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Research Article

Changes in growth, antioxidant, anti-Alzheimer, and antidiabetic potential of lamb's lettuce *Valerianella locusta* grown hydroponically and on soil in response to salinity

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Abstract

The purpose of this study was to provide new insights into the effects of salinity on growth and nutritional value of lamb's lettuce *Valerianella locusta* grown in two different culture systems, hydroponic and soil, and subjected to 0 and 50 mM NaCl for 3 weeks. Salinity treatment reduced shoot dry weight (DW) by 50% in both growing media, root DW by 41% only on hydroponics, relative growth rate (RGR) also decreased, and K^+/Na^+ ions ratio in *Valerianella locusta* grown both hydroponically and in soil. Salinity also lowered shoot total phenolic content (TPC), total flavonoids content (TFC), radical scavenging activity (ABTS), anti-amylase, and anti-acetylcholinesterase (AChE) activities. In contrast, it promoted shoot total antioxidant activity (TAA) in both growing systems. When comparing both growing systems, soil-grown *Valerianella locusta* was found to have significantly higher TPC (41.6 and 28.1 mg GAE g^{-1} DW) and TFC (39.6 and 35.6 mg CE g^{-1} DW) for control and salt treated shoots, respectively. Further, it showed a better TAA and ABTS scavenging ability, as well as superior anti-α-amylase (94.3 and 39.5 mg ACE. g^{-1} DW) and anti-AChE (307.4 and 228.3 µg DE. g^{-1} DW) activities, under control and salt stress conditions respectively. Additionally, soil-grown *Valerianella locusta* showed better K^+/Na^+ ions homoeostasis compared to the hydroponically-grown. This study highlighted two main points: first, it revealed that lamb's lettuce is a sensitive crop to be grown on saline lands, and second it underlined the distinct differences in growth aspects and nutritional quality between hydroponics and soil cultivation. Additionally, this study is the first to shed some light on the interesting medicinal quality of lamb's lettuce as a leafy vegetable.

Keywords: antioxidant activity; hydroponics; phenolics; salinity; soil; *Valerianella locusta*

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Introduction

Over the past two decades, rapid population growth, together with environmental degradation, significantly increased the requirement for sustainable agricultural for crop production, usually affected by abiotic and biotic constraints (Le *et al.,* 2020). Soil salinity is an environmental stress that has many damaging effects on the agronomic traits and nutritional quality of several crops (Galieni *et al.,* 2015). Worldwide, around one billion hectares of land are salt-damaged, which corresponds to more than 6% of the world's total land area (Petropoulos *et al.,* 2017). In the Mediterranean region, soil salinization may worsen at increasing rates in the upcoming decades given the expected rise in irrigated lands and the increasing scarcity of good quality water (Machado and Serralheiro 2017).

In fact, salinity induces osmotic stress, which leads to reduced water and mineral uptake, reduced photosynthesis yield, as well as the overproduction of reactive oxygen species (ROS). As a result, plants suffer yield and nutritional value loss (Paranychianakis and Chartzoulakis 2005; Parida and Das 2005). Therefore, over the years, there has been a considerable effort to understand and improve the salinity tolerance in major food crops, including leafy vegetables.

Leafy vegetables are important source of vitamins, minerals and phenolic compounds, known as powerful antioxidants highly needed for human health (Natesh *et al.,* 2017). Clinical evidence has shown that eating leafy vegetables is positively associated with a high therapeutic capacity in aging and oxidative-lesions related pathologies such as neurodegenerative diseases, like Alzheimer disease, cardiovascular diseases, diabetes, and cancer (Ratnam *et al.,* 2006; Shin *et al.,* 2020).

Many studies have explored the impact of salt stress on growth and nutritional quality of numerous leafy vegetable crops. For instance, salinity stress at different NaCl concentrations decreased growth parameters (fresh and dry weight of shoots and roots, shoot length, and leaf number), as well as phytochemical compound concentrations (ascorbic acid, total phenols, and flavonoids) in lettuce (Shin *et al.,* 2020). Another study found a similar degrading effect on total phenolic concentrations in spinach (Ferreira *et al.,* 2018).

Furthermore, in spite of the large number of works, studies usually tend to be only carried out either in hydroponics or in soil, setting aside the possible physiological and nutritional quality differences in plants grown on floating systems and on the soil. For example, in non-saline conditions, Lei and Engeseth (2021) reported that giant Caesar lettuce revealed different morphology, texture, antioxidant capacity, and functional quality aspects when grown hydroponically and on soil. Tavakkoli *et al.* (2012) further reported that under salt stress conditions, growth parameters reductions were greater under hydroponics than that in soil, and the uptake of Na^+ and Cl^- was also more pronounced.

Lamb's lettuce (*V. locusta* L., Caprifoliaceae family), also known as corn salad, is an annual winter leafy vegetable, with a Mediterranean origin where it can be found in some regions of Europe, North Africa, and America (Berger *et al.,* 2021; Martyniak-Przybyszewska 2005). The production of this lettuce is important in Italy, France, Netherlands, Germany, and Belgium, with France being the leading producer (75% of world production) with a yield that can reach up to 43,000 tons (Kolton and Baran 2008; Verdin *et al.,* 2018). In Germany, lamb's lettuce ranks third in terms of consumption after tomatoes and cucumbers (Muminovic *et al.,* 2004). Lamb's lettuce is known for its richness in vitamin C, fatty acids (α-linolenic acid), and phenolic compounds (Ferrante *et al.,* 2009; Ramos-Bueno *et al.,* 2016). It was demonstrated to contain one phenolic acid (chlorogenic acid), and four flavonoids (rutin, luteolin, kaempferol-3-o-rutinoside, and genistein) (Ramos-Bueno *et al.,* 2016).

The aim of this study is to investigate the effect of salt stress on physiological and biochemical parameters of lamb's lettuce *V. locusta* grown in hydroponics and on soil to give new insights about the effect of salinity on its biomass production, relative growth rate (RGR), ion homeostasis (Na^+ and K^+ uptake and accumulation), and to evaluate the variations of its phenolic compounds contents (TPC and TFC), its antioxidant capacity

(Total antioxidant activity and radical scavenging ability) as well as its anti-α-amylase, and anti-acetylcholinesterase (AcHE) inhibitory activities under control and salt stress conditions.

Materials and Methods

Plant material and growth conditions

In this study, *V. locusta* was grown on two culture systems; hydroponics and soil. Seeds were first sterilized in sodium hypochlorite 5% (w/v) for 10 min, then sown in Petri dishes at 25 °C for the hydroponic culture, and in pots filled with a mixture of peat (2/3) and sand (1/3) washed with HCl 1% and then three times with distilled water, with one seed per pot, for the soil culture.

For hydroponics, seedlings (after 9 days of germination) were transferred on plastic pots, one seedling per pot, filled with a quarter-strength Hoagland nutrient solution containing 0.625 mM Ca (No_3), 0.625 mM KNO_3, 0.25 mM Mg SO_4, 0.125 mM KH_2 PO_4, for macronutrients, and 5.10^{-5} mM Mn SO_4, $2.5.10^{-4}$ Cu SO_4, $.5.10^{-4}$ Zn SO_4, $2.5.10^{-5}$ $(NH4)_6MO_7O_{24}$, 5.10^{-3} H_3BO_3, for micronutrients, and 0.1 mM Fe-EDTA, and kept in a growth chamber under controlled conditions (16-8 h day-night photoperiod, 25-27 °C temperature, and 50-70% relative humidity). The soil culture was carried out under natural conditions with an average photoperiod of 14-10 h day-night, with an average temperature and relative humidity of 21 °C and 69% during the day, and 11 °C and 40% at night.

Following 21 days of growth, an initial harvest was made for both growing systems (12 plants) and NaCl treatment was initiated to provide final concentrations of 0 mM (control) and 50 mM. The treated seedlings were grown for an additional 21 days. Every four days, the hydroponic system's nutrient solutions were aerated and renewed, whereas the soil system was watered every two days with the provided concentrations. After 41 days, harvest occurred for both cultures.

