochemical determinations, as indicated in the following sections.

2.3. Effects of LE on Tomato Photosynthetic Activity

Some aspects of the photosynthetic processes were monitored on intact and fully expanded leaves in the early morning after 2 h of light exposure. To this end, the MultispeQ device (PHOTOSYNQ INC., East Lansing, MI, USA) linked to the web platform PhotosynQ (http://www.photosynq.org) was used [22]. In particular, the following parameters were studied: the quantum yield of PSII (Phi2), the fraction of light that can be lost via non-regulated processes (PhiNO) or released as non-photochemical quenching (PhiNPQ), the fraction of PSII centers which are in the open state (qL), the maximal quantum efficiency of PSII (Fv/Fm), the dark-interval relaxation kinetics of P700 (P700 DIRK), PSI photosynthetic reaction center proteins in open state (PSI open centers) and oxidized state (PSI oxidized centers), the total non-photochemical quenching (NPQt), the linear electron flow between photosystems (LEF), the total electrochromic shift (ECSt) and the proton conductivity of the thylakoid membrane (gH^+).

2.4. LE Treatment Effect on Tomato Growth at the Shoot and Root Level

Shoot development was evaluated by measuring shoot height and number of leaves. Furthermore, the leaf thickness was recorded. At the root level, biomass production was investigated, and the phenotyping was carried out on the scanned root using RhizoVision Explorer v2.0.3.0, according to Seethepalli et al. [23], measuring the total root length (cm), number of root tips, diameter (mm), surface area (cm^2) and volume (cm^3). Finally, the fresh mass of shoots and roots was recorded, and the dry weight was determined after oven-drying the samples at 60 °C to constant weight.

2.5. Leaf Biochemical Analysis (Chlorophyll and Carotenoid Contents, TPC, TFC and Soluble Carbohydrates)

Chlorophyll a (Chl a), chlorophyll b (Chl b) and carotenoid contents were ascertained by extracting 0.5 g of fresh leaf samples in 5 mL of methanol. This suspension was then centrifuged (20,000 rpm, 5 min). According to Venkatachalam et al. [24], the resulting supernatant was analyzed spectrophotometrically.

Finally, the total phenolic content (TPC), total flavonoid content (TFC) and soluble carbohydrates were determined by extracting 0.25 g of fresh leaf samples in 2.5 mL of methanol, then centrifuging at 6000 rpm (20 min). The Folin–Ciocalteu method was adopted for TPC, and the phenols content was referred to as the gallic acid equivalent (GAE) g^{-1} [25]. TFC was determined spectrometrically, according to Atanassova et al. [26], and was expressed as mg of catechin equivalents (CE) g^{-1}. Soluble carbohydrates were evaluated using the anthrone method, according to Al Murad and Muneer [27]. The supernatant (50 µL) from the methanolic extract was transferred to the solution with 950 µL of distilled water, and 2.5 mL of 0.2% anthrone reagent was added. The solutions were heated (100 °C, 10 min) to complete the reaction with anthrone, and, after cooling, the absorbance of the samples was measured spectrometrically at 620 nm. The total soluble carbohydrates were expressed as mg g^{-1} fresh weight (FW).

2.6. Statistical Analyses

The experiment was carried out according to a completely randomized design with four treatments (control, LE 0.1, 0.5 and 1.0%) and five replicates per treatment. The full dataset was subjected to statistical analysis through a one-way analysis of variance (ANOVA), and significant differences were assayed using Duncan's test at the $p < 0.05$ probability level [28]. The data presented in the tables represent the mean value ± standard deviation.

