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Stimulatory Effect of an Extract of *Lemna minor* L. in Protecting Maize from Salinity: A Multifaceted Biostimulant for Modulating Physiology, Redox Balance, and Nutrient Uptake

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Abstract: Water and soil salinization significantly reduce crop yields. Among the strategies developed to counteract salt stress, biostimulants can maintain crop productivity, reversing its impact. In this context, there is interest in finding new substances that could act as biostimulants. Recently, the biostimulatory potential of *Lemna minor* L. (duckweed) extracts has been shown. This work aimed to highlight whether an extract from duckweed (Lemna extract—LE) could protect maize grown in salinity, exploring the mechanisms induced to improve crop resistance. Plants were grown by applying two concentrations of NaCl (150 and 300 mM), and some physiological, morphological, and biochemical traits were studied in control and salt-stressed samples, treated or not with LE. Salinity decreased shoots, roots, pigment, and soluble protein. LE prompted ameliorative changes at the root level and increased photosynthetic pigment and soluble protein. Furthermore, concerning the oxidative impairment provoked by salt stress, LE enhanced the cellular redox state, contrasting H_2O_2 and MDA accumulation and positively affecting the activity of superoxide dismutase (SOD—EC 1.15.1.1) and catalase (CAT—EC 1.11.1.6). The assessment of some mineral nutrients showed that LE stimulated their acquisition, especially for the highest salt dosage, explaining some benefits found for the parameters investigated.

Keywords: salt stress; biostimulant; duckweed extract; oxidative stress; plant mineral nutrition; stress-adaptive mechanisms

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1. Introduction

Anthropogenic activities with high environmental impact are causing the progressive degradation of natural resources, and water and soil are among the most affected. High salinity levels of soil and water are considered environmental stresses of concern, as they can lead to the loss of agricultural soil and a sharp reduction in crop yields [1,2]. Mediterranean countries are particularly affected by salinity, as many coastal agricultural areas are experiencing the progressive salinization of soils and waters [3]. A substantial contribution to this phenomenon is related to effects brought about by extreme events caused by climate change, such as flooding and rising sea levels [4]. About 7% of the earth surface is affected by salinity, which has degraded more than 900 million hectares of total arable land on a planetary scale [2]. Salinity determines annual economic losses of USD 27 billion to agricultural production [2]. Although natural geochemical processes also cause this phenomenon, it has been estimated that about 30% of irrigated land has problems with human-induced salinity [4]. This percentage is expected to grow, and salinity could affect 50% of the total farmland by 2050 [5].

Agriculture is among the anthropogenic activities most affected by soil and water salinization [6]. This is due to the impact of salinity on soil health and quality. As for crops, salinity causes physiological, morphological, and biochemical alterations, leading to severe decreases in crop productivity [7,8]. Salt causes stunted growth, impaired nutrition, and reduced water availability, resulting, when in excessive concentrations, in tissue necrosis and plant death [5,9,10]. In addition to decreasing the productivity of crops [11], salinity also threatens biodiversity, determining long-term changes in the ecosystems [12].

Among the mechanisms of salt toxicity, osmotic and ionic impairments, decreased photosynthesis, and enzymatic activities have been documented [13,14]. Furthermore, salt stress interferes with the mobilization of carbohydrates, proteins, and hormones, and alters root development and morphology [15]. Salinity affects photosynthetic and accessory pigments and reduces the leaf area, even though, in some cases, this can be considered an adaptation to minimize water loss for transpiration [16]. High salt concentrations generally determine the overproduction of reactive oxygen species (ROS), mainly in chloroplasts and mitochondria [17]. Among ROS, superoxide anion (O_2^-) and hydrogen peroxide (H_2O_2) are particularly harmful for their reactivity, as they can degrade proteins, DNA, lipids, and many metabolites [17].

Some adaptive mechanisms have been proposed to explain plant tolerance to salinity. In some cases, plants avoid or limit salt uptake at the root level, preventing its accumulation and toxicity in the aerial part [18]. This strategy seems to operate at not excessively high salt concentrations [19]. In addition, plants can increase K^+ in leaves, as it results in osmotic adjustments [20]. Finally, the coordinated action of the antioxidant machinery is considered of primary importance and may allow plants to limit oxidative stress damage. Indeed, salt-tolerant species show a higher content of molecules and enzymes with antioxidant activity, which can also be induced following salt stress [10].

Several strategies have been developed to offer effective and environmentally friendly solutions to increase crop tolerance to salt stress. Among them, biostimulants are finding increasing applications in agriculture. Biostimulants, by definition, are materials capable of raising crop productivity by enhancing plant nutrition, nutrient utilization, and primary and secondary metabolism [7]. They can also increase crop resistance to abiotic stresses [7]. Biostimulants have been grouped into two main groups: microbial and non-microbial [21]. The latter include plant extracts (plants and algae), protein hydrolysates (mainly of plant origin), fulvic and humic substances, and some organic and inorganic compounds [22,23].