Determination of growth parameters

The Dry Weights (DW) of shoot and root samples were determined after desiccation at 60 °C for 72 h.

Relative Growth Rate (RGR) was calculated using Hunt (1978) method:

RGR (day -1) = (Ln (DW2)-Ln (DW1))/Δt, where DW1 and DW2 are the DW of the initial and final harvest samples respectively and Δt is the salt treatment duration between the final and the initial harvest.

Sodium and potassium concentrations

Na^+ and K^+ were extracted from shoots and roots grounded dry matter using sulphuric acid (H_2SO_4 1N) (Zorrig *et al.,* 2019), and then assayed by flame emission spectrophotometry (BWB-XP). K^+/Na^+ ratio was also determined for both organs.

Extraction of total phenolics content

Shoot extracts were obtained by macerating 1 g of dry leaf ground dry matter in 10 ml of 80% ethanol under magnetic stirring for 6 hours. The extracts were then kept for 24 h at 4 °C, then filtered through a Whatman no. 4 filter paper, and evaporated to dryness using a rotary evaporator. The water fraction of each extract was evaporated with nitrogen gas (N_2). The dry tailings were used to prepare solutions of concentrations ranging from 1 to 5 mg ml^{-1}.

Quantification of total phenolics content

Total phenolic content (TPC) was assayed using Folin-Ciocalteu reagent, following the method of Dewanto *et al.* (2002). An aliquot of diluted sample fraction was added to 20 µl of distilled water and 5 µl of Folin-Ciocalteu reagent. The mixture was shaken and incubated for 3 min before adding 41 µl of Na_2CO_3 (7%).

After incubation in the dark for 90 min, the absorbance was read at 760 nm versus a prepared blank. TPC were expressed as mg gallic acid equivalents per gram dry weight (mg GAE g^{-1} DW) through the calibration curve of Gallic acid. All samples were analysed in triplicate.

Quantification of total flavonoids content

Total flavonoids content (TFC) was measured using a colorimetric assay developed by Zhuang *et al.* (1992). An aliquot of diluted sample was added to 5 µl of Na NO_2 solution (5%) and mixed for 6 min before adding 10 µl of $AlCl_3$ (10%). After 5 min, 33 µl of NaOH solution (1 M) and 100 µl of distilled water were added, thoroughly mixed, and the absorbance of the mixture was finally determined at 510 nm. TFC were expressed as mg catechin equivalents per gram dry weight (mg CE g^{-1} DW), through the calibration curve of catechin. All samples were analysed in triplicate.

Total antioxidant activity

TAA of the ethanolic extracts was evaluated through colorimetric assay described by Prieto *et al.* (1999) based on a green phosphate/Mo^{5+} complex. An aliquot (100 µl) of diluted extract was combined with 1 ml reagent solution (sulphuric acid 0.6 M, 28 mM sodium phosphate, and 4 mM ammonium molybdate). Mixtures were incubated at 95 °C bath for 90 min then cooled to room temperature. Their absorbance was measured at 695 nm. Ethanol was used instead of sample for blank. TAA was expressed as mg gallic acid equivalent per gram dry weight (mg GAE g^{-1} DW). All samples were analysed in triplicate.

Radical scavenging activity

The ABTS cation radical scavenging activity was determined according to Re et *al.* (1999). ABTS was produced by the reaction between 5 ml ABTS solution (7 mM) and 5 ml potassium persulphate solution (2,5 mM) stored in the dark at room temperature for 12 to 16 h. Before usage, this solution was diluted with ethanol 80 % to get an absorbance of 0.7 at 734 nm. In a final volume of 200 µl, the reaction mixture contained 150 µl ABTS solution and 50 µL of *V. locusta* extract at various concentrations ranging from 40 to 200 µl. The mixture was homogenized and after 20 min its absorbance was recorded at 734 nm. Similarly, the reaction mixture of standard group was made using 150 µL ABTS solution and 50 µL BHT. Results were expressed as inhibitory concentration IC_{50} (µg ml^{-1}) of the radical ABTS scavenging ability. The IC_{50} values expressed represent the inhibitory concentrations necessary to neutralize 50% of free radicals. The lower the IC_{50} value, the greater the anti-free radical activity of the extract.

Anti-α-amylase activity

The anti-α-amylase activity was measured using an adapted method described by Kim *et al.* (2011), with slight modifications. Briefly, 40 µl of diluted *V. locusta* shoot extracts were incubated for 10 min at 37 °C with 40 µl of α-amylase enzyme solution (1U) and 400 µl of sodium phosphate buffer (0.02 M, pH 6.9) and mixed thoroughly. After incubation, 50 µl of starch solution and another 400 µl of sodium phosphate buffer was added, and then further incubated for 15 min at 37 °C. Finally, 50 µl of 3,5-dinitrosalicyclic acid colour reagent was added to the mixture. Tests were carried out in triplicate and absorbance was measured at 603 nm. The absorbance of the blank (extraction solvent instead of tested samples and α-amylase solution) and control (extraction solvent instead of tested samples) samples was also determined. A standard curve was prepared similarly replacing the tested samples with acarbose. The α-amylase inhibitory activity (I%) was calculated as follows: (I%) = [(AC-AT)/AC] *100; where AC and AT are the absorbance values of the control and the tested sample, respectively, and results were expressed as mg Acarbose Equivalent per gram of Dry Weight (mg ACE g^{-1} DW).

Anti-acetylcholinesterase activity

The inhibition of acetylcholinesterase (AChE) by shoot extracts of *V. locusta* was determined by slightly modifying the method of Eldeen *et al.* (2005). The assay consisted of adding 20 µl of tested extracts to 25 µl of the enzyme solution (AChE; 0.28 U ml^{-1}) and incubating the mixture for 15 min at 37 °C. Then, 100 µl of AChI (acetylthiocholine iodide; 0.15 mM), 500 µl of DTNB (5,5′ -dithiobis-2-nitrobenzoic acid; 0.3 mM), and 355 µl of Tris-HCl buffer (50 mM, pH 8; containing 0.1% bovine serum albumin) were added to the mixture and incubated for another 30 min at 37 °C. The absorbance was measured at 405 nm. A standard curve was prepared similarly replacing the tested samples with donepezil. The AChE inhibitory activity (I%) was calculated as follows: (I%) = [(AC-AT)/AC] *100; where AC is the absorbance of the solution without sample and AT is the absorbance of the tested sample. Results were expressed as µg Donepezil Equivalent per gram Dry Weight (µg DE g^{-1} DW).

Statistical analysis

All reported results were the means of three to six biological replicates. Data were subjected to a one-way analysis of variance (ANOVA, CoStat software) and means were evaluated according to Duncan's test at a significance level $P \leq 0.05$. A Principal Component Analysis (PCA) was carried out using XLSTAT software v. 2014 (Addinsoft, Paris, France), considering variables centred on their means and normalized with a standard deviation of 1.

Results

Salinity impact on plant growth

After 3 weeks of exposure to 50 mM of NaCl, shoot DW of *V. locusta* decreased by 50% when plants were grown in hydroponic or soil culture systems (Figure 1a). Roots DW decreased by 41% when plants were grown in hydroponic medium, while it remained significantly maintained on soil (Figure 1b).

Salt stress (50 mM NaCl) dropped the shoot/root ratio by 20% and 60% for hydroponic and soil cultures, respectively (Figure 1c). In addition, salt stress decreased shoot RGR of *V. locusta* by 13% and 16% on both hydroponic and soil growing systems, respectively (Figure 1d). Root RGR was reduced by 14% in hydroponic medium, but it increased by 6% in soil (Figure 1e).