3. Results

3.1. Effects of LE on Tomato Photosynthetic Activity

LE improved photosystem II in tomato plants. In particular, the quantum yield (Phi2) increased significantly in all plants treated, compared to the untreated ones, with the LE 0.1 and 0.5% concentrations being the most effective (Figure 1). In parallel, the light energy dissipated through non-regulated mechanisms (PhiNO) was reduced by LE, with the highest difference recorded for the dosage of 0.5%. Non-photochemical photoprotective quenching (PhiNPQ) was also significantly decreased in treated plants proportionally to the LE dosage applied. LE applications affected the open state rate of photosystem II centers (qL), particularly for LE 0.5 and 1.0%. Differently, LE treatments did not affect the Fv/Fm ratio. Concerning photosystem I, the dark-interval relaxation kinetics of P700 (P700 DIRK) underwent a considerable reduction in all samples on which the LE was applied. In addition, the centers of the photosystem I found in an open state (PSI open centers) were significantly higher in plants treated with LE 0.5% than the control samples. As for the oxidized state of the photosystem I (PSI oxidized centers), LE caused a dose-dependent increase, with the 1.0% concentration being the most effective.

Compared to control samples, the linear electron flow (LEF) increased for all the LE treatments, with the highest values reached by 0.5 and 1.0%. In addition, the total amount of non-photochemical quenching (NPQt) was lowered by LE 1.0% (Figure 2).

The LE reduced the total electrochromic shift (ECSt), regardless of the dosage applied, while no differences were detected for the proton conductivity of the thylakoid membrane (gH^+) (Figure 2).

