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Alleviating saline-calcareous stress in *Atriplex nummularia* seedlings by foliar spraying with silymarin-enriched bee-honey solution

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ABSTRACT

When the plant is subjected to stress conditions on saline-calcareous soil, it is severely damaged and may die. Overcoming these saline and calcareous stresses is a challenge for the sustainability of human and animal feeding. During 2020 and 2021, the potential enhancing impacts of treating *Atriplex nummularia* seedlings with a 1% bee-honey solution enriched with 200 µg silymarin per L of solution (SBH) on plant growth, physiological and biochemical traits, antioxidant systems, and nutritional value under saline-calcareous soil stress conditions were investigated. Foliar spraying of *A. nummularia* with SBH significantly improved plant growth, root activity, photosynthetic efficiency, leaf cell integrity, soluble protein, osmo-regulators, different antioxidants, and nutritional status, while markers of oxidative stress (H_2O_2 and $O_2^{\bullet-}$) and oxidative damage (lipid peroxidation and electrolyte leakage), total phenols, and Na^+ levels were suppressed. The results of this investigation recommended the utilization of SBH for producing satisfactory plant productivities under saline-calcareous soil conditions.

1. Introduction

The land cultivated with crops for human food in Egypt is not sufficient for the requirements of human food security. Therefore, to expand crop cultivation for animal feeding, it is necessary to exploit new arable areas using tolerant plants and external bio-stimuli. Sustainability of crop productivity is achieved primarily by overcoming various eco-stressors, including high $CaCO_3$ and salinity. Cultivation of disreputable soils constrains agricultural productivity due to their low fertility, nutritional imbalance, high EC_e, and unavailability of water and nutrients [1,2].

Calcareous soils are predominant in dry climates [3]. The common properties of calcareous soils (e.g., availability of water and nutrients; Mg, Zn, Cu, Fe, N, P, and K) are adversely influenced by high pH value (7.5–8.5) and carbonate content [1]. These undesirable conditions inhibit plant growth and production via overproducing ROS, negatively influencing physio-biochemical attributes, osmoregulation, and antioxidant defense systems [1,4,5]. Otherwise, saline soils are common, particularly in dry regions. Salinity stress causes malnutrition, "physiological drought", and osmotic stress. It restricts plant growth and production via the impacts of overproducing ROS on physio-biochemical indices and defense systems in plants [6–10]. The adverse impacts on plants are exacerbated by the combination of saltiness and calcareousness (saline-calcareous soil), and the soil becomes unproductive. Therefore, it is essential to use plants that are tolerant of such soils along with treating them foliarly with bio-stimulators to increase their tolerance further to stress conditions.

Foliar treatment with a silymarin-enriched bee-honey solution (SBH) as an efficient bio-stimulator would be expected to further improve plant growth and production. The use of SBH notably suppresses ROS and enhances plant growth and productivity by enhancing physio-biochemical attributes, nutritional status, and antioxidant defense systems. Enriching this bio-stimulator (bee-honey) with silymarin (Sim; a powerful antioxidant) elevates its efficiency and maximizes plant growth and productivity under severe stress conditions [11–17].

A. nummularia Lindl is a halophyte and animal feed [18,19]. This plant is more suitable to grow under severe soil conditions but loses more of its production and quality [20–23]. But by treating the *A. nummularia* with SBH, it can produce a high ratio of forage (dry matter), providing forage for livestock efficiently.

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No information is available on the impact of SBH applied as foliar spraying in minimizing the adverse effects of saline-calcareous soil stress on the quantity and quality of forage yield of *A. nummularia*. This research work hypothesized that the use of SBH as foliar spraying will efficiently enhance the growth and productivity of *A. nummularia*. Therefore, the aim of the study was to explore the enhancing influences of *A. nummularia* treatment with SBH on plant growth, photosynthetic machinery, membrane integrity, oxidative stress markers, osmotic regulators, non-enzymatic and enzymatic antioxidant activities, and nutritional status under the adverse conditions of saline-calcareous soil.

2. Materials and Methods

2.1. Location and dates of planting plant material and soil analysis

Standard 2.5-month-old *Atriplex nummularia* Lindl transplants were secured from a nursery of the Egyptian Ministry of Agriculture for two field trials in 2020 and 2021 using two different sites in the same experimental location. The trials were conducted on private farm soil under reclamation at Fayoum Governorate (29° 36′N; 30° 40′E), Egypt. In both seasons, transplantation was performed on March 17 and 20 and experiments ended on June 17 and 20, respectively.

Before transplantation in both seasons, samples were collected at 20-cm depth from the experimental soils to analyze soil properties applying the [24] and [25] procedures, and the data are presented in Table 1.