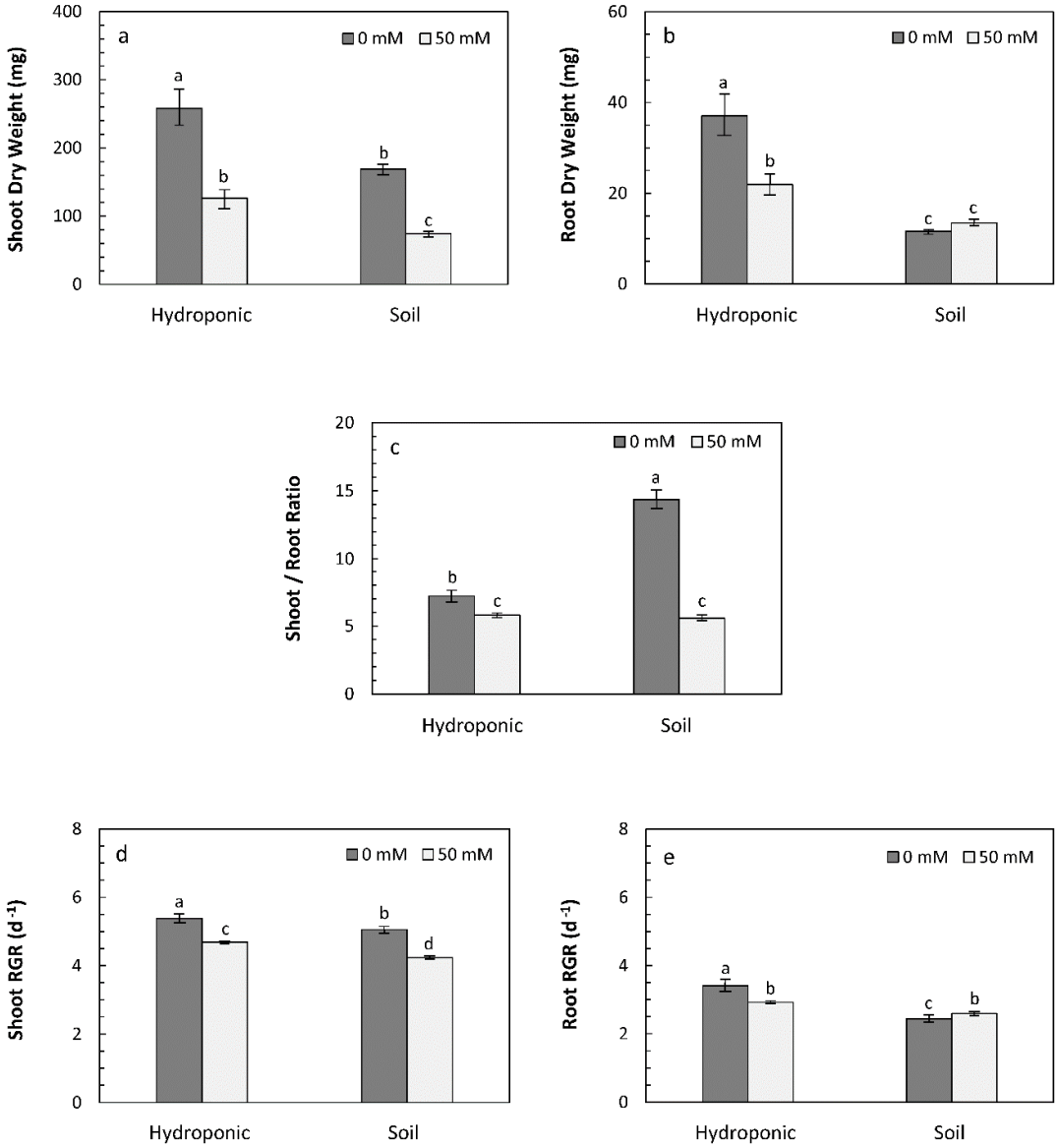

Figure 1. Dry weight (DW) (a and b), relative growth rate (RGR) (d and e) in shoots and roots and shoot/root ratio (c) of hydroponically and soil-grown *V. locusta* after 41 days of growth with or without 50 mM NaCl

Bars are means of six replicates ± Standard Error. Bars marked with different letters, in each panel, are significantly different according to Duncan's multiple-range test at $p \leq 0.5$

Effect of salinity on the mineral profile

Accumulation of Na^+ increased in shoots and roots of *V. locusta* treated with NaCl (50 mM) for 3 weeks (Figure 2a-b). The accumulation of Na^+ in roots was more pronounced for both growing systems compared to the control (Figure 2b). However, Na^+ contents were 3 to 4 times much higher in shoots and roots of lamb's lettuce grown on hydroponics, compared to the soil system (Figure 2a-b).

In addition, NaCl treatment alerted K^+ uptake for both organs, growing in hydroponic or soil systems. Shoot level of K^+ was reduced by 45% in *V. locusta* grown hydroponically, whereas it remained at steady levels

in plants grown in soil (Figure 2c). In roots, NaCl treatment reduced K^+ content by 26% in plants growing both in hydroponic and soil systems (Figure 2d).

The K^+/Na^+ ratio is a selectivity indicator used to determine the extent to which these ions are transported to shoot as well as their accumulation in roots. In this study, the results showed that adding NaCl increased the K^+/Na^+ ratio in shoots and roots of *V. locusta* grown on both hydroponic and soil systems, when compared to control (Figure 2e-f). However, this increase was 2 to 3 times greater in shoot and root of *V. locusta* grown in soil.