It should be pointed out that there is an ever-increasing interest in finding new bioactive and natural substances that can benefit crops, focusing on plant extracts for their relevant bioactive properties. Some plants can have a noticeable content of bioactive compounds that can enhance cultivar performance under normal conditions and biotic or abiotic environmental stresses [8,24].

Recent studies have been conducted on the biostimulatory potential of an aqueous extract obtained from *Lemna minor* L. (duckweed), a small freshwater aquatic species (average frond dimensions of about 5 mm). In particular, this extract promotes benefits in crops grown in normal and stressful conditions [25–28]. To explain such effects, metabolomic analyses of duckweed extracts have revealed a broad spectrum of bioactives [25,27]. Nevertheless, no studies have been conducted on duckweed extracts to verify their possible effect on salinity-grown maize plants, one of the most important cereal crops globally. Therefore, the present work aimed to highlight whether this extract resulted in benefits in maize raised under saline conditions. To this end, the LE effects on various mechanisms related to the crop's resistance to such abiotic stresses were explored, paying particular attention to the antioxidant metabolism and the plant's ability to absorb certain macro- and micro-nutrients.

2. Materials and Methods

2.1. Duckweed Growth Conditions

Duckweed was collected from a natural freshwater pond located near the city of Perugia (Italy). Collected plants were surface-sterilized with 0.5% NaClO and gently rinsed twice with distilled water. Afterward, plants were transferred to polyethylene trays (10 L volume) and allowed to grow, according to Panfili et al. [29], in a growth chamber at 24 ± 1 °C, light intensity of 100 µmol m^{-2} s^{-1}, and a photoperiod of 8/16 h (light/dark). Duckweed was harvested every two weeks before the renewal of the growing medium.

2.2. Preparation of Duckweed Extract (LE)

Harvested duckweed was rinsed thoroughly with water and dried at 40 °C until reaching constant weight. Then, 1 g of dried sample was ground to a powder and extracted in 100 mL of distilled water. The obtained suspension was mixed with an orbital shaker for 12 h and then filtered. The resulting liquid phase was brought to 100 mL and designated as the 1% *w*/*v* duckweed extract (LE) used in the trials described in Section 2.3. This concentration was chosen based on the results of a previously published work [25].

2.3. Maize Growth Conditions and Treatments

Maize (*Zea mays* L., hybrid ISH302V) seeds were surface-sterilized with 0.5% NaClO and then rinsed several times with distilled water. They were then placed in pots (1 L volume) containing a mixture of peat and perlite (3:1 *v*/*v* ratio). Tap water was added up to 75% of the field capacity and maintained constant throughout the trial by irrigating the pots daily and checking the soil humidity using a hygrometer. After three days in the dark, pots were placed in a growth chamber at 24 ± 1 °C, light intensity of 300 µmol m^{-2} s^{-1}, and a photoperiod of 12/12 h (light/dark). Seedlings were treated 6 days after sowing (DAS) by a foliar spray application of LE (2.5 mL per plant). The salinity stress was imposed at 7 DAS at two different levels by adding 150 mM or 300 mM NaCl solutions to the pots. In detail, the treatments were as follows: control, 150 mM NaCl, 150 mM NaCl + LE, 300 mM NaCl, and 300 mM NaCl + LE. Plants were harvested at the third leaf stage (21 DAS) and submitted to the determinations described in the following sections. In addition, the shoot height and length of the third fully expanded leaf of each seedling were recorded. Then, the leaf area was estimated using ImageJ 1.50i software (National Institutes of Health, NIH, USA) [25]. As for root analysis and phenotyping, roots were copiously washed with tap water, gently dried, and scanned on the same day of harvesting. Then, the scanned images were analyzed to determine the total root length, number of tips, ramification number, diameter, surface area, and volume using RhizoVision Explorer v2.0.3.0 software (open-source software), according to Seethepalli et al. [30].

2.4. Pigment and Soluble Protein Content Determination

Next, 0.5 g of fully developed leaf samples were extracted with 5 mL of methanol (MetOH), and this suspension was then centrifuged (20,000 rpm, 5 min) to determine chlorophyll a (Chl a), chlorophyll b (Chl b), and carotenoid contents. According to Venkatachalam et al., the resulting supernatant was analyzed spectrophotometrically [31]. In addition, total chlorophyll content (TotChl) was calculated as the sum of Chl a and Chl b. Total soluble protein determination was performed through spectrophotometry, according to Bradford [32], using 0.1 g of fresh leaf samples extracted with 0.5 mL of 50 mM phosphate buffer.