Table 1. Some initial physicochemical properties of the experimental soil (0–20 cm depth)

Properties	Units	Values
Particle size distribution		
Sand		83.20 ±7.42
Silt	%	8.10 ±0.81
Clay		8.70 ±0.84
Texture class	Loamy sand	
Physico-chemical properties		
pH (in soil paste at 25°C)		8.19 ±0.70
ECe	dS m^{-1}	8.16 ±0.74
OM	%	0.52 ±0.02
$CaCO_3$		32.8 ±2.74
Na^+	meq L^{-1}	64.3 ±5.48
Ca^+		25.0 ±2.10
K^+		24.2 ±2.15
P		3.24 ±0.24
N		11.4±1.04
Fe	mg kg^{-1}	3.10 ±0.31
Mn		2.08 ±0.18
Zn		1.42 ±0.10
Cu		1.11 ±0.08

Values are means (±SE). ECe= Electrical conductivity in soil paste extract at 25°C, OM= Organic matter, and $CaCO_3$= Calcium carbonate.

2.2. Experimental setup and treatments

The designs followed for the trials were completely randomized plots (CRD). Three replicates were planned for each treatment and each replicate included five transplants. In each season, a total area of 78 m^2 was plowed and leveled. The soil of this area was thoroughly mixed with 6.7 kg of $(NH_4)_2SO_4$, 6.7 kg of $CaH_6O_9P_2$, and 5.0 kg of K_2SO_4 fertilizers. The experimental area was divided into 6 plots for three replicates for both control and silymarin-enriched bee-honey (SBH) treatments. The area of each plot was 6.0 × 1.50 = 9 m^2, and the plots were separated by borders of 2 m each. In each plot, three rows were 1.5 m wide and the distance between the transplants on each row was 1.2 m. The *A. nummularia* transplants were transplanted at a rate of 1 $hill^{-1}$. 50 days after transplantation, seedling leaves were sprayed with SBH and the control seedlings were sprayed with distilled water. The bee-honey used in this study was analyzed prior to its use and the results are presented in Table 2.

The SBH was prepared [12,15,26] by adding 10 mL to each L distilled water and enriched with 200 µg Sim per L of solution. The SBH (1.0% bee-honey solution enriched with 200 µg silymarin per L of solution) was nominated for this principal study because it conferred the best findings among many treatments (0.5% bee-honey solution, 1.0% bee-honey solution, 1.5% bee-honey solution, 1.0% bee-honey solution enriched with 100 µg silymarin per L of solution, 1.0% bee-honey solution enriched with 200 µg silymarin per L of solution, and 1.0% bee-honey solution enriched with 300 µg silymarin per L of solution) applied in a preliminary study (data not shown). Furthermore, seedling leaves were sprayed with SBH twice again at 35 and 55 days after transplantation. As a surfactant, Tween-20 was added in a few drops to the solutions, which were sprayed with 20-L dorsal sprayers.

The appropriate system designed to irrigate the *A. nummularia* seedlings in this investigation was drip irrigation. The drip emitters were 120 cm apart with 4 L h^{-1} discharges utilizing electric timers. Suitable run times were applied according to the water requirements of the seedlings.

2.3. Sampling

Trials were ended 90 days after transplantation in both the 2020 and 2021 seasons. At this date, 5 seedlings were randomly selected from each treatment to assess growth traits, levels of oxidative stress markers, and osmo-regulators. Another 5 randomly selected seedlings were used to determine various enzymatic and non-enzymatic antioxidants. The nutrient contents and physiological indices were assessed using the remaining 5 seedlings in each treatment.

Table 2. Physico-chemical composition of raw clover honey (on a fresh weight basis)

Property/Component	Unit	Value
Osmoprotectants:		
Proline	(mg kg^{-1} FW)	48.22 ± 1.452
Total soluble sugars	(%)	81.90 ± 2.621
Amino acids		0.344 ± 0.010
Sugar fractions:		
Fructose	(%)	43.80 ± 1.112
Glucose		26.14 ± 0.524
Maltose		3.694 ± 0.012
Sucrose		4.182 ± 0.021
Mineral nutrients:		
Potassium (K)	(g kg^{-1} FW)	0.462 ± 0.003
Phosphorus (P)		0.052 ± 0.000
Magnesium (Mg)		0.086 ± 0.001
Calcium (Ca)		0.074 ± 0.001
Sulphur (S)		0.078 ± 0.001
Iron (Fe)	(mg kg^{-1} FW)	0.071 ± 0.001
Manganese (Mn)		8.395 ± 0.058
Zinc (Zn)		5.486 ± 0.042
Copper (Cu)		4.584 ± 0.035
Iodine (I)		80.27 ± 2.122
Sodium (Na)		41.28 ± 1.214
Selenium (Se)		0.947 ± 0.007
Antioxidants and Vitamins:		
Silymarin	(mg kg^{-1} FW)	0.008 ± 0.000
Ascorbic acid (vitamin C)		25.02 ± 0.708
Thiamine (B1)		0.151 ± 0.001
Riboflavin (B2)		0.179 ± 0.001
Niacin (B3)		1.668 ± 0.011
Pantothenic acid (B5)		1.079 ± 0.010
Pyridoxine (B6)		2.302 ± 0.018
Folate (B9)		0.203 ± 0.002
DPPH radical-scavenging activity	(%)	89.84 ± 2.112

Values presented in the table are means (n = 3 for all measures) ± standard error.