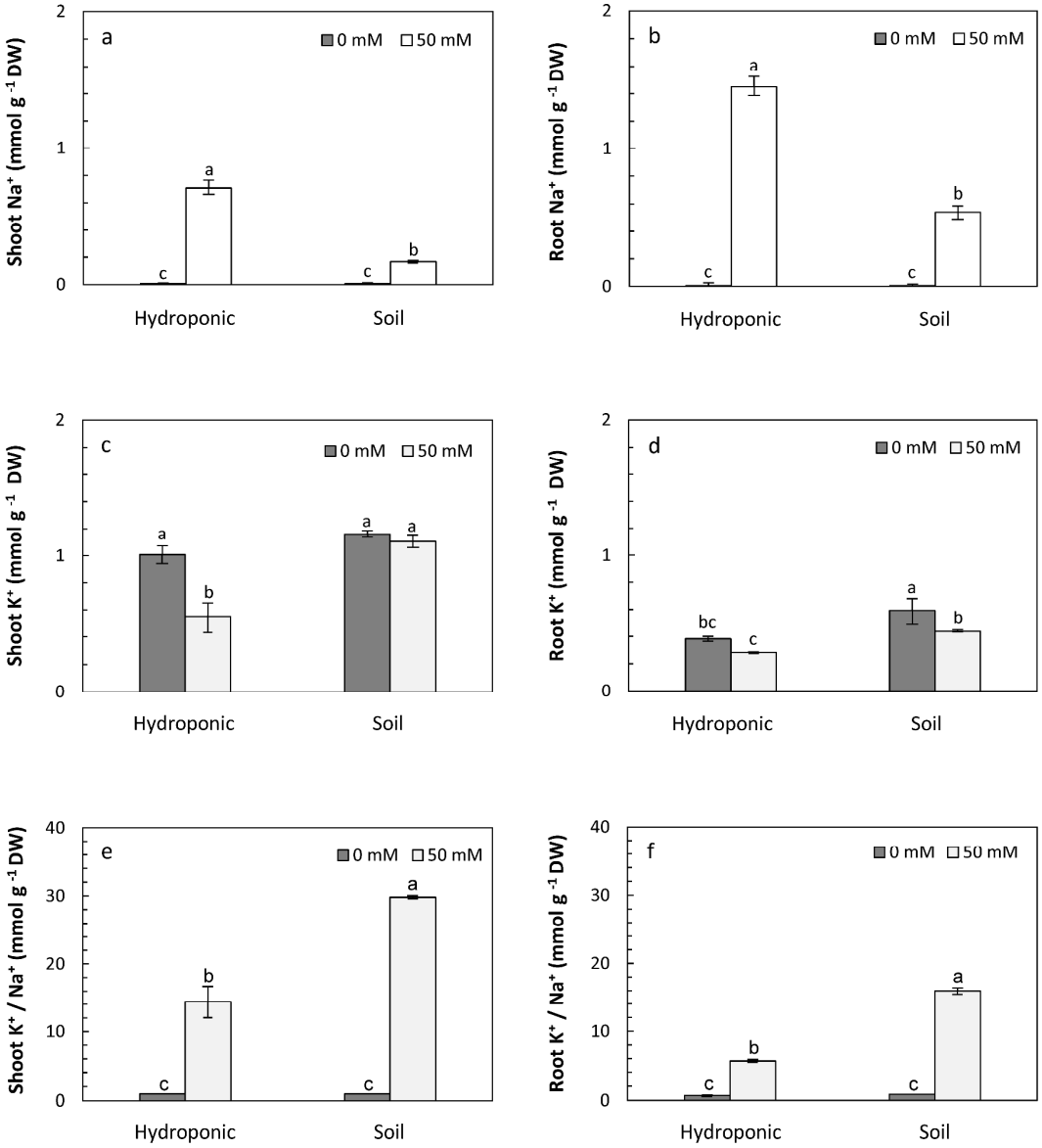

Figure 2. Effect of salinity on Na^+ (a and b), K^+ (c and d), and K^+/Na^+ ratio (e and f) in shoots and roots of hydroponically and soil-grown *V. locusta* with 50 mM NaCl after 41 days

Values are means of six biological replicates ± standard error. Bars marked with different letters, in each panel, are significantly different according to Duncan's multiple-range test at $p \leq 0.5$

Effect of salinity on phenolic and flavonoids accumulation

TPC and TFC were only evaluated in shoots of *V. locusta*. Results revealed that TPC decreased in shoots of *V. locusta* by 12 and 32%, in hydroponic and soil culture systems, respectively (Figure 3a). Similar but not the same, TFC decreased by 28% only in the shoots of lamb's lettuce growing under hydroponic system (Figure 3b). Regardless of the treatments, shoots of soil-grown lamb's lettuce held the highest TPC and TFC, when compared to those growing in the hydroponic system (Figure 3a-b).

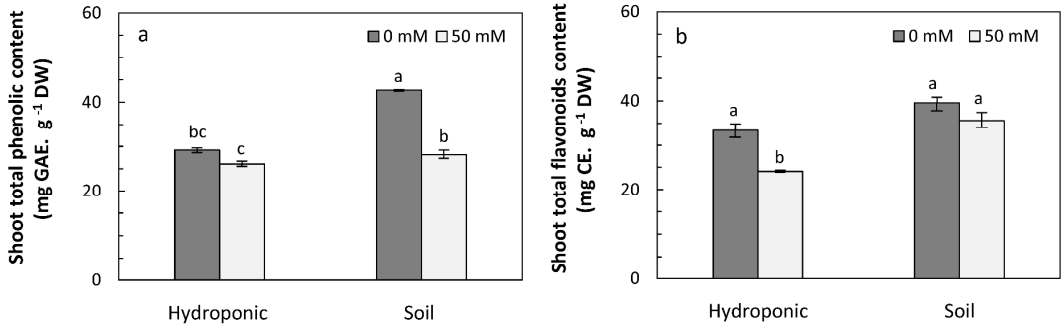

Figure 3. Effect of salinity on total phenolic (a) and total flavonoids (b) content in shoots of *V. locusta* plants grown hydroponically and on soil with 50 mM of NaCl for 41 days

Means of three biological replicates ± standard error. Bars marked with different letters, in each panel, are significantly different according to Duncan's multiple-range test at $p \leq 0.5$

Effect of salinity on total antioxidant activity and radical scavenging activity

In contrast to the TPC decrease, TAA was significantly increased in salt treated shoots of *V. locusta*, when compared to control (i.e., 142.1 and 161.36 mg GAE. g^{-1} DW for shoot extracts of *V. locusta* hydroponically and soil-grown, respectively) (Figure 4a). However, shoot extracts of soil-grown lamb's lettuce showed the highest TAA in the presence or the absence of NaCl (50 mM).

Salinity decreased the ABTS radical scavenging activity in shoots of lamb's lettuce on both growing systems, when compared to control (Figure 4b). However, in the absence or presence of NaCl, shoots of *V. locusta* growing in soil showed the highest ABTS scavenging activity as recorded by the lowest IC_{50} values (i.e., 54 µg ml^{-1} and 61 µg ml^{-1} for control and treated conditions, respectively), compared to those of hydroponic culture (i.e. 104 µg ml^{-1} and 124 µg ml^{-1} for control and treated conditions, respectively).

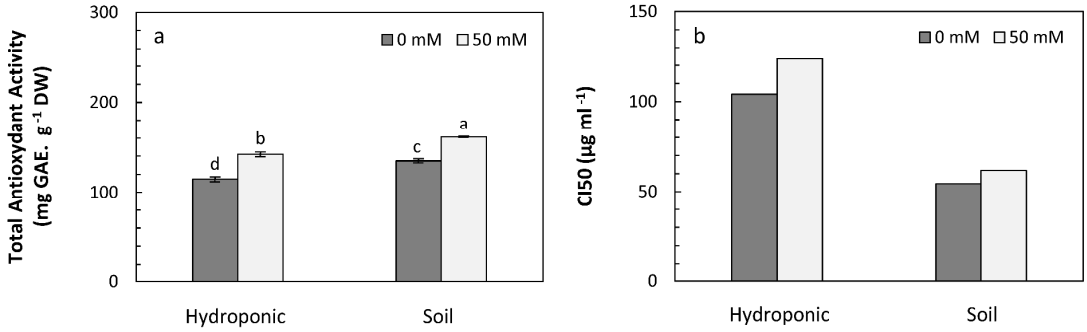

Figure 4. Effect of salinity (NaCl 50 mM) on shoots total antioxidant activity (a) and radical scavenging capacity (b) of hydroponically and soil-grown *V. locusta*

Means of three biological repeats ± standard error. Bars marked with different letters, in each panel, are significantly different according to Duncan's multiple-range test at $p \leq 0.5$

Anti-α-amylase and anti-AChE activity

The addition of NaCl 50 mM significantly reduced the inhibitory activity of α-amylase and AChE enzymes in shoots extracts of *V. locusta* grown hydroponically and in soil (Figure 5a-b). However, in both control and treated conditions, shoot extracts of soil-grown lamb's lettuce showed a significantly better inhibitory activity of both enzymes when compared to that of hydroponically-grown.