2.5. H_2O_2, MDA, SOD, CAT, TPC, TFC, and Anthocyanin Determination

Hydrogen peroxide (H_2O_2) content was assessed by extracting 0.25 g of fully developed leaves with 2.5 mL of 0.1% trichloroacetic acid (TCA), following a published procedure [33]. Malondialdehyde (MDA) content was ascertained using the extract, prepared as described for H_2O_2 determination. Then, the supernatant fraction was incubated with a 20% TCA and 0.5% thiobarbituric acid (TBA) solution, according to Heath and Packer [34].

Fresh leaf samples were extracted with a 50 mM phosphate buffer solution containing 1 mM of EDTA and 1% (*w*/*v*) PVPP to evaluate the activity of superoxide dismutase (SOD) and catalase (CAT), as described by Rezazad Bari et al. [35]. A spectrophotometric assay was performed according to Beyer and Fridovich [36] to determine SOD activity, which was expressed in units per mg of protein^{-1}. According to Camilo dos Santos et al. [37], CAT activity was determined and expressed in μmol s^{-1} mg of protein^{-1}.

Total phenolic content (TPC), total flavonoid content (TFC), and anthocyanin content were determined using the methanolic extract described in Section 2.4. The Folin–Ciocalteu method was adopted for TPC determination, and phenols content was expressed as gallic acid equivalent (GAE) g^{-1} [38]. TFC was determined spectrometrically, according to Atanassova et al. [39], and was expressed as mg of catechin equivalents (CE) g^{-1}. Anthocyanin content was estimated following a published procedure [40].

2.6. Maize Mineral Nutrient Determination

Maize samples consisting of stems and leaves were dried at 60 °C until reaching constant weight. A 0.1 g dry sample was digested at 90 °C for 90 min with 5 mL of 65% HNO_3 and 1 mL of 35% H_2O_2. After cooling, the volume was brought to 20 mL with water and the suspension was filtered. Na, K, Ca, Mg, Fe, Mn, and Zn were measured using inductive coupling plasma spectrometry (ICP), according to Hansen et al. [41].

2.7. Statistics

Experiments were carried out according to a completely randomized design with five treatments (control, 150 mM NaCl, 150 mM NaCl + LE, 300 mM NaCl, and 300 mM NaCl + LE) and four replicates for each experimental group. The statistical study was carried out using one-way ANOVA. Using Duncan's test, significant differences were identified at $p < 0.05$.

3. Results

3.1. Impact of Duckweed Extract on Maize Growth

Salinity determined detrimental effects on the development and growth of maize plants. In detail, 300 mM NaCl decreased the shoot height compared to control samples (Figure 1). In contrast, plants treated with LE showed higher values than plants stressed with salt alone. In addition, at 150 mM NaCl, they did not show significant differences compared to the untreated control group. Salt induced a dose-dependent reduction in leaf area, and the LE treatment did not influence this parameter. Shoot fresh weight was decreased by both NaCl levels, but LE entailed a recovery if compared to untreated samples. LE increased dry weight at the lower salt concentration compared to values observed for plants stressed with salt alone. At the higher salt concentration, the dry weight did not statistically differ from the values shown by control samples.

Root analysis revealed that salt stress caused diffused decreases in several morphological parameters, with some recovery prompted by the LE treatment (Table 1). Regarding the total root length and number of tips, the higher salt level decreased these parameters, regardless of LE treatment. However, applying LE at the lower NaCl level restored the values shown by the control. Salinity decreased the ramification number in all treatments, except for plants grown at a higher salt dosage and treated with the extract, which did not differ from the control. Root diameter and fresh weight were reduced by 300 mM NaCl but were unaffected by 150 mM NaCl. Regarding root volume, it has to be highlighted that 150 mM NaCl alone decreased this parameter, whereas the LE treatment maintained the plants in line with the control group. Finally, the root area was not influenced by either salt stress level.