2.4. Assessment of growth traits, root activity, and photosynthesis efficiency

Growth traits of *A. nummularia* seedlings were evaluated using shoot system. Seedling shoots were quickly cleaned smoothly using tap water and then deionized water and air-dried. After determination of shoot length and shoot fresh weight, shoot dry weight was evaluated after drying at 70 °C until the weights remained constant.

The procedure detailed in Rehman et al. [27] was applied to assess *A. nummularia* root activity using Na-P buffer (pH 7.0), α-naphthylamine, ρ-aminobenzene sulfonic acid, and $NaNO_2$. SPAD index was evaluated utilizing a Chl meter (SPAD 502, Minolta, Osaka, Japan). A handy PEA Chl fluorometer (Hansatech Instruments Ltd., Kings Lynn, UK) was functioned to measure Chl-fluorescence. The formula Fv/Fm = (Fm – F0)/Fm of Maxwell and Johnson [28] was applied to evaluate Fv/Fm. The procedure of Clark et al. [29] was applied to compute the PI of photosynthesis.

2.5. Stability of membranes and markers of oxidative stress

Leaf tissue RWC was evaluated based on the [30]. procedures. Two cm-diameter discs were prepared from leaf blades (without midribs) and weighed (Fwt) immediately. In the dark for a whole day, the discs were fully saturated in deionized water. Before recording turgid weight (Twt), the surfaces of discs were softly dried from water and the discs were dried for 48 h under 70 °C to record dry weight (Dwt). Then, the following formula was applied:

$$RWC\ (\%) = [(Fwt - Dwt) / (Twt - Dwt)] \times 100$$

Twenty discs were prepared from leaf blades (without midribs) to evaluate total ions leaked from the disc tissues [31]. The discs were exposed to soaking in deionized water to record EC0. Then, EC1 was recorded after 30-min heating (for discs + water) at 45 °C – 55 °C. After boiling for 10 min, EC2 was taken. Then, EL was computed from the following formula:

$$EL\ (\%) = [(EC1 - EC0) / EC2] \times 100$$

Level of MDA [32], and contents of two oxidative stress markers [$O_2^{\bullet-}$ ([33] and H_2O_2 [34]] were determined. Content of MDA (μmol g^{-1} FW) was assessed utilizing 155 mM^{-1} cm^{-1}; the extinction coefficient. H_2O_2 content (μmol g^{-1} FW) was colorimetrically determined at 390 nm and was computed based on convenient standard curves. Fragments (1 × 1 mm, 0.1 g) of samples were prepared to assess content of $O_2^{\bullet-}$ (μmol g^{-1} FW). The fragments were flooded utilizing 10 mmol K-P buffer (pH 7.8). After mixing the buffer with NBT (0.05%) and NaN_3 (10 mM), the mixture was stored for one hour at 25 °C and for 15 min at 85 °C. After rapid cooling, the absorbance was recorded at 580 nm.

2.6. Osmo-regulators contents determinations

Free proline was extracted utilizing toluene solution [35]. The readings of absorbance were recorded at 520 nm and the leaf content of Pro (μg g^{-1} FW) was computed applying convenient standard curves. By applying the professional procedures of Irigoyen et al. [36], ethanol (96%) was utilized to extract soluble sugar and then the content (mg g^{-1} DW) was determine. One hundred microliters of the extract were reacted with 0.15 g anthrone (a reagent prepared, freshly, in 100 mL H_2SO_4, 72%). The mixture was then exposed to 10-min boiling. After cooling, the absorbance was taken at 625 nm. The procedures of Bradford [37] was applied to assess TS protein content (mg g^{-1} DW).