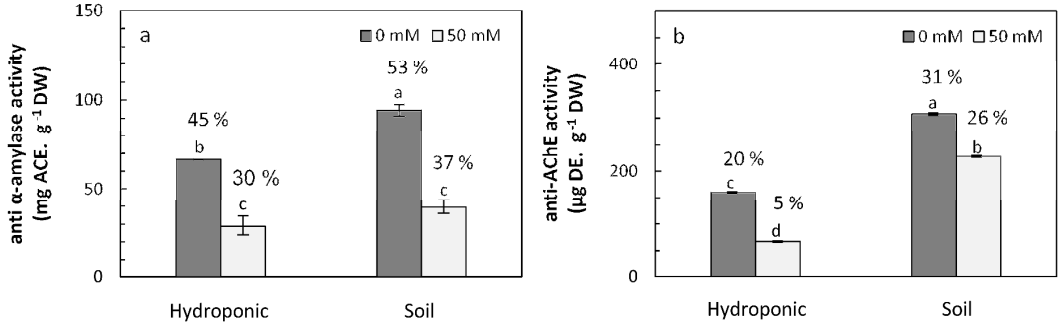

Figure 5. Anti-α-amylase and anti-AChE activities in shoot extracts of hydroponically and soil-grown *V. locusta* under 0 mM and NaCl 50 mM

Means of three biological repeats ± standard error. Bars marked with different letters, in each panel, are significantly different according to Duncan's multiple-range test at $p \leq 0.5$

Correlation Analysis and Principal Component Analysis (PCA)

For a better understanding of the obtained data, the trait-by-trait analyses were used by correlation analysis (Tables 1 and 2), as well as by Principal Component Analysis (PCA) (Figure 6a and b). The results obtained the correlation analysis showed a perfect match with our trait-by-trait analyses. In the absence of NaCl, the performance of *V. locusta* on both growing systems, was positively correlated with the shoot and root DW, RGR activity and K^+ content, shoot TPC and TFC, TAA, anti-α-amylase, and anti-AChE activities. Following the addition of NaCl 50 mM, a positive correlation was recorded regarding Na^+ content in shoots and roots of lamb's lettuce grown hydroponically and in soil, proving the accumulation of this ion in both organs. Further, these analyses confirmed the negative effect of NaCl 50 mM, previously observed on both growing systems. Indeed, negative correlations were recorded upon Na^+ accumulation in shoots and roots, with; growth parameters (DW and RGR), shoot and root K^+ content, shoot TPC and TFC, and anti-α-amylase and AChE activities. Interestingly, a positive correlation was recorded between NaCl (50 mM) treatment and shoot TAA on both growing systems, further proving that the stimulating effect of the TAA was indeed a result of Na^+ accumulation in shoots.

Table 1. Pearson's correlation matrix analysing 0 mM and 50 mM NaCl treatments and different studied parameters variables were centred on their means and normalized with a standard deviation of 1

Variables	Hydronic system		Soil system		
	0 mM	50 mM	0 mM	50 mM	
SDW	**0.80 ****	**-0.80 ****	**0.97 *****	**-0.97 *****	1
RDW	**0.67 ***	**-0.67 ***	-0.54	0.54	0.9
Shoot/Root Ratio	**0.70 ***	**-0.70 ***	**0.97 *****	**-0.97 *****	0.8
Shoot RGR	**0.80 ****	**-0.80 ****	**0.97 *****	**-0.97 *****	0.7
Root RGR	**0.58 ***	**-0.58 ***	-0.54	0.54	0.6
Shoot Na^+	**-0.97 *****	**0.97 *****	**-0.93 *****	**0.93 *****	0.5
Root Na^+	**-0.98 *****	**0.98 *****	**-0.95 *****	**0.95 *****	0.4
Shoot K^+	**0.76 ****	**-0.76 ****	0.30	-0.30	0.3
Root K^+	**0.88 *****	**-0.88 *****	0.49	-0.49	0.2
Shoots K^+/Na^+	**-0.88 *****	**0.88 *****	**-0.99 *****	**0.99 *****	0.1
Roots K^+/Na^+	**-0.99 *****	**0.99 *****	**-0.99 *****	**0.99 *****	0
STPC	**0.91 ***	**-0.91 ***	**0.98 *****	**-0.98 *****	-0.1
STFC	**0.92 ****	**-0.92 ****	0.49	-0.49	-0.2
TAA	**-0.97 ****	**0.97 ****	**-0.98 *****	**0.98 *****	-0.3
Anti-α-amylase activity	**0.98 ***	**-0.98 ***	**0.99 ****	**-0.99 ****	-0.4
Anti-AChE activity	**0.98 *****	**-0.98 *****	0.75	-0.75	-0.5
					-0.6
					-0.7
					-0.8
					-0.9
					-1

Values represent correlation coefficients (R). Correlations are presented in red (negative correlations) and green (positive correlations). SDW: Shoot dry weight; RDW: Root dry weight; Shoot/Root ratio; Shoot RGR: Shoot Relative Growth Rate; Root RGR: Root Relative Growth Rate; Shoot Na^+; Root Na^+; Shoot K^+; Root K^+; Shoot K^+/Na^+ ratio; Root K^+/Na^+ ratio; STPC, Shoot Total Phenolic Content; STFC, Shoot Total Flavonoid Content; TAA: Total Antioxidant Activity; anti-α-amylase activity; anti-AChE activity: anti-acetylcholinesterase activity.
Values in bold represent the statistically significant correlations at 0.05 (*), 0.01 (**), and 0.001 levels (***)

Table 2. Pearson's correlation matrix between plant growth, shoots and roots nutrients content; shoot total phenolic and flavonoid content; antioxidant activity; anti-α-amylase activity and anti-acetylcholinesterase activity in lamb's lettuce (*V. locusta*) grown hydroponically (Table 2a) and on soil (Table 2b) with 0 or 50 mM NaCl. Correlations are displayed in green (positive) and red (negative); colour intensity is proportional to the correlation coefficient. SDW: Shoot dry weight; RDW: Root dry weight; Shoot/Root ratio; Shoot RGR: Shoot Relative Growth Rate; Root RGR: Root Relative Growth Rate; Shoot Na^+; Root Na^+; Shoot K^+; Root K^+; Shoot K^+/Na^+ ratio; Root K^+/Na^+ ratio; STPC, Shoot Total Phenolic Content; STFC, Shoot Total Flavonoid Content; TAA: Total Antioxidant Activity; anti-α-amylase activity; anti-acetylcholinesterase (AChE) activity.

Table 2a

Variables	SDW	RDW	Shoot/Root Ratio	Shoot RGR	Root RGR	Shoot Na^+	Root Na^+	Shoot K^+	Root K^+	Shoot K^+/Na^+	Root K^+/Na^+	STPC	STFC	STAA	Anti-α-amylase activity	Anti-AChE activity	
SDW	**1**	**0.97 *****	0.25	**0.99 *****	**0.93 *****	**-0.76 ****	**-0.83 *****	0.40	**0.76 ****	**-0.76 ****	**-0.79 ****	**0.92 ****	0.81	**-0.89 ***	**0.98 ***	**0.95 ****	1
RDW		**1**	0.02	**0.97 *****	**0.99 *****	**-0.64 ***	**-0.73 ****	0.28	**0.60 ***	**-0.62 ***	**-0.64 ***	**0.88 ***	0.80	**-0.87 ***	**0.99 ***	**0.92 ****	0.9
Shoot/Root Ratio			**1**	0.25	-0.08	**-0.65 ***	-0.58	**0.59 ***	**0.69 ***	**-0.69 ***	**-0.74 ****	**0.89 ***	0.76	**-0.87 ***	0.76	**0.90 ***	0.8
Shoot RGR				**1**	**0.94 *****	**-0.75 ****	**-0.82 ****	0.38	**0.73 ****	**-0.77 ****	**-0.78 ****	**0.89 ***	**0.82 ***	**-0.89 ***	**0.99 ****	**0.93 ****	0.7
Root RGR					**1**	-0.56	**-0.65 ***	0.20	0.51	-0.55	-0.56	**0.83 ***	0.77	**-0.82 ***	**0.98 ***	**0.86 ***	0.6
Shoot Na^+						**1**	**0.98 *****	**-0.86 *****	**-0.86 *****	**0.74 ****	**0.92 *****	-0.77	**-0.84 ***	**0.90 ***	**-0.95 ***	**-0.95 ****	0.5
Root Na^+							**1**	**-0.81 ****	**-0.85 *****	**0.78 ****	**0.93 *****	**-0.88 ***	**-0.92 ***	**0.96 ****	**-0.98 ***	**-0.97 *****	0.4
Shoot K^+								**1**	**0.69 ***	-0.41	**-0.68 ***	0.48	0.61	-0.66	**0.95 ***	0.78	0.3
Root K^+									**1**	**-0.76 ****	**-0.87 *****	0.81	0.56	-0.74	0.91	**0.85 ***	0.2
Shoot K^+/Na^+										**1**	**0.94 *****	**-0.96 ****	**-0.95 ****	**0.98 *****	**-0.99 ***	**-0.95 ****	0.1
Root K^+/Na^+											**1**	**-0.93 ****	**-0.94 ****	**0.98 *****	**-0.98 ***	**-0.97 ****	0
STPC												**1**	**0.85 ***	**-0.90 ***	**0.95 ***	**0.91 ***	-0.1
STFC													**1**	**-0.97 ****	0.90	**0.87 ***	-0.2
STAA														**1**	-0.92	**-0.93 ****	-0.3
Anti-α-amylase activity															**1**	0.92	-0.4
Anti-AChE activity																**1**	-0.5
																	-0.6
																	-0.7
																	-0.8
																	-0.9
																	-1