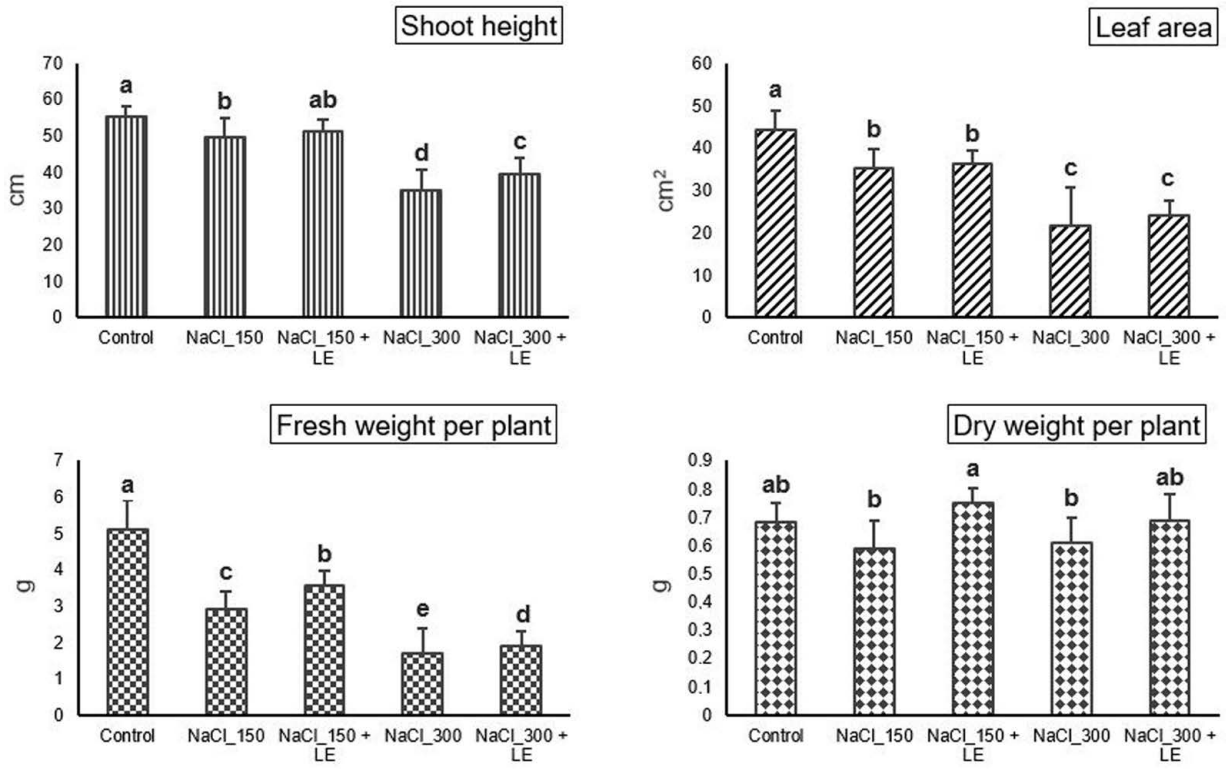

Figure 1. Shoot analysis of maize samples grown in salinity and treated or not with LE. Different letters are statistically significant according to Duncan's multiple comparison test ($p < 0.05$).

Table 1. Root analysis of maize samples grown in salinity and treated or not with LE.

Treatment	Total Length (cm)	Number of Tips	Ramification Number	Diameter (mm)	Root Area (cm^2)	Volume (cm^3)	Fresh Weight (g)
Control	131 ± 11 a	206 ± 16 a	373 ± 60 a	1.44 ± 0.16 a	53.6 ± 7.9 ab	3.17 ± 0.85 a	1.35 ± 0.14 a
NaCl_150	107 ± 16 c	158 ± 44 b	274 ± 72 b	1.42 ± 0.07 a	49.6 ± 9.4 ab	2.53 ± 0.65 bc	1.15 ± 0.16 a
NaCl_150 +LE	140 ± 26 a	196 ± 41 a	263 ± 80 b	1.43 ± 0.09 a	55.8 ± 10.9 a	3.01 ± 0.60 ab	1.17 ± 0.09 a
NaCl_300	120 ± 24 bc	164 ± 37 b	274 ± 108 b	1.17 ± 0.11 b	47.2 ± 10.0 b	2.34 ± 0.62 c	0.85 ± 0.19 b
NaCl_300 +LE	121 ± 19 bc	164 ± 23 b	310 ± 70 ab	1.22 ± 0.08 b	45.4 ± 6.5 b	2.10 ± 0.45 c	0.86 ± 0.12 b

Different letters are statistically significant according to Duncan's multiple comparison test ($p < 0.05$).

3.2. Pigment Content and Soluble Protein Content

At the end of the experiment, pigment contents were recorded, revealing a decrease in Chl a content in plants treated with salt alone at both concentrations, whereas LE allowed plants to show values not significantly different from those shown by the control samples (Table 2). Regarding Chl b, its content was unaffected by 150 mM NaCl but was decreased by 300 mM NaCl. On the contrary, plants treated with LE did not differ from control samples. In addition, both salt concentrations reduced TotChl, but LE reverted this effect, raising the content to values that were not different from those of the control samples. As for carotenoids, the content of this pigment was unaffected by all the treatments. Furthermore, the soluble protein content was unaffected by 150 mM NaCl and decreased by 300 mM NaCl. However, LE significantly increased the protein content for samples treated with this last NaCl concentration, compared to those treated with salt alone.