2.7. Antioxidant compounds contents determinations

A 5% solution of HPO_3 (ice-cold) including 1 mM EDTA was utilized for homogenization of leaf tissue to extract AsA. AsA content (μM g^{-1} FW) was determined after exposing the produced homogenates to 20-min centrifugation process at 4,000 × g. The produced supernatants were utilized to evaluate AsA content [38]. The procedures of Yu et al. [39], which supported with a minor modification [40] were applied to determine GSH content (μM g^{-1} FW). GSH standard curves were utilized to compute the content of GSH. Total phenolic (TPh) contents (μM g^{-1} DW) was quantified applying the Folin-Ciocalteu method [41]. Absorbance reading values were taken at 725 nm. TPh contents (mg gallic acid equivalents; GAE g^{-1} DW) were computed from a standard curve prepared using gallic acid. Content of silymarin (Sim) was assessed utilizing the Thermo Fisher Scientific HP 1100 Liquid Chromatograph (Waltham, MA, USA) [42,43]. Leafy samples were extracted in a "Soxhlet apparatus" with 200 ml of CH_3OH for each. Then, extracts were evaporated to dryness and reconstituted in HPLC grade CH_3OH (25 mL). The CH_3OH was then utilized to dilute the reconstituted samples, which were used to quantify the contents of Sim (μg g^{-1} DW).

2.8. Activities of Enzymatic Antioxidants Assays

All following steps were performed at 4 °C. Extracts were prepared for enzymes by homogenizing 200 mg freeze-dried leaves in 2 ml of 0.1 M K-P buffer (pH 7.0) utilizing a cold mortar. The extraction buffer was received an EDTA solution (0.1 mM). To assay activity of APX, the extraction buffer was also received 2 mM AsA. The homogenates were filtered through nylon clothes. Then, the filtrates were exposed to 15-min centrifugation at 12,000 ×g. Each EE was utilized immediately or stored under −25 °C until use.

Activity of SOD (Unit g^{-1} protein) was assessed by determining SOD capacity to inhibit reducing of NBT photochemical [44]. The amount of enzyme required to block 50% NBT photoreduction is equal to 1 unit of SOD activity. All following enzyme activities were expressed as μmol H_2O_2 min^{-1} g^{-1} protein. Activity of CAT was assessed by noting the decrease in absorbance readings at 240 nm as a result of the H_2O_2 degradation (ε=36 M^{-1} cm^{-1}) [45]. Activity of APX was assessed applying the procedures of Nakano and Asada [46], by noting oxidation of AsA that identified as a decrease in absorbance readings at 290 nm (ε=2.8×10^{-3} M^{-1} cm^{-1}). The Foster and Hess [47] procedures were utilized to assay GR activity, which was monitored (at 340 nm for 3 min) as a change in the absorbance of a mixture; 0.1 mL EE, 100 mmol K-P buffer (pH 7.0), 0.1 mmol EDTA, 0.5 mmol NADPH, and 0.1 mmol GSSG.

2.9. Contents of nutrient elements

After drying and powdering, the leaf samples were digested in perchloric acid mixed with nitric acid (1: 3, v/v, respectively), to evaluate leaf content of nutrients. The "micro-Kjeldahl" (Ningbo Medical Instruments Co., Ningbo, China) was utilized to determine N content [48]. The blue-color-method detailed in Jackson [49] was applied to evaluate content of P. In this method, Mo was utilized for reducing molybdo-phosphoric in H_2SO_4 to

exclude As. The Perkin-Elmer, Model 52-A, Flame Photometer (Glenbrook, Stamford, CT, USA) was utilized to assess K^+ and Na^+ contents Page et al. [24]. Ca^{2+}, Zn, Mn, and Fe contents were assessed using an Atomic Absorption Spectrophotometer [50].

2.10. Analysis of data

After testing for error variance homogeneity [51], the tow-way ANOVA was utilized to statistically analyze all data through the GLM procedure of Gen STAT (version 11) (VSN International Ltd., Oxford, UK). Differences among and between means were tested utilizing the LSD test [52] at the 0.05 ($p \leq 0.05$) probability levels.

3. Results

Since all data of the 2020 season match the corresponding data of the 2021 season, the average was processed for the data of the two seasons. This study examined the potential enhancing impacts of foliar spraying with silymarin-enriched bee-honey solution (SBH) on saline-calcareous-stressed *A. nummularia* seedlings.

3.1. Growth traits, root activity, photosynthetic efficiency, and leaf tissue stability

Supplementing the *A. nummularia* seedlings with SBH notably increased shoot length, shoot fresh weight, shoot dry weight, root activity, SPAD index, Fv/Fm, performance index (PI), relative water content (RWC) and membrane stability index (MSI) compared to the control. The increases caused by SBH are shown in Tables 3 and 4.

Table 3. Growth traits and root activity of *Atriplex nummularia* Lindl seedlings treated with silymarin-enriched bee-honey (SBH) under saline calcareous soil conditions. All values are the average of the 2020 and 2021 seasons.