Table 2b

Variables	SDW	RDW	Shoot/ Root Ratio	Shoot RGR	Root RGR	Shoot Na^+	Root Na^+	Shoot K^+	Root K^+	Shoot K^+/Na^+	Root K^+/Na^+	STPC	STFC	STAA	Anti-α-amylase activity	Anti-AChE activity	
SDW	**1**	-0.44	**0.97 *****	**0.99 *****	-0.42	**-0.90 *****	**-0.93 *****	0.21	**0.63 ***	**-0.97 *****	**-0.96 *****	**0.98 *****	0.50	**-0.93 ****	**0.99 ****	0.63	**1**
RDW		**1**	**-0.61**	-0.42	**0.99 *****	0.52	0.32	-0.11	-0.18	0.53	**0.58 ***	-0.58	0.15	0.65	-0.27	-0.37	**0.9**
Shoot/Root Ratio			**1**	**0.97 *****	**-0.60 ***	**-0.91 *****	**-0.91 *****	0.19	0.53	**-0.97 *****	**-0.97 *****	**0.98 *****	0.44	**-0.97 ****	**0.98 ***	0.68	**0.8**
Shoot RGR				**1**	-0.41	**-0.90 *****	**-0.95 *****	0.19	0.58	**-0.97 *****	**-0.96 *****	**0.98 *****	0.52	**-0.94 ****	**0.99 ****	0.67	**0.7**
Root RGR					**1**	0.54	0.34	-0.08	-0.10	0.53	0.58	-0.58	0.13	0.66	-0.24	-0.36	**0.6**
Shoot Na^+						**1**	**0.90 *****	-0.29	-0.42	**0.92 *****	**0.92 *****	**-0.97 ****	-0.44	**0.88 ***	-0.88	-0.46	**0.5**
Root Na^+							**1**	-0.34	-0.41	**0.95 *****	**0.92 *****	**-0.91 ***	-0.59	**0.91 ***	**-0.99 ****	-0.72	**0.4**
Shoot K^+								**1**	0.00	-0.28	-0.32	0.47	0.50	-0.60	0.51	**0.88 ***	**0.3**
Root K^+									**1**	-0.49	-0.50	0.76	0.33	-0.56	**0.97 ***	0.20	**0.2**
Shoot K^+/Na^+										**1**	**0.99 *****	**-0.98 *****	-0.49	**0.98 *****	**-0.99 ****	-0.75	**0.1**
Root K^+/Na^+											**1**	**-0.96 ****	-0.47	**0.97 ****	**-0.99 ****	-0.76	**0**
STPC												**1**	0.50	**-0.95 ****	**0.97 ***	0.62	**-0.1**
STFC													**1**	-0.43	0.50	0.62	**-0.2**
STAA														**1**	**-0.96 ***	-0.78	**-0.3**
Anti-α-amylase activity															**1**	0.69	**-0.4**
Anti-AChE activity																**1**	**-0.5**
																	-0.6
																	-0.7
																	-0.8
																	-0.9
																	-1

Values in bold represent the statistically significant correlations at 0.05 (*), 0.01 (**), and 0.001 levels (***)

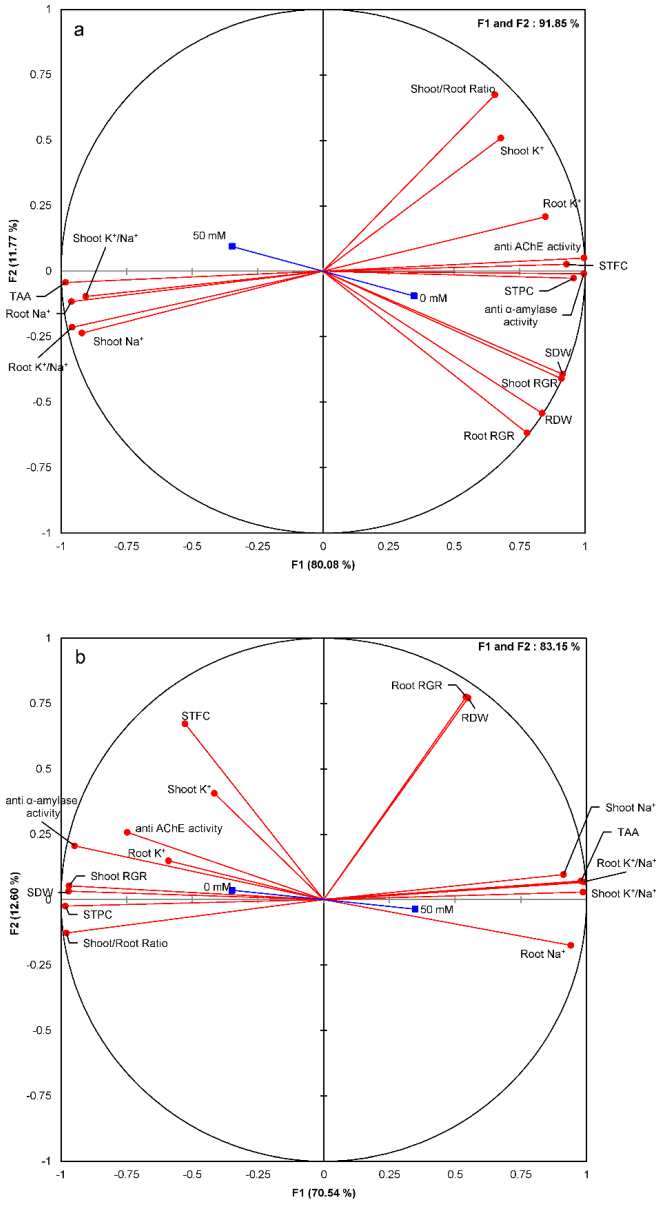

Figure 6. Principal Component Analysis (PCA) of all parameters studied in *V. locusta* grown hydroponically (a) and on soil (b). Red circles represent different analysis parameters. Blue squares represent control (0 mM) and NaCl treatment (50 mM). All studied parameters and the different treatments are projected onto the F1-F2 principal factorial plane that explains 74.99 % of the variation. SDW: Shoot dry weight; RDW: Root dry weight; Shoot/Root ratio; Shoot RGR: Shoot Relative Growth Rate; Root RGR: Root Relative Growth Rate; Shoot Na^+; Root Na^+; Shoot K^+; Root K^+; Shoot K^+/Na^+ ratio; Root K^+/Na^+ ratio; STPC, Shoot Total Phenolic Content; STFC, Shoot Total Flavonoid Content; TAA: Total Antioxidant Activity; anti-α-amylase activity; anti-AChE activity: anti-acetylcholinesterase activity

Discussion

The obtained results showed that under non-stress conditions, *V. locusta* grown on hydroponics exhibited an overall better growth performance (shoot and root DW and RGR) compared to that grown on soil. Manzocco *et al.* (2011) reported similar results, with a higher yield in hydroponically-grown lamb's lettuce than that of soil-grown. In fact, soilless systems have been proven an effective way to improve growth and nutritional quality of vegetable crops and their shelf life. This is particularly due to the several advantages that hydroponics offers over open field crop production, such as light and temperature management, modulating mineral levels according to the plant needs, as well as guaranteed efficient water uptake, which are considered as primordial growth conditions (Sambo *et al*., 2019).