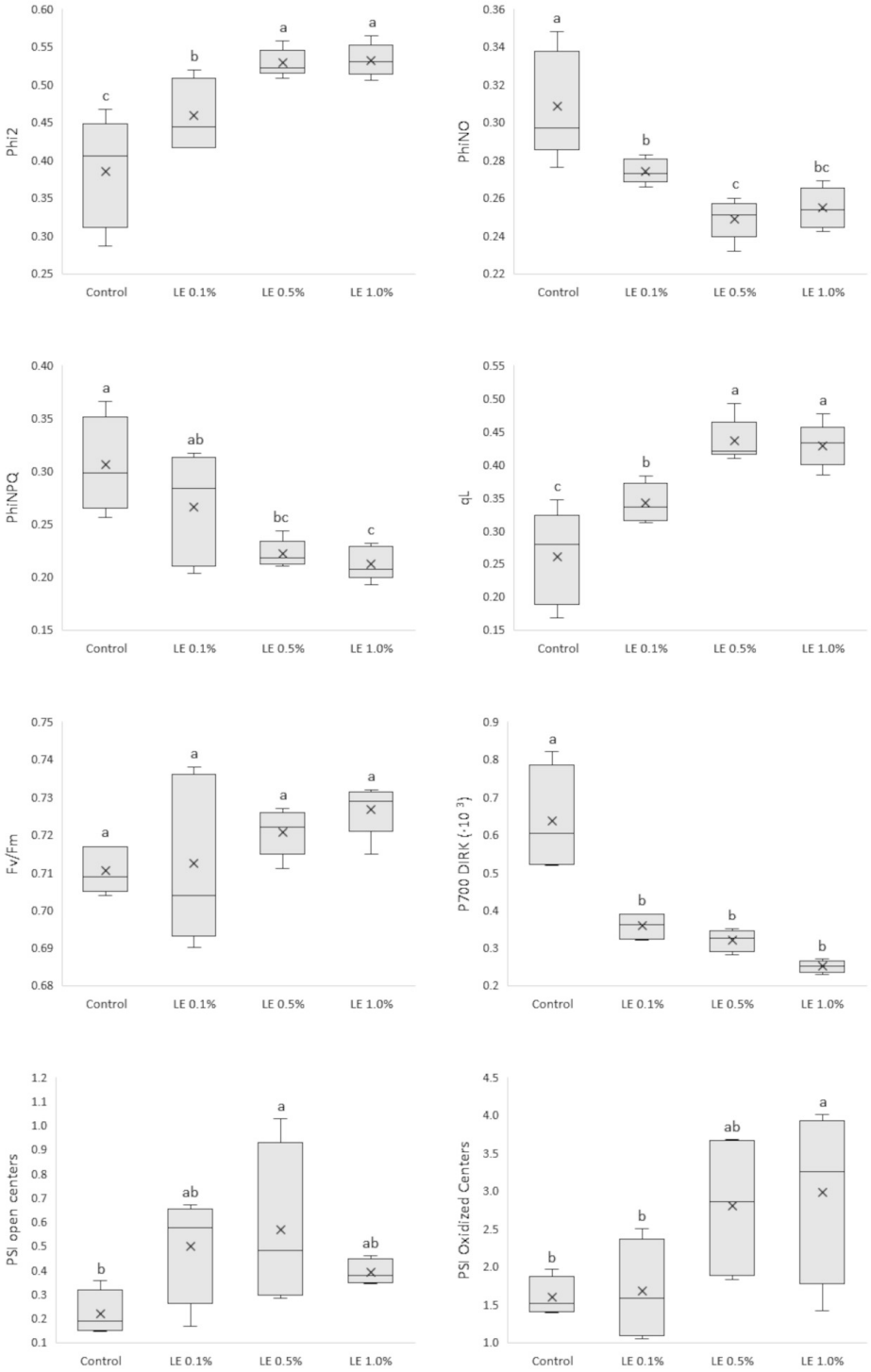

Figure 1. Effect of different duckweed extract concentrations (LE 0.1, 0.5 and 1.0%) on Phi2 (the efficiency of PSII), PhiNO (the non-regulated dissipation of light energy), PhiNPQ (the photo-protective non-photochemical quenching), qL (the open state of PSII), Fv/Fm (the photochemical efficiency of PSII), P700 DIRK (the dark-interval relaxation kinetics of P700), PSI open centers and PSI oxidized centers. Different letters indicate statistically different values, according to Duncan's multiple comparison test ($p < 0.05$).

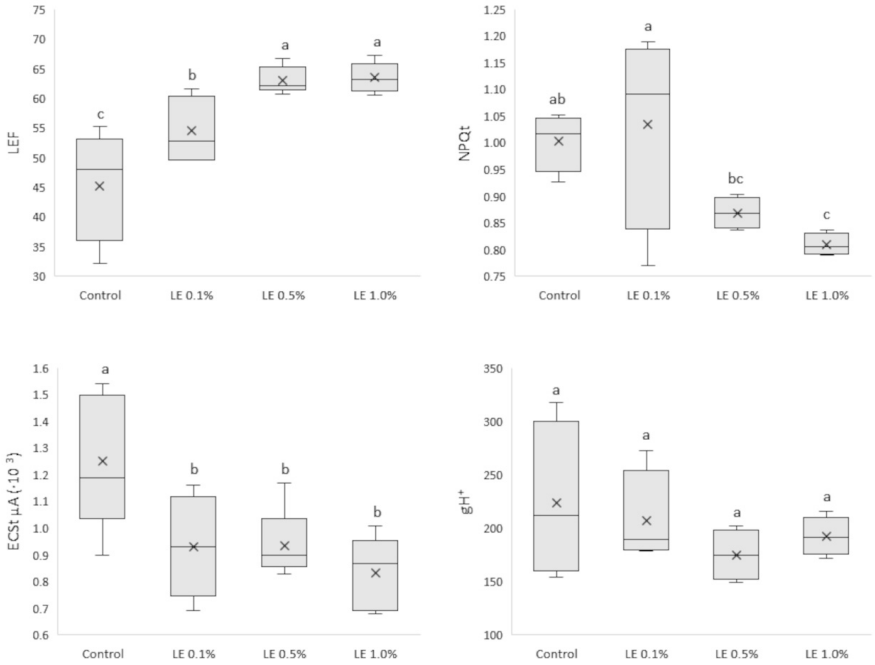

Figure 2. Effect of different duckweed extract concentrations (LE 0.1, 0.5 and 1.0%) on LEF (linear electron flow), NPQt (total non-photochemical quenching), ECSt (total electrochromic shift) and gH^+ (proton conductivity of the thylakoid membrane). Different letters indicate statistically different values, according to Duncan's multiple comparison test ($p < 0.05$).

3.2. LE Treatment Effect on Tomato Growth at the Shoot and Root Level

LE treatments affected the growth of tomato seedlings at both aerial and root levels. Plants treated with 1.0% concentration showed more leaves than those untreated (Table 1). Shoot height and leaf thickness were not influenced by any of the treatments. On the other hand, analyzing the shoot fresh and dry weights, it can be pointed out that all LE applications prompted tomato plants to produce more biomass than the control.