Treatment	Parameters			
	Shoot length (cm)	Shoot fresh weight (kg)	Shoot dry weight (kg)	Root activity (μg α-NA g^{-1} fresh root h^{-1})
Control	63.6±5.2b	3.56±0.29b	1.27±0.11b	78.4±2.2b
SBH	71.4±6.3a	3.98±0.36a	1.41±0.12a	85.7±2.6a
% of control	+12.3	+11.8	+11.0	+9.3

Values are means (±SE). Means followed by different lowercase letters in each column are significantly different according to the LSD test ($p \leq 0.05$). α-NA= α-naphthylamine and SBH= Bee-honey solution (1%) enriched with 200 μg silymarin per L of solution. The samples were taken 75 days after transplantation of 75-day-old transplants.

Table 4. Efficiency of photosynthetic machinery and leaf integrity of *Atriplex nummularia* Lindl seedlings treated with silymarin-enriched bee-honey (SBH) under saline calcareous soil conditions. All values are the average of the 2020 and 2021 seasons.

Treatment	Parameters				
	SPAD index	F_v/F_m	PI (%)	RWC (%)	MSI (%)
Control	50.8±2.0b	0.78±0.02b	16.4±0.32b	77.4±4.0b	65.2±3.4b
SBH	58.1±2.4a	0.84±0.03a	18.4±0.38a	84.6±4.3a	71.8±3.8a
% of control	+14.4	+7.7	+12.2	+9.3	+10.1

Values are means (±SE). Means followed by different lowercase letters in each column are significantly different according to the LSD test ($p \leq 0.05$). SPAD= soil-plant analysis development, Fv/Fm= PSII efficiency; PSII maximum quantum yield, PI= performance index, RWC= relative water content, MSI= membrane stability index, and SBH= Bee-honey solution (1%) enriched with 200 μg silymarin per L of solution. The samples were taken 75 days after transplantation of 75-day-old transplants.

3.2. Oxidative stress biomarkers, oxidative damage, phenolics, and Na^+

Foliar spraying of the *A. nummularia* seedlings with SBH markedly decreased electrolyte leakage (EL), and the contents of malondialdehyde (MDA), hydrogen peroxide (H_2O_2), superoxide ($O_2^{\bullet-}$), total phenolic compounds, and Na^+ compared to the control. The reductions induced by SBH are shown in Table 5.

Table 5. Levels of oxidative stress biomarkers, oxidative damage, phenolics, and Na^+ of *Atriplex nummularia* Lindl seedlings treated with silymarin-enriched bee-honey (SBH) under saline calcareous soil conditions. All values are the average of the 2020 and 2021 seasons.

Treatment	Parameters					
	EL	MDA level	H_2O_2 level	$O_2^{\bullet-}$ level	TPh	Na^+
	%	μmole g^{-1} FW			mg GAE g^{-1} DW	g kg^{-1} DW
Control	23.2±0.6a	1.32±0.03a	4.16±0.06a	2.52±0.04a	10.44±0.18a	41.2±1.8a
SBH	18.2±0.4b	0.91±0.02b	2.74±0.03b	1.88±0.03b	7.88±0.13b	35.7±1.5b
% of control	−21.6	−31.1	−34.1	−25.4	−24.5	−13.3

Values are means (±SE). Means followed by different lowercase letters in each column are significantly different according to the LSD test ($p \leq 0.05$). EL= Electrolyte leakage, MDA= malondialdehyde, H_2O_2= hydrogen peroxide, $O_2^{\bullet-}$= superoxide, and SBH= Bee-honey solution (1%) enriched with 200 μg

silymarin per L of solution. The samples were taken 75 days after transplantation of 75-day-old transplants.

3.3. Osmoregulators and non-enzymatic antioxidants

Supplementation of the *A. nummularia* seedlings with SBH considerably increased the contents of soluble protein, soluble sugars, free proline, ascorbate, glutathione, and Sim compared to the control. The increases stimulated by SBH are shown in Table 6.

Table 6. Osmoregulators and non-enzymatic antioxidant levels of *Atriplex nummularia* Lindl seedlings treated with silymarin-enriched bee-honey (SBH) under saline calcareous soil conditions. All values are the average of the 2020 and 2021 seasons.

Treatment	Parameters					
	Protein	Sugars	Free proline	AsA	GSH	Sim
	g kg^{-1} DW		µM g^{-1} DW	µM g^{-1} FW		mg kg^{-1} DW
Control	101.6±4.5b	15.2±0.2c	122.2±2.2c	1.38±0.02c	0.74±0.01c	10.2±0.5c
SBH	178.9±6.0a	24.4±0.3b	172.8±2.9b	1.89±0.03b	0.96±0.02b	28.2±1.3b
% of control	+76.1	+60.5	+41.4	+37.0	+29.7	+176.5

Values are means (±SE). Means followed by different lowercase letters in each column are significantly different according to the LSD test ($p \leq 0.05$). TPh= Total phenolic compounds, TS sugars= total soluble sugars, AsA= Ascorbate, GSH= Glutathione, Sim= Silymarin, and SBH= Bee-honey solution (1%) enriched with 200 µg silymarin per L of solution. The samples were taken 75 days after transplantation of 75-day-old transplants.