Further, results showed that salt stress (50 mM NaCl) significantly reduced the shoot dry weight (DW) and relative growth rate (RGR) of *V. locusta* grown in both hydroponic and soil systems. This decrease was confirmed by the strong negative correlation with NaCl treatment (Table 2), particularly with shoot Na^+ content in hydroponics (r = -0.76 **) and soil-grown *V. locusta* (r = -0.90 ***) (Table 2 a and b). In fact, the presence of salt in soil causes osmotic stress, which leads to reduced water uptake and turgor pressure, resulting in stomatal closure, reduced photosynthesis, reduced mineral uptake, and therefore the decline in growth and nutritional quality of food crops (Adhikari *et al.,* 2019). Results also showed that salt stress did not affect the root DW of soil-grown *V. locusta*; in fact, it showed a significant increase in RGR compared to control. In contrast, both root DW and RGR decreased in hydroponically-grown *V. locusta* with salt stress. These results suggest a better tolerance and growth performance of lamb's lettuce in soil under salt stress.

Under salt stress, shoot/root biomass ratio is also an indicator of salt tolerance. In this study, salinity decreased the shoot-to-root ratio in both growing systems, which was confirmed with a negative correlation with NaCl treatment (Table 1). The observed decrease, was more pronounced in soil-grown *V. locusta* (60%), compared to hydroponics (20%). He *et al.* (2019) explained that decreased shoot/root ratio under salt stress signify the higher growth promotion effect in roots, which is considered as an adaptation mechanism to osmotic stress. This result seems to support that *V. locusta* has rather a better tolerance to salt stress when grown on soil.

Further results of this study showed that Na^+ contents were 3 to 4 times much higher in shoots and roots of hydroponically-grown lamb's lettuce. In fact, the uptake characteristics of Na^+ in hydroponics and soil culture differs. The difference is related to the effect of the solid soil matrix on ions uptake. In fact, in soil, ion adsorption occurs between soil-colloidal particles (clays, sesquioxides and amorphous minerals) and Na^+, making its uptake difficult for roots, while in hydroponics, plant roots meet nutrients, including Na^+, solely via the hydroponic solution; therefore, making Na^+ uptake by roots occur more easily (Tavakkoli *et al*., 2010). These results put hydroponic systems at a disadvantage when using them to assess crops salinity tolerance in research, comparing to soil cultures.

Besides, in both hydroponics and soil systems, *V. locusta* accumulated Na^+ largely at roots level compared to shoots. This finding indicates that *V. locusta* restricted Na^+ buildup in shoots, given the fact that its accumulation in leaves disrupts the ionic balance required for normal cell metabolic function (Teakle and Tyerman, 2010; Kamran *et al.,* 2020). In a previous study, Yan et *al.* (2015) found similar results in honeysuckle *Lonicera japonica*, a species of the same family as *V. locusta* (Caprifoliaceae family). They showed that the lower leaf Na^+ concentration in honeysuckle depended on the restriction of Na^+ transport from root to leaf.

The reduction of plant growth under salt stress is a common feature connected with osmotic stress and macronutrient uptake, like K^+ (Munns and Tester, 2008). In fact, most plants use K^+ to ensure proper osmotic adjustment. Therefore, under salt stress, maintaining a high cytosolic K^+/Na^+ ratio is one of the key determinants of plant tolerance to salinity (Zhang *et al.,* 2010). In this study, NaCl (50 mM) had no effect on shoot K^+ content of soil-grown *V. locusta*. However, it reduced K^+ content by 45% in shoots of hydroponically-

grown *V. locusta*. Shoots Na^+ content negatively correlated with shoot K^+ content (r = -0.86 ***) only in hydroponics (Table 2a).

In roots, K^+ content decreased under salt stress on both growing systems, which is due to the interactions between Na^+ and K^+ at the absorption sites in roots.

Besides, soil-grown *V. locusta* under salt stress maintained a higher K^+/Na^+ ratio in both roots and shoots when compared to hydroponics, which affirms a relatively better ability of *V. locusta* to tolerate salt stress when grown on soil. Tavakkoli *et al.* (2012) also reported an overall better tolerance performance to salt stress in barley grown in soil compared to hydroponics.

One important aspect of our study was to investigate the effect of salt stress on the accumulation of bioactive compounds in lamb's lettuce shoots grown hydroponically and in soil. In fact, under salt stress conditions, plants are exposed to oxidative stress, which calls them to synthesize secondary metabolites, including phenolic compounds, as a non-enzymatic antioxidant mechanism of defense (Król *et al.,* 2014).

Results revealed that under control conditions, shoots of soil-grown *V. locusta* also showed higher TPC than in hydroponically-grown *V. locusta*. Salinity, however, significantly decreased TPC in shoots of *V. locusta* grown in hydroponics (r = -0.91 *) and soil (r = -0.98 ***) (Table 1). Compared to other studies that used a similar quantification method for different vegetables, the current findings are in agreement with those reported for lettuce, spinach, artichoke, and tomato (Rezazadeh *et al.,* 2012; Ferreira *et al.,* 2018; Ben-Abdallah *et al*., 2019; Shin *et al*., 2020). However, it contradicts the findings of a recent study conducted on *V. locusta* by Hernández *et al.* (2021), with no effect reported on TPC in all imposed salt treatments (15, 30, and 60 mM NaCl) when compared to control. In fact, the production of phenolics under salt stress conditions relies significantly on plant tolerance to salt stress (Karray-Bouraoui *et al.,* 2011). There are numerous possible mechanisms for the regulation, accumulation and degradation of phenolic compounds in plant tissues (Zheng *et al.,* 2006). Besides, the nutritional properties of vegetables are highly influenced by agricultural management practices and climatic conditions (Chandra *et al.,* 2014; Fernandes *et al.,* 2021). For instance, mineral nutrition in plants is one of the main factors influencing secondary metabolites production (Skrypnik *et al.,* 2019). Under salt stress, Rezazadeh *et al.* (2012) linked the decrease in secondary metabolite production with the impaired uptake of nutrients, mainly phosphorus and potassium, which are essential elements for the synthesis of secondary metabolites including phenolic compounds.

On the other hand, TFC remained stable in shoots of soil-grown lamb's lettuce at 50 mM NaCl, whereas it decreased in hydroponics. This decrease was negatively correlated with the heavier Na^+ accumulation previously reported in shoots of hydroponically-grown lamb's lettuce compared to the soil-grown (r = -0.92**) (Table 1). These findings further indicate that lamb's lettuce has a better tendency to tolerate salt stress when grown in soil.

More importantly, when comparing both growing systems, shoots of *V. locusta* grown in soil showed higher TPC and TFC when both untreated and treated with NaCl (50 mM), demonstrating the difference between soil cultures and floating systems mainly related to different environmental and growing conditions, and showing advantages of soil culture.

The antioxidant activity of phenolics is mainly due to their redox properties, that make them act as free radical scavengers (Sgherri *et al.,* 2010). In this study, results revealed that salt stress induced an increase in the TAA of shoot extracts obtained from both hydroponic and soil systems. This significant increase was positively correlated with the presence of NaCl (50 mM) in both media (Table 2) and the accumulation of Na^+ in shoots (Table 2 a and b). However, it was inconsistent with the decrease of the TPC. Rezazadeh *et al.* (2012) also reported that despite the decrease in TPC and TFC in artichokes grown under salt stress, there was a dramatic increase in TAA compared to the control. To explain this, Djeridane *et al.* (2006) elucidated that the phenolic fraction does not incorporate all the antioxidant compounds in plant tissues. In addition, the synergism between the TAA and the bioactive molecules in the extracts would probably make the antioxidant activity not

only depend on the quantity of such molecules, but also on their structure and nature. Moreover, the overall antioxidant activity is a complex process that depends on several modes of polyphenol interactions, which may participate to different degrees due to their different ratios among phenolic classes. In fact, Rasool *et al.* (2013) explained that the distinction in polyphenols subclasses production under salt stress is related to a differential synthesis of these compounds relative to plant necessities to adapt to salt stress.