Table 2. Pigments and soluble protein content in maize samples grown in salinity, treated or not with LE.

Treatment	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	TotChl (mg g^{-1} FW)	Car (mg g^{-1} FW)	Soluble Protein (mg g^{-1} FW)
Control	2.83 ± 0.10 a	1.94 ± 0.19 a	4.78 ± 0.26 a	1.53 ± 0.41 a	4.65 ± 1.11 a
NaCl_150	2.55 ± 0.10 b	1.62 ± 0.18 ab	4.17 ± 0.08 b	1.49 ± 0.05 a	4.10 ± 0.35 ab
NaCl_150 +LE	2.77 ± 0.05 a	1.88 ± 0.25 ab	4.65 ± 0.25 a	1.59 ± 0.03 a	3.80 ± 0.40 ab
NaCl_300	2.58 ± 0.05 b	1.52 ± 0.22 b	4.11 ± 0.18 b	1.47 ± 0.17 a	3.14 ± 0.21 c
NaCl_300 +LE	2.78 ± 0.08 ab	1.93 ± 0.20 a	4.65 ± 0.21 a	1.46 ± 0.09 a	3.70 ± 0.06 b

Different letters are statistically significant according to Duncan's multiple comparison test ($p < 0.05$).

3.3. H_2O_2, MDA, SOD, CAT, TPC, TFC, and Anthocyanin

Determinations of the oxidative status (H_2O_2 and MDA), enzymatic (SOD and CAT), and non-enzymatic antioxidants (TPC, TFC, and anthocyanin) of maize plants subjected to all the different treatments were carried out. Samples treated with NaCl at 150 and 300 mM showed higher levels of H_2O_2. Differently, samples subjected to salt stress and treated with LE showed values similar to those of the control samples (Figure 2). In the case of MDA, the plants stressed with salt alone had higher values than those treated with LE.

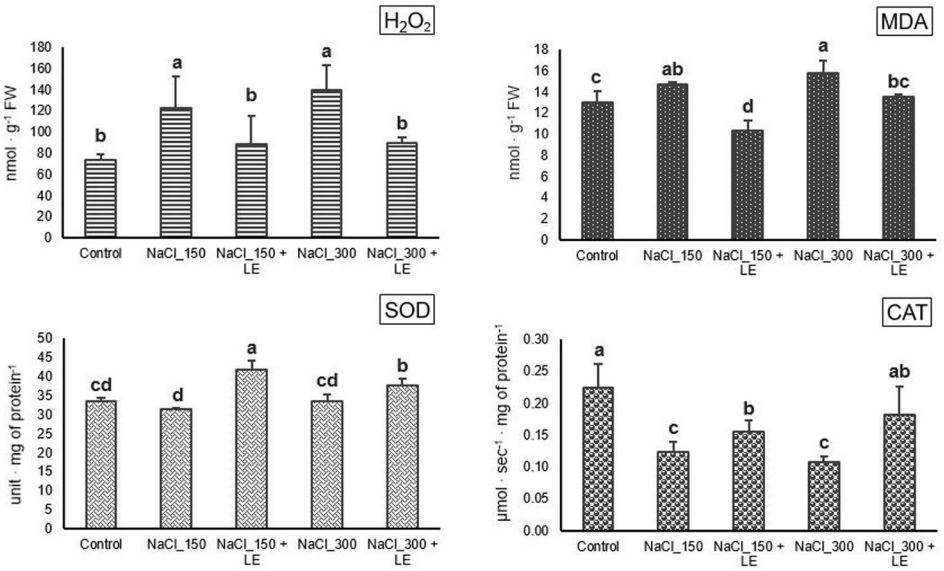

Figure 2. H_2O_2, MDA, and activity of superoxide dismutase (SOD) and catalase (CAT) determined in maize samples grown in salinity and treated or not with LE. Different letters are statistically significant according to Duncan's multiple comparison test ($p < 0.05$).

In fact, LE-treated samples showed MDA values similar to or lower than those observed for the control samples. As for the enzyme activities, salt stress alone did not increase SOD activity compared to control samples. On the contrary, when stressed plants were treated with LE, SOD activity reached higher values than those found in the control.