Table 1. Shoot analyses of tomato plants untreated (control) and treated with different duckweed extract concentrations (LE 0.1, 0.5 and 1.0%).

	Shoot Height (cm)	Number of Leaves (number)	Leaf Thickness (mm)	Fresh Weight per Plant (g)	Dry Weight per Plant (g)
Control	13.83 ± 0.50 a	33.5 ± 1.7 c	0.40 ± 0.07 a	6.25 ± 0.37 b	0.84 ± 0.14 b
LE 0.1%	14.65 ± 0.59 a	38.3 ± 1.7 a	0.62 ± 0.30 a	7.13 ± 0.31 a	1.07 ± 0.06 a
LE 0.5%	14.70 ± 0.59 a	38.8 ± 0.5 a	0.53 ± 0.10 a	7.21 ± 0.14 a	1.15 ± 0.02 a
LE 1.0%	14.48 ± 1.06 a	35.8 ± 1.0 b	0.53 ± 0.14 a	6.97 ± 0.34 a	1.02 ± 0.03 a

Different letters indicate statistically different values, according to Duncan's multiple comparison test ($p < 0.05$).

As for root phenotyping, the data highlighted that the number of root tips increased proportionally with the LE concentration, with all treatments being significantly higher than the control samples (Table 2). In addition, the extract increased the surface area and volume. Regarding the former, plants treated with LE 0.1 and 0.5% performed better than the control samples, while those belonging to LE 1.0% did not differ from the untreated samples. As for the root volume, the extract effectively increased this trait at 0.5 and 1.0% concentrations, and 0.1% was in line with the control. No treatment influenced the total length and average diameter of the roots. Finally, the root fresh weight per plant was higher than that shown by the control samples for all the samples treated with LE, regardless of the concentration applied. Regarding the dry weight per plant, it can be noted that only the LE 0.5% differed from the control.

Table 2. Root analyses of tomato plants untreated (control) and treated with different duckweed extract concentrations (LE 0.1, 0.5 and 1.0%).

	Total Length (cm)	Root Tips (Number)	Diameter (mm)	Surface Area (cm^2)	Volume (cm^3)	Root Fresh Weight (g)	Root Dry Weight (g)
Control	5094 ± 792 a	727 ± 91 c	0.73 ± 0.07 a	95 ± 20 b	2.46 ± 0.66 b	1.33 ± 0.10 b	0.25 ± 0.03 b
LE 0.1%	6051 ± 710 a	994 ± 130 b	0.69 ± 0.03 a	132 ± 14 a	3.46 ± 0.52 ab	2.93 ± 0.26 a	0.29 ± 0.02 ab
LE 0.5%	5806 ± 265 a	1061 ± 65 ab	0.72 ± 0.06 a	132 ± 6 a	3.74 ± 0.39 a	3.24 ± 0.13 a	0.32 ± 0.01 a
LE 1.0%	6110 ± 782 a	1216 ± 89 a	0.71 ± 0.03 a	120 ± 6 ab	3.73 ± 0.86 a	3.19 ± 0.27 a	0.30 ± 0.03 ab

Different letters indicate statistically different values, according to Duncan's multiple comparison test ($p < 0.05$).

3.3. Leaf Biochemical Analysis (Chlorophyll and Carotenoid Contents, TPC, TFC and Soluble Carbohydrates)

Some biochemical aspects were investigated in plants treated with LE (Table 3). For chlorophyll a, LE increased its content in the treated samples at the dosages of LE 0.5 and 1.0%. Regarding chlorophyll b, LE-treated plants showed higher values than the control for all dosages applied. Carotenoids did not differ in LE-treated samples, while the flavonoids (TFC) content increased in LE 0.5%-treated plants. Finally, the contents of phenols and soluble carbohydrates were not affected by treatments with the extract.

Table 3. Chlorophyll a (Chl a), chlorophyll b (Chl b), carotenoid content, total phenols (TPC), total flavonoids (TFC) and soluble carbohydrates in tomato plants untreated (control) and treated with different duckweed extract concentrations (LE 0.1, 0.5 and 1.0%).