When comparing both growing systems, lamb's lettuce grown in soil showed higher TAA than that grown in hydroponics, under untreated and treated conditions, which further proves that soil appears to be a better environment for efficient growth and better nutritional quality for *V. locusta*.

Important scavenging activity of the ABTS radical was observed in this study under control conditions, with an IC_{50} of 104 µg ml^{-1} and 54 µg ml^{-1} for hydroponics and soil shoot extracts, respectively, which shows a very promising antiradical potential of the ethanolic shoot extracts of this salad. While the scavenging properties were decreased by salt stress in both media, *V. locusta* grown on soil still possessed a better scavenging ability of the free radical ABTS (IC_{50}= 62 µg ml^{-1}) compared to hydroponically-grown *V. locusta*.

Several studies have shown the importance of a phenolic-rich diet, including phenolic acids and flavonoids, in the prevention of several types of diseases, such as diabetes and Alzheimer disease. Inhibition of α-amylase, a key pancreatic enzyme involved in the hydrolysis of oligosaccharides into monosaccharides, is a therapeutic way to manage blood glucose levels by diminishing the absorption of monosaccharides by the intestines, therefore reducing postprandial hyperglycaemia (Ali *et al.,* 2020). Under control conditions, our data on α-amylase inhibitory activity showed a strong ability of *V. locusta* shoot extracts to inhibit the activity of this enzyme, with inhibition activities of 45% (66.7 mg ACE. g^{-1} DW) and 53% (94.3 mg ACE. g^{-1} DW) for hydroponics and soil cultures, respectively. The inhibition of α-amylase positively correlated with shoot TPC in hydroponics (r = 0.95 *), and in soil-grown lamb's lettuce (r = 0.91 *). In comparison with another research that used similar quantification method, it was found that shoots of *V. locusta* had greater inhibitory activity than that of the ethanolic extract of Pelargonium, an aromatic plant widely known for its pharmacological properties (12.21 mg ACE. g^{-1} DW) (Ali *et al.,* 2020). Interestingly, when compared to a marketed polyherbal formulation for diabetes (D-Diabetes smart powder), which consists of a blend of nineteen medicinal herbs, shoots of lamb's lettuce showed an impressive inhibitory activity of α-amylase. In fact, the control shoot extracts of soil and hydroponically-grown lamb's lettuce showed a greater inhibitory activity of 53% and 45%, respectively, compared to 40% inhibition by the marketed polyherbal formulation (Kamtekar *et al.,* 2014).

Following the addition of 50 mM NaCl in both media, the inhibition activity of shoot extracts decreased in both growing systems, demonstrated by a significant negative correlation for hydroponics (r = -0.98 *), and for soil (r = -0.99 **) (Table 1). Although non-significant, the shoot extract of soil-grown lamb's lettuce treated with salt still exhibited greater inhibitory ability of α-amylase (37%), compared to the hydroponically-grown (30%).

Inhibition of AChE, the enzyme responsible for the hydrolysis of the neurotransmitter acetylcholine, is implicated in the treatment of numerous neurodegenerative illnesses, including Alzheimer disease. The inhibition of AChE makes it possible to raise synaptic acetylcholine concentrations, thus helping relieve Alzheimer's symptoms (Ayaz *et al.,* 2017). Data showed that control shoot extracts of *V. locusta* showed inhibition percentages of 20% (160.3 µg DE. g^{-1} DW) and 31% (307.4 µg DE. g^{-1} DW) of the AChE enzyme activity, respectively for hydroponics and soil mediums. Adding NaCl significantly reduced the inhibition capacity of shoot extracts on both growing systems.

When further comparing both mediums, significant differences in anti-α-amylase and anti-AChE activities were revealed between extracts from hydroponically-grown and soil-grown lamb's lettuce. In fact, control and treated shoot extracts of soil-grown *V. locusta* showed a higher inhibition capacity of both enzymes' activity, which might be attributed to a higher total phenolic content. El Adib *et al.* (2015) found similar results comparing two argan varieties. They revealed that the variety with the highest contents of phenolic compounds in its leaves had a higher α-amylase enzyme inhibitory activity. Furthermore, Zheng *et al.* (2006) explained that

different production systems used for *Echinacea angustifolia*, a medicinal plant commonly used to prevent colds and other respiratory tract infections, resulted in different concentrations of caftaric acid, chlorogenic acid, cichoric acid and echinacoside acid. This underlines the effect of production systems on phenolic subclasses synthesis and thus explains the strong influence on its antioxidant and biological activities. Previous studies have shown that many phytochemical substances, mainly phenolic acids and flavonoids, exhibit significant antidiabetic and neuroprotective activities. In fact, several flavonoid compounds, such as rutin, myricetin, kaempferol and quercetin, have already been reported to have hypoglycemic effects by inhibiting the α-amylase enzyme (Yao *et al.,* 2013). Szwajgier (2014) showed that flavonoids; luteolin, kaempferol, and rutin are also strong inhibitors of AChE catabolic activity. Among the flavonoids that *V. locusta* leaves contain, Ramos-Bueno *et al.* (2016) revealed the presence of rutin and kaempferol. In addition, Hernández *et al.* (2021) recently revealed the presence of luteolin and quercetin. On the other hand, Xiao *et al.* (2013) and Orhan *et al.* (2007) proved that chlorogenic acid have a higher inhibiting capacity of α-amylase and AChE compared to other phenolic acids. In fact, phenolic acids reflect the major fraction of phenolic compounds in *V. locusta* leaves, with chlorogenic acid being on top (Długosz-Grochowska *et al.,* 2016). Hernández *et al.,* 2021 has further proved that chlorogenic acid makes up 57% of the total phenolic concentration. Given the fact that no published data is yet available about the anti-α-amylase and anti-AChE activities of phenolic extracts from *V. locusta*, it could be concluded that the presence of rutin, kaempferol, and chlorogenic acid might be the main cause of the anti- α-amylase and anti-AChE activities. Upon these results, more studies are certainly needed to further investigate the nutritional quality of shoot extracts of *V. locusta*.

Conclusions

In conclusion, results clearly demonstrated that *V. locusta*'s sensitivity to salt stress significantly differed between hydroponics and soil systems. Soil-grown *V. locusta* exhibited greater tolerance to salt stress, as it showed higher TPC and TFC, better antioxidant activities (TAA and ABTS scavenging ability), and also greater therapeutic activity against diabetes (enhanced inhibition of the α-amylase enzyme) and Alzheimer's disease (enhanced inhibition of the AChE enzyme), which significantly highlights the differences between solution and soil cultures in assessing the salinity tolerance of crops. Under control conditions, all cited parameters were also higher in soil-grown *V. locusta*. Additionally, results pointed out interesting therapeutic potential in this salad, as little research explored it.

Based on this study's outcomes, further investigations are required to better comprehend *V. locusta*'s response to salt stress in both growth systems and to delve deeper into its nutritional benefits by examining additional biological activities and a comprehensive phenolic profile.

Authors' Contributions

Conceptualization: NKB and SBA; Data curation: FB and SBA; Formal analysis: FB and MF; Funding acquisition: NKB and WZ; Investigation: FB, SBA, and NKB; Methodology: FB, SBA, IBHA and AR; Project administration: NKB and WZ; Resources: NKB and SBA; Software: WZ and SBA; Supervision: NKB; Validation: NKB and WZ; Visualization: FB and MF; Writing - original draft: FB and MF; Writing - review and editing: FB and MF.

All authors read and approved the final manuscript.

Ethical approval (for researches involving animals or humans)

Not applicable.

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The listed authors contributed directly and intellectually to the manuscript and provided their approval for publication.

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Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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