Compared to untreated samples, the CAT activity was significantly reduced in plants treated with 150 and 300 mM NaCl. However, when plants stressed with salt were treated with LE, CAT activity recovered and showed higher values than in samples treated with salt alone. Furthermore, TPC was higher in plants stressed with 300 mM salt alone and in plants treated with LE, while the 150 mM NaCl concentration showed similar data compared to the control (Figure 3). Regarding TFC, a general decrease in this parameter was observed in samples raised in salinity, regardless of whether LE was applied. Finally, the anthocyanin content was generally higher in plants treated with LE, regardless of the salt concentration applied. LE-treated samples showed values that were not statistically different or higher than those found for the control samples.

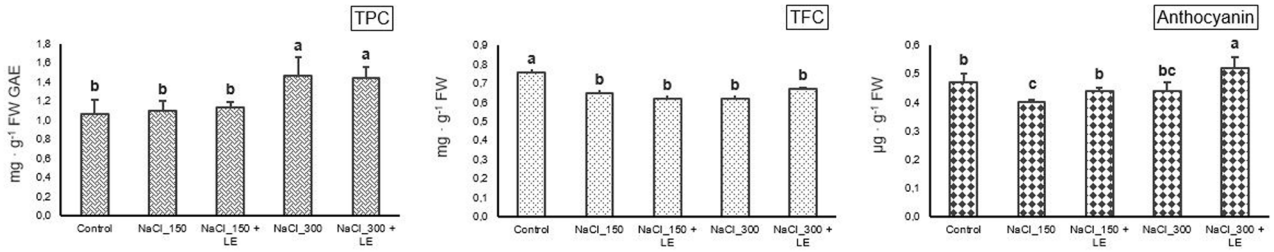

Figure 3. Total phenols (TPC), flavonoids (TFC), and anthocyanin content detected in maize samples grown in salinity and treated or not with LE. Different letters are statistically significant according to Duncan's multiple comparison test ($p < 0.05$).

3.4. Maize Mineral Nutrient Content

Na content was increased by NaCl, in a dose-dependent manner, demonstrating higher values than those of control samples, regardless of LE application (Table 3). K was unaffected by any treatment, while salinity reduced Mg for both NaCl concentrations. When samples were treated with LE, the plants showed increased Mg values, significantly higher than those exhibited by the salt-stressed samples. As for Ca content, in general, none of the treatments affected the content of this nutrient in maize shoots. Relatively to the content of Fe and Mn, no significant differences were recorded at the lowest salt dosage, regardless of the LE application or not, compared to the untreated controls. At 300 mM, LE application increased the content of the two elements, which reached the same values exhibited by the control samples for Fe and higher for Mn. Finally, concerning Zn, a general increase was observed in the saline-reared samples compared to the control samples. Nonetheless, it was observed that at the higher salt concentration, LE promoted a higher nutrient uptake than that found in samples grown in salt alone.

Table 3. Macro- and micro-nutrient content ascertained in maize samples grown in salinity and treated or not with LE.

Treatment	Na	K	Mg	Ca	Fe	Mn	Zn
	mg g^{-1} DW					µg g^{-1} DW	
Control	0.69 ± 0.05 c	9.1 ± 1.19 ab	6.53 ± 0.60 a	13.87 ± 0.13 ab	116 ± 9 ab	157 ± 24 bc	64 ± 7 c
NaCl_150	11.87 ± 2.24 b	8.14 ± 0.57 b	4.70 ± 0.35 c	12.08 ± 0.39 b	120 ± 23 ab	184 ± 27 ab	125 ± 19 ab
NaCl_150 +LE	13.33 ± 2.95 b	9.10 ± 0.82 ab	5.82 ± 0.14 b	12.52 ± 1.33 b	112 ± 12 b	165 ± 39 abc	153 ± 29 a
NaCl_300	32.05 ± 5.54 a	8.99 ± 1.77 ab	5.51 ± 0.46 bc	12.35 ± 1.06 b	89 ± 7 c	123 ± 16 c	117 ± 8 b
NaCl_300 +LE	33.70 ± 5.60 a	10.75 ± 1.40 a	6.30 ± 0.63 a	14.75 ± 1.74 a	142 ± 17 a	218 ± 44 a	154 ± 22 a

Different letters are statistically significant according to Duncan's multiple comparison test ($p < 0.05$).

4. Discussion

This study examined the impact of an aqueous extract obtained from *Lemna minor* L., a free-floating aquatic species with a significant bioactive substance content [25–27], in maize grown at two high-salinity concentrations. Therefore, the objective was to ascertain the biostimulant potential of a biological resource that is easily accessible to enhance maize tolerance to salt and understand the mechanisms by which any eventual protective action was exerted.