	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Carotenoids (mg g^{-1} FW)	TPC (mg GAE g^{-1} FW)	TFC (mg CE g^{-1} FW)	Soluble Carbohydrates (mg g^{-1} FW)
Control	0.99 ± 0.06 c	0.26 ± 0.03 b	0.25 ± 0.02 a	1.95 ± 0.47 a	1.42 ± 0.14 b	1.22 ± 0.29 ab
LE 0.1%	1.07 ± 0.06 bc	0.39 ± 0.05 a	0.27 ± 0.03 a	1.79 ± 0.11 a	1.42 ± 0.10 b	0.94 ± 0.07 b
LE 0.5%	1.24 ± 0.05 a	0.44 ± 0.08 a	0.30 ± 0.02 a	2.02 ± 0.09 a	1.64 ± 0.04 a	1.32 ± 0.24 a
LE 1.0%	1.13 ± 0.05 b	0.38 ± 0.02 a	0.30 ± 0.03 a	1.74 ± 0.08 a	1.49 ± 0.03 b	1.07 ± 0.03 ab

Different letters indicate statistically different values, according to Duncan's multiple comparison test ($p < 0.05$).

4. Discussion

Finding natural and functional biobased solutions, such as biostimulants, can decisively improve cropping systems and increase crop performance in normal conditions and their tolerance to environmental stress. In addition, biostimulants can allow for the reduction in use or replacement of synthetic chemical compounds, which can have a high environmental impact [29]. Biostimulants have gained prominence and are considered an innovative agronomic tool because they can improve crop performance, help plants cope with environmental pressure and have economic and environmental benefits [7]. This becomes particularly important when considering the effects of climate change on cropping systems and the challenges of meeting growing food demand on a global scale.

Biostimulants can be used in both horticultural and cereal crops, and to date, research is increasingly focusing on new biostimulants obtained from plants, such as simple extracts. In fact, in cases where they are rich in bioactive compounds, they can effectively promote the growth and traits of the crops to which they are applied. Furthermore, if these biostimulant materials can be obtained from non-food and invasive plant species, this solution becomes relevant, cheap and smart, and aligns with the main concepts of the circular economy [30].

In this frame, some studies have highlighted the richness of duckweed, a free-floating aquatic invasive species, in terms of substances that can promote the development of crops, such as maize and olive, under normal and abiotic stress conditions [11,14–16]. However, despite their agronomic and food importance, the LE has never been tested for horticultural crops. For the above, this study reports the results of experiments on tomato plants grown

under normal conditions and treated with different concentrations of LE. Our research has shown that according to a general dose–response type trend, LE promoted and stimulated a range of beneficial effects in tomato plantlets. In particular, the photosynthetic machinery was affected by the treatments (Figures 1 and 2). This benefit is worth mentioning as it enables plants to more efficiently utilize light energy and transform it into chemical energy, thus impacting biomass production and crop productivity [31]. LE improved the efficiency of PSII and PSI, which enhanced the ability of the photosystems to intercept light for biosynthetic purposes. The increase in Phi2 (the amount of light used for photochemical biosynthesis) indicates a higher ability of PSII to absorb electromagnetic radiation for photosynthesis. PSII is considered an indicator of the efficiency of plants in utilizing light for carbon dioxide assimilation by crops [32]. This benefit was associated with a decrease in PhiNPQ, the energy that plants do not use for photochemical reactions and disperse mainly as heat, and PhiNO, which represents the fraction of energy that can give rise to oxidative stress, thus negatively impacting the crop [33]. It has been documented that a marked decrease in photosystem II efficiency and an increase in PhiNPQ and PhiNO can occur due to abiotic environmental stresses and are associated with reductions in crop yield [33]. In addition, in support of the above beneficial effects, LE induced increases in PSII active centers (qL) without damaging the photosystems, as indicated by the Fv/Fm ratio, representing the integrity of PSII [34]. LE also had a positive impact on PSI function and activity, as revealed by P700 DIRK (i.e., PSI relaxation kinetics) and the increase in PSI open centers and P700 oxidized, thus indicating an enhanced ability of PSI to proceed with the transport of electrons transmitted by PSII, and then used for the reduction of $NADP^+$ to NADPH [35].