3.4. Enzymatic antioxidants

Providing the *A. nummularia* seedlings with SBH significantly increased the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) compared to the control. The increases of enzyme activities stimulated by SBH are shown in Table 7.

Table 7. Activities of antioxidant enzymes of *Atriplex nummularia* Lindl seedlings treated with silymarin-enriched bee-honey (SBH) under saline calcareous soil conditions. All values are the average of the 2020 and 2021 seasons.

Treatment	Parameters			
	SOD	CAT	APX	GR
	Unit g^{-1} protein	µmol H_2O_2 min^{-1} g^{-1} protein		
Control	35.4±0.5c	18.5±0.4c	21.6±0.4c	15.4±0.2c
SBH	46.8±0.7b	24.8±0.6b	31.1±0.7b	19.4±0.3b
% of control	+32.2	+34.1	+44.0	+26.0

Values are means (±SE). Means followed by different lowercase letters in each column are significantly different according to the LSD test ($p \leq 0.05$). SOD= Superoxide dismutase, CAT= Catalase, POD= Peroxidase, APX= Ascorbate peroxidase, GR= Glutathione reductase, and SBH= Bee-honey solution (1%) enriched with 200 µg silymarin per L of solution. The samples were taken 75 days after transplantation of 75-day-old transplants.

3.5. Nutritional status

Foliar spraying of the *A. nummularia* seedlings with SBH noticeably increased the contents of macro- and micronutrients (N, P, K^+, Fe, Mn, Zn, and Ca^{2+}) compared to the control. The increments caused by SBH are shown in Table 8.

Table 8. Nutritional status of *Atriplex nummularia* Lindl seedlings treated with silymarin-enriched bee-honey (SBH) under saline calcareous soil conditions. All values are the average of the 2020 and 2021 seasons.

Treatment	Parameters						
	N	P	K	Fe	Mn	Zn	Ca^{2+}
	g kg^{-1} DW			mg kg^{-1} DW			g kg^{-1} DW
Control	18.2±0.6c	1.98±0.08c	19.4±0.7c	792±16c	494±10c	291±6c	4.42±0.36d
SBH	23.4±0.8b	2.50±0.14b	25.6±1.0b	890±20b	564±14b	352±9b	5.28±0.44c
% of control	+28.6	+26.3	+32.0	+12.4	+14.2	+21.0	+19.5

Values are means (±SE). Means followed by different lowercase letters in each column are significantly different according to the LSD test ($p \leq 0.05$). DW= Dry weight, N= Nitrogen, P= Phosphorus, K= Potassium, Fe= Iron, Mn= Manganese, Zn= Zinc, Ca^{2+}= Calcium, Na^+= Sodium, and SBH= Bee-honey solution (1%) enriched with 200 µg silymarin per L of solution. The samples were taken 75 days after transplantation of 75-day-old transplants.

4. Discussion

To maintain adequate growth factors like fresh water and essential nutrients for crop growth, growth requirements must be secured. In this study, *Atriplex nummularia* seedlings were grown in defective (saline calcareous) soil and faced some stresses. Among them, salinity (EC = 8.16 dS m^{-1}), osmotic

stress, physiological drought (FC = 11.6%), high pH (8.19), high $CaCO_3$ content (32.8%), low CEC (4.62 meq 100^{-1} g soil), nutrient deficiency, etc. These stresses certainly make the soil less productive. Accordingly, to get a suitable and satisfactory crop production from this tested defective soil, a salt-tolerant plant such as *A. nummularia* should be used and treated (leafy spraying) with a biostimulant, especially the tested SBH (1% bee-honey solution enriched with 200 µg Sim per L of solution). Biostimulants have been used successfully to overcome many stresses and have been documented as effective treatments for stressed plants [11,12,53,54].

By foliar spraying of *A. nummularia* with SBH, an economic productivity (biomass as a forage yield) could be obtained under saline-calcareous soil conditions. No information is available on the leafy treatment of *A. nummularia* seedlings with SBH to mitigate the harmful impacts of the saline-calcareous stress on plant performance. Lately, a number of articles have documented positive alterations in growth, physio-biochemical attributes, and defense systems in some crop plants after being treated with SBH under stress [17,55,56]. These works signalize the noteworthiness of plant biostimulators, especially those enriched with Sim. However, this study contains impressive findings, including a noticeable increase in the performance of *A. nummularia* plant (a forage yield) and defensive systems along with nutritional balance due to the application of SBH to the plant. In this study, efforts were made to obtain a large growth of *A. nummularia* (a forage yield) to maintain sustainable agricultural development and animal production under afflicted soils.