Our experiments showed that salt affected the aerial part of the plant, causing reductions in shoot length and fresh and dry weight (Figure 1) due to the toxic and osmotic effects caused by salinity [42] and its documented impact on the photosynthetic machinery [43–45]. The duckweed extract reversed the salt effect on the above parameters, and the results obtained aligned with what has been reported for substances with biostimulant action, which promote plant growth and adaptability to this stress [9,46]. Regarding the effect of salt on the leaf area, the reductions observed, regardless of the application or not of LE, can be the consequence of the stress [10], even though, for non-excessive salt concentrations, this can be considered a response that reduces water loss by transpiration [9].

The effect of salinity on roots is of primary importance because they are the first part of the plant to sense this stress, and morphological and anatomical changes can reveal adaptive mechanisms [9]. Our results showed that salt stress generally affected all the parameters investigated for roots (Table 1). Despite this, LE exerted beneficial effects on roots, and this action is worth mentioning since the improvements in root length and the number of tips allow plants to increase their capacity to explore the soil for water and nutrient acquisition. However, the highest salt dosage generally reduced the diameter and fresh weight, and the LE was ineffective in contrasting this effect. It is known that salt can target these two traits, altering root development [47].

The chlorophyll content is susceptible to environmental stresses, and species that increase or maintain unaffected the content of pigments are more salt tolerant [48]. This places the chlorophyll content among the most interesting biochemical markers for understanding plant behavior in salinity. It should be noted that the decreases in chlorophyll may be associated with oxidative damage or interference in their biosynthesis. Impairment in chlorophyll can result in a decline in photosynthetic activity [49]. Our experiment evidenced that salt stress significantly reduced chlorophyll a and total chlorophyll, while LE contrasted this (Table 2). The effect of the extract on this parameter is worth mentioning and aligns with plant extracts showing biostimulant activity in inducing chlorophyll biosynthesis [25,26]. The protective role of duckweed on chlorophyll can be attributed to improved mineral nutrition and the high content in the extract of antioxidant- and biostimulant-acting substances [27,50].

Furthermore, LE increased the soluble protein content in maize grown at the highest salt concentration (Table 2). The syntheses of substances acting as osmolytes are considered among the responses promoted by plants to deal with salinity, as this can help to contrast the osmotic and oxidative damages [49]. Soluble proteins can exert this action, and the increase observed in samples treated with LE indicates that the extract positively affected protein biosynthesis. In general, a higher soluble protein content has been reported in rice and barley plants grown in salinity, and this benefit allows for readjustments of metabolism functional to overcoming stress [49].

One of the worst effects of Na accumulation is the onset of oxidative stress [17]. This harmful condition is due to the overproduction of reactive oxygen species (ROS). For their reactivity, ROS can degrade lipids, proteins, DNA, and other molecules that perform vital functions. Among ROS, H_2O_2 is of primary importance, as at physiological concentrations, it can function as a signal molecule that regulates many processes [10]. In contrast, the accumulation of H_2O_2 can be toxic and impair cellular functions [8]. Furthermore, MDA is an aldehyde that accumulates as a lipid peroxidation product during oxidative perturbations [17]. Nonetheless, a certain amount of MDA is produced in chloroplasts and mitochondria, as these organelles are characterized by high electron flux processes [51]. For the above reasons, we determined the H_2O_2 and MDA content to understand how the cellular redox status was affected by salt treatments and to point out any eventual benefits prompted by LE. Our experiments evidenced that LE significantly reduced H_2O_2 and MDA, suggesting that this extract stimulated the antioxidative metabolism (Figure 2). The H_2O_2 and MDA reductions aligned with the results observed in other studies that employed plant extracts to prompt benefits in crops [52].

Plant extracts with biostimulant action can mitigate oxidative stress by regulating antioxidant enzymes, maintaining redox cellular balance, and reducing lipid peroxidation [53]. Therefore, to shed light on these aspects and the mechanisms that allowed the reductions in MDA and H_2O_2, the activity of superoxide dismutase (SOD) and catalase (CAT) was assessed in all the samples, as these enzymes are among the most important that plants use to remove ROS [54]. SOD protects cells by disproportioning O_2^- to O_2 and H_2O_2, and CAT removes H_2O_2 in glyoxysomes and peroxisomes [10]. These actions reduce H_2O_2 and lipid peroxidation products [7]. Our experiment highlighted that LE increased SOD and maintained higher CAT activity than samples treated with salt alone (Figure 2). This helped plants to mitigate the oxidative stress caused by salinity. In addition, the decline of CAT

experienced by the samples treated with NaCl alone was particularly indicative, and this suggested that the stress hampered the capacity of this plant to remove H_2O_2. Antioxidant enzymes can significantly decrease their activity in response to excessively severe abiotic stresses [10]. Finally, SOD and CAT activity results agreed with the reductions in H_2O_2 and MDA observed for LE-treated samples.