Thanks to a highly regulated mechanism, the linear electron flow (LEF) through the photosystems is associated with the formation of a proton electrochemical gradient across the thylakoid membrane that results in a proton motive force (*pmf*), which is then used for ATP synthesis [36]. The improved activity of the two photosystems in LE-treated tomato plants resulted in increased LEF (Figure 2), reflecting LE capacity to improve the ability of the plants to extract electrons from H_2O and transfer them through the PSs, reaching a higher NADPH production.

The overall decrease in NPQt, the total energy dissipated as heat by the photosynthetic machinery, aligns with the increased photosynthetic efficiency (Figure 2). Increasing NPQt attenuates energy transmission between photosystems in stress situations, but its reduction is worth mentioning under normal conditions as it assumes positive significance [37]. Photosynthesis is a highly regulated and coordinated flow of electrons associated with the translocation of protons from the stroma to the lumen, thus forming an electrochemical gradient exploited by ATP-synthase to produce chemical energy as ATP [36,38]. Indeed, the decrease in electrochromic shift (ECSt) (Figure 2) reveals that LE stimulated ATP production, as the amplitude of ECSt is proportional to the proton motive force (*pmf*), and its decrease indicates a concomitant consumption of the electrochemical energy by ATPase to synthesize ATP [36]. Accordingly, when plants suffer from environmental stressors or, for instance, there is a depletion in phosphate, the ATP synthesis can slow down with a *pmf* accumulation across the membrane and ECSt increase [39]. Finally, the gH^+, the thylakoidal membrane conductivity, did not change compared to the control samples. This parameter can slightly increase during high-intensity radiation but is relatively stable over a wide light-intensity range [37]. On the other hand, gH^+ may vary mainly in conditions such as decreasing CO_2 levels or during different light treatments, and it may also reflect metabolic alterations due to environmental stress [37].

Biostimulants can activate multifaceted aspects in the treated plants, but photosynthesis enhancements and biomass production have been very often recorded, both under normal and biotic and abiotic stress conditions [40]. In particular, bioactive compounds can improve plant efficiency by enhancing specific aspects of the photosynthetic machinery, improving its efficiency in capturing light and modulating electron and proton transfer in chloroplasts [41]. For instance, it has been found that biostimulant seed pretreatment

ameliorated the photochemistry of PSII in soybean. In particular, the biostimulant treatment resulted in a more efficient use of light energy in photochemical reactions rather than the induction of photoprotective processes (decreases in NPQ) [42]. Regarding LE, it has already been observed that this species can prompt general benefits to photosynthesis, mainly affecting the stomatal aperture, due to its wide range of bioactive, signal and regulatory molecules capable of influencing metabolic processes [14]. Our previous studies have shown that the phytochemical profile of LE reveals the presence of molecules with biostimulatory activity, such as auxins [11,15,16]. The presence in LE of such compounds explains the effects on the photosynthetic traits mentioned above; it is well known that the exogenous application of auxins induces photosynthetic activity [15]. Auxins can also positively influence transpiration and stomatal conductance [43]. In addition, Lemnaceae have a significant content of antioxidant metabolites [44], which can also induce photosynthesis, such as phenolic compounds [15], as evidenced by the extract used in this research.

Regarding aerial biomass production, data showed that all the LE concentrations generally promoted the stimulating effects. Indeed, the treatments increased the number of leaves and shoot fresh and dry weight (Table 1). Root phenotyping also showed an inductive effect in response to all concentrations investigated, with a consistent increase in root tip number and root fresh weight (Table 2). In addition, LE generally affected root surface area, volume, and for LE 0.5%, even dry weight. The phytochemical profile of LE should be considered to explain these effects in connection with the activation found in the functionality of the photosynthetic machinery [11,15,16]. In particular, in a previous study, we ascertained in LE, as already mentioned above, a significant number of auxins and related compounds that can activate root and aerial biomass production and photosynthesis [15,43]. In addition, the high content in LE of antioxidants, mainly phenolic compounds [15], can promote positive responses in the plant by improving crop functional traits and photosynthesis [14]. Our data also showed that LE modulated and improved root architecture. All these effects agree with the bibliography that documents that biostimulants can generally improve root tissues and their architecture and organization [45,46]. Also, in agreement with the effects recorded, the high content of phytohormones and glucosinolates found in LE [11,14–16], given their stimulating activity on the root system, justifies what was observed in this study. Finally, LE has a noticeable proline content, which can stimulate biomass production at shoot and root levels and crop resistance to biotic and abiotic stresses [14–16].