The soil stress under study considerably affect the performances and returns of plants through affecting different metabolic processes by stimulating abnormal generation of reactive oxygen species (ROS). Under these stresses, plants attempt to avert damage through stimulating different specific strategic mechanisms such as ionic homeostasis, osmotic adaptation, and adoption of different components of their antioxidant defenses [15–17,55–59]. Consistent with Abou-Sreea et al. [17] and Alharby et al. [16] findings, our study indicated the efficacy of SBH in alleviating the saline-calcareous-stress effects in *A. nummularia* plant due to their richness in various plant growth stimulators and anti-stressors such as various essential nutrients, osmo-regulators, antioxidants, vitamins, and plant hormones as efficient mechanisms. Besides, the high antioxidant activity of SBH (89.8%) due to its richness in various antioxidants conferred pivotal mechanisms to prevent, totally or partially, the ROS production and oxidative stress damage and thus decreasing the lipid peroxidation in cell membranes, increasing tissue cell turgor and integrity, and elevating membrane stabilities in plant cells under the stress tested [60–64]. These positive findings were conferred due to the repaired antioxidant defense mechanisms, and ionic balance, all of which repaired the photosynthetic machinery and thus the satisfactory performance of the *A. nummularia* plant. As a pivotal mechanism, the presence of Sim as a good enrichment perfected the efficacy of the tested SBH as early reported by Abou-Sreea et al. [17] and Alharby et al. [16]. They explored an effective role of Sim as a conducive metabolite to support the SBH, in this study, in elevating the antioxidant defense mechanisms of the *A. nummularia* seedlings and thus elevating its stress-resistance and its performance under the saline-calcareous-stress tested.

Our findings signalize that all the vital components present in SBH, including the Sim contributed efficiently to the improvement of the *A. nummularia* seedling ability to adapt well to multi-stress and withstand stress harmful conditions through optimizing different plant metabolic processes, cellular division and elongation, and biomass accumulation, allowing plants to perform well [16,17,65,66]. The noticeable enhanced growth of the *A. nummularia* seedling (length and dry weight of shoots) under soil multi-stress by foliar spray with SBH may be attributed to the increased root activity as an effective mechanism to increase root uptake of water and nutrients from the defective soil tested. Plant roots can perceive the soil physicochemical constraints and correspondingly modify their development, so that they can maintain plant nutritional and signaling functions under abiotic stress. In this concern, Ghosh and Xu [67] documented a proteomic analysis on stressed plant roots that revealed molecular and cellular mechanisms specific to multi-stress conditions in which transmembrane water and/or ion channel proteins are available in abundance, signalizing alterations in ionic and/or osmotic balance. Ghosh and Xu [67] added that multi-stress conditions stimulate higher levels of proteins implicated in the primary root metabolism, indicating promoted energy demand during stress conditions. These affirmative influences are conferred by different signaling pathways that affect plant adaptive responses to stress (e.g., cell turgor and integrity, membranes stabilities, osmotic adaptation, and different antioxidant mechanisms) along with gene regulation and expression, contributing to tolerance to stress and thus the efficiency of photosynthesis machinery and plant growth [16,17,56,68–70]. In SBH-treated *A. nummularia* seedling, induction of metabolic pathways integrated with phenylpropanoid synthesis may elucidate the mitigation of stress influences in the plant [71]. Lemoine et al. [72] demonstrated that the formation of the cell protoplasm is promoted by vital organic nutrients, amino acids including proline, and sugars, which are components of SBH used in this study. These bio-stimulators also contain plant hormones, including IAA, GA_3, and CKs, which stimulate rapid cellular division, elongation, and multiplication [12,59,73]. Additionally, the high antioxidant capacity along with Sim endows the *A. nummularia* seedling with additional antioxidant capacity as a pivotal mechanism to mitigate the multi-stress impacts via regulating the plant's adaptive responses to the multi-stress tested, reflecting efficient plant growth and development. All these are in agreement with Abou-Sreea et al. [17] and Alharby et al. [16].

The noticeable reinforcement of photosynthesis indices (e.g., SPAD, PI, and Fv/Fm) by SBH added more photosynthetic substances (e.g., sugars, amino acids including proline, etc.), in addition to those that penetrated plant leaves by SBH application to offer some pivotal mechanisms. These increased photosynthetic substances can be contributed to osmotic adaptation, cell integrity, and enhanced relative water content (RWC) and membrane stability index (MSI), which all contributed to lower oxidative stress markers. These positive findings, including SPAD chlorophyll, have delayed leaf senescence (data not shown) in favor of a longer period of healthy photosynthesis. As a consequence, the metabolism of the plant was reinforced by the SBH treatment due to its protective influences on different systems of photosynthesis machinery under the multi-stress tested in this study. In addition, a vigorous correlation was found in this study between the reinforced antioxidants and osmo-regulators and the ability of stressful *A. nummularia* plants to survive. This finding is in agreement with previous reports [57,74,75].