After the two antioxidant enzymes, total flavonoids, phenols, and anthocyanin contents were determined for their involvement as antioxidants in scavenging ROS [55]. Despite this, we found no remarkable effect on TPC and TFC in response to LE (Figure 3). However, decreases in TFC and increases in TPC at the highest salt concentration, regardless of LE application, were ascertained. This last finding indicates that plants reacted to the most severe salt stress concentration by increasing the phenols content, highlighting the involvement of this class of metabolites in dealing with salt stress and ROS [56]. Anthocyanins are essential biomolecules, as they show multiple functions acting in removing ROS and protecting plants from stress-raised photoinhibition [57]. In addition, decreases in H_2O_2 and MDA have been correlated with anthocyanin levels [58]. Our results evidenced that LE increased the anthocyanin content, thus contributing to maintaining a proper cellular redox status (Figure 3). These results align with other studies, showing that biostimulants can maintain or increase the production of anthocyanin [59,60].

Salinity can interfere with plant mineral nutrition, impairing crop development and biomass production. Therefore, the ultimate objective of the study was to ascertain the content of some mineral nutrients in the plants subjected to the different treatments. In addition, a material to be considered a biostimulant and plant growth promoter should demonstrate the ability to stimulate crop nutrition [7]. Additionally, these determinations were carried out since some tolerant species can uptake more potassium (K) for its involvement in salinity tolerance. This element maintains cellular functions, osmotic balance, and water uptake, and it reduces sodium (Na) acquisition, thus decreasing the Na/K ratio. Crops often activate such a strategy for dealing with salt stress [61]. Our experiment showed that plants grown in salinity conditions had higher concentrations of Na (Table 3). In addition, LE did not modify the Na/K ratio, which increased in plants exposed to salt stress (data not reported). These results allowed us to exclude the involvement of increased levels of K among the mechanisms activated by the plant studied to tolerate salt stress better and to exclude that the LE affected the Na/K ratio.

Differently, magnesium (Mg) increased following LE treatments, and this aligns with what has been found for chlorophyll a and total chlorophyll. Mg is an essential element for plant nutrition and development, and it is necessary for plant growth and productivity, being part of chlorophyll and playing a central role in photosynthesis [62]. The other elements investigated, iron (Fe), manganese (Mn), and zinc (Zn), are essential micronutrients involved in numerous biological processes. For instance, Fe takes part in photosynthesis, respiration, and chlorophyll biosynthesis, and is a component of the heme and Fe-sulfur clusters [63]. Mn is part of the photosystem II (PSII) and is involved in regulating antioxidant functions, and it is a co-factor for about 6% of known metalloenzymes [64]. Zn regulates some enzymes involved in proteins, chlorophyll, carbohydrates, and nucleic acid synthesis [65]. In the case of the treatment conducted with the highest salt concentration, LE increased the plant content of these three elements. This effect explained the benefits of biomass production for the cited involvement of these nutrients in relevant biological processes. Moreover, the LE impact on Zn content for the highest salt concentration explained the increase in soluble protein content for the involvement of this element in its biosynthesis. Finally, it has been documented that an increase in Zn acquisition is essential under salinity conditions, as this element exerts a protective action by preventing cellular damage due to oxidative stress [66]. In particular, Kavian et al. [67] reported that increases in Zn stimulated the activity of antioxidant enzymes, including SOD and CAT, thus improving plant resistance to salt stress.

5. Conclusions

This study demonstrated that an extract derived from an aquatic species, duckweed, might be offered as an efficient biostimulant for maize plants cultivated in salinity conditions. Specifically, the extract improved aerial biomass production and stimulated root development, photosynthetic pigment, and soluble protein. Furthermore, the duckweed extract improved the cellular redox state in salt-treated samples through the induction or maintenance of the main enzymes involved in the antioxidant responses. In general, the latter effect is relevant in promoting the resistance of cultures to different abiotic and biotic stresses. Therefore, the enzyme and non-enzymatic antioxidant responses and the estimation of the content of other ROS appear to be of pivotal importance in understanding the effect of biostimulants. We also noted that the extract affected anthocyanin content and plant mineral nutrition. This last aspect is worth mentioning because it aligns with one of the most documented benefits promoted by biostimulants.

This study corroborates the need to find the response to increasingly pressing abiotic environmental stresses related to climate change in bioactive-rich plants to obtain new materials with biostimulant action. This strategy is significant because the biostimulants thus far obtained are low in cost, beneficial and protective on crops, and are entirely eco-friendly. Finally, as the last aspect, the importance of investigating biochemical, physiological, and nutrition responses in the case of other maize varieties subjected to LE treatments should be emphasized. Indeed, this makes it possible to validate the extent of the benefits demonstrated in this study by Lemna extracts.

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