In addition to the above determinations, the content of chlorophylls, total phenols and flavonoids and soluble carbohydrates were analyzed in tomato plants treated with LE (Table 3). Regarding the pigments, the two highest dosages of LE significantly increased the content of chlorophylls a and b, while it did not affect carotenoids. Chlorophyll is a key pigment that plays a crucial role in photosynthesis, as it absorbs light in the visible region and uses it in reaction centers to support this anabolic process of chemical energy production [47]. This result is in line with what was found for photosynthesis and can be justified as an effect attributable to the bioactives in LE [11]. The induction in photosynthetic pigment content aligns with other studies in which crops treated with different plant extracts manifested significantly higher chlorophyll values than control untreated samples [48,49].

The treatments generally had no significant effects on phenol and flavonoid content, except for the LE 0.5% dosage, which enhanced TFC (Table 3). An increase in flavonoid content is relevant since these molecules play molecular regulatory roles in the cell and are involved in the defensive response to biotic and abiotic stresses and plant acclimatization [50,51]. In addition, there is a growing interest in increasing the content of these biomolecules in crops, given their protective action for human health. Flavonoids can exert numerous benefits, as they can exhibit antioxidant, anti-inflammatory, anticancer and antiviral properties, as well as neuroprotective and cardioprotective action [52]. The last aspect investigated in our experimentation was the content of soluble carbohydrates, as a variation in them may indicate possible treatment-related stress responses, as soluble

carbohydrates are involved in protective osmoregulatory processes. The results reveal that LE did not affect their content.

5. Conclusions

High environmental impacts characterize current agricultural systems. Therefore, solutions need to be found to improve their sustainability and, at the same time, increase their productivity to meet the growing global demand for food in the context of climate change. A smart and suitable way to increase the performance of crops is the development of new biostimulants, as these are ecological tools that can also help crops cope with challenging climatic conditions. In this context, one strategic way is to obtain plant extracts rich in bioactive compounds from non-food and/or invasive species. This study showed that it is possible to obtain a biostimulant from LE, a widespread free-floating aquatic species that can promote many benefits in tomato plants. LE stimulated photosynthesis by increasing the ability of photosystems I and II to intercept light and reducing the amount lost as heat or potentially toxic to chloroplasts. Furthermore, LE stimulated some physiological and biochemical aspects correlated with it, such as linear electron flow, ATP synthesis and pigment content. All these inductive effects resulted in increased biomass production and improved root traits. The results indicated that all concentrations of LE promoted substantial benefits in treated tomato samples, but 0.5% was the most effective in influencing the crop. In light of the above, this study demonstrated how natural resources such as duckweed can be obtained and developed with a convenient application in agriculture to increase the productivity of cropping systems, making them more sustainable. Nonetheless, studies like ours conducted on a laboratory scale must necessarily be followed by others in the field to verify the beneficial effects on crops throughout their life cycle. However, it is well known that positively conditioning the early stages of the plants has essential effects on their entire life cycle.

From a future perspective, we emphasize the strategic importance of the research as a key and crucial step that should help more attention be paid to identifying and obtaining useful materials to be applied in agriculture, with low or absent environmental impact and the characteristic of eco-sustainability. In this sense, an intelligent path is the development of innovative and effective biobased materials from unexploited biological resources. This will reduce the use of synthetic chemicals in agriculture that strongly impact the environment and ecosystems and mitigate the emission of greenhouse gases. Conversely, a not-so-easy substantial paradigm shift is needed in the manufacturing world for a real ecological transition, based on abandoning the current linear economy approach in favor of a circular one.

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