In an integration with sugars and protein, this investigation confirmed the action of proline as an efficient ROS-scavenger, contributing to adjusting the osmosis and protecting enzymes. Together with the proline mechanism, Rosa et al. [68] and Desoky et al. [9] also stated that sugars contribute greatly to mitigate the stress noticeably by adjusting the osmosis, signalizing a pivotal mechanism for plant adaptations under stress conditions. higher contents of the osmo-regulators in the *A. nummularia* seedling by SBH treatment enhanced photosynthetic pigments to stimulate carbohydrates metabolism, which creates new strong relationships between sources and elevating accumulation of dry matter to mitigate stress conditions [74,76]. The findings we have found signalize that the *A. nummularia* plant water status depends mostly on root activity and shoot biomass. Therefore, a plant that has higher root activity and shoot biomass can uptake and keep higher water in favor of tolerance to the multi-stress under study. As a convenient signal, the RWC vs. shoot biomass can be utilized to differentiate the non-specific and specific characteristics for more tolerance to multi-stress in the *A. nummularia* seedlings [77]. This investigation signalizes a vigorous relationship between the accumulation of the shoot biomass and the leaf tissue RWC under multi-stress due to SBH treatments.

The oxidative stress markers (H_2O_2 and $O_2^{\bullet-}$ levels) and their consequences (MDA level and EL) were noticeably decreased by the SBH treatment in favor of growth and photosynthetic efficiency of the *A. nummularia* seedling. To scavenge H_2O_2 and $O_2^{\bullet-}$ and minimize EL and MDA level, the activities of different antioxidants as important mechanisms were reinforced under multi-stress conditions and further reinforced by the SBH treatment. The high levels of AsA, GSH, TPh compounds, and Sim under multi-stress conditions help the *A. nummularia* seedling withstand the examined multi-stress as vigorous mechanisms along with other pivotal mechanisms via other bioactive plant ingredients, morpho-structural alterations and elevated secondary metabolites. The high levels of non-enzymatic antioxidants were reached in parallel with the reinforcement in the activities of antioxidant enzymes (SOD, CAT, APX, and GR) in the *A. nummularia* seedling treated with SBH under both stress-free and stress conditions. The reinforced activities of different antioxidants conferred the plant more antioxidative capacity (89.8%) to withstand the multi-stress influences in the plant through scavenging ROS; $O_2^{\bullet-}$ (by SOD as the first defense line and H_2O_2 (by CAT, POD, or APX; [78]. Abou-Sreea et al. [17] and Alharby et al. [16] confirm the findings we reached.

As a pivotal mechanism, the noticeable increase in the *A. nummularia* root activity under multi-stress by the SBH treatment helped increase in the nutrient absorption (N, P, K, Ca, Fe, Mn, and Zn) by roots along with reduced uptake of Na^+ ions. The *A. nummularia* seedling produced from the defective (saline-calcareous) soil has many advantages if it is used for animal feeding. It has a high nutritional value; high proteins (about 22.0%), sugars (about 3.2%), amino acids, vitamins C, and essential nutrients (e.g., N, P, K, Fe, Mn, Zn, and Ca), while TPh compounds, and Na^+ ions are less.

5. Conclusions

The findings of the current study indicate that foliar treatment of *Atriplex nummularia* with a novel bio-stimulator (1% bee-honey solution enriched with 200 µg silymarin per L of solution) increased plant tolerance, suppressed saline-calcareous-stress influences, and enhanced *Atriplex nummularia* seedling productivity. The findings indicate that the exploitation of a bee-honey solution enriched with silymarin, as a promising safe and eco-friendly strategy, displayed many advantages through suppressing the influences of soil stress, and raising the productivity of *Atriplex nummularia*. Instead of chemicals, the use of natural bio-stimulators, such as a silymarin-enriched bee-honey solution to suppress the stress influences still needs further research to explore its precise mechanisms with the aim of raising its efficiency to convince farmers to use it.

Author Contributions

Conceived and designed the paper: MMR, DAMMT, and SMAA. Analyzed the data: MMR, DAMMT, SMAA, and IAAM. Contributed reagents//analysis tools: DAMMT. Wrote the paper: MMR, DAMMT, SMAA, and IAAM. Revised the paper: MMR, SMAA, and IAAM. All authors read and approved the final manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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