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Attenuation of saline-calcareous stress in *Atriplex nummularia* seedlings by treating the soil with an acidified compost

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ABSTRACT

Saline-calcareous soils induce many stresses in plants when grown on them, including salinity, high carbonate, and limitation of water and nutrients, all of which severely damage plants that may die. Attenuating these stresses is a challenge for human and animal feeding sustainability. During 2020 and 2021, the potential promoting impacts of treating saline-calcareous soil with an acidified leguminous compost (AcC) on plant growth, physiological and biochemical traits, antioxidant systems, and nutritional value of *Atriplex nummularia* seedlings were investigated. Treating the tested saline-calcareous soil with AcC at a rate of 2 kg per m^2 significantly improved *A. nummularia* 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 AcC for producing satisfactory plant productivities under saline-calcareous soil conditions.

1. Introduction

In Egypt, the soils cultivated with human food crops are not enough for human food security requirements. Thus, to increase the area of cultivation of crops for animal feed, it is necessary to reclaim new arable soils with tolerant forage plants and to apply effective fertilization of these soils. Sustainability of crop productivity is performed essentially by attenuating various stresses, including high $CaCO_3$ and salinity. Cultivation of saline-calcareous 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 [3,4]. 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 [1,4]. These undesirable conditions inhibit plant growth and production via overproducing ROS, negatively influencing physio-biochemical attributes, osmoregulation, and antioxidant defense systems [1,5]. On the other side, 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 these soils with a suitable fertilization program (a highly efficient compost)[1,11].

Owing to its various benefits, leguminous compost (LC) can be utilized to repair damaged soils [12–14]. As an easy-to-use and cost-effective nutrient resource, LC can repair defective soils by enhancing fertility, availability and uptake of water and nutrients, as well as chelation ability [15–17]. Humic and sulfuric acids, acidic substances, contribute to repair the reclaimable soils and improve their physicochemical characteristics, which are reflected in the satisfactory production of crops [1,18]. Acidified compost (AcC) is obtained by combining the acidic substances with LC. The importance of AcC is because it has low pH and EC, and high contents of organic matter and nutrients. AcC is more efficient in overcoming saline-calcareous soil stress conditions, reflecting ameliorated plant growth and production through suppressing ROS and improving physio-biochemical indices, nutrient status,

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and plant antioxidant systems [14,19].

A. nummularia Lindl is a halophyte and animal feed [20,21]. This plant is more suitable to grow under severe soil conditions but loses more of its production and quality [22–25]. But by treating *A. nummularia* with SBH, it can produce a high ratio of forage (dry matter), providing forage for livestock efficiently.

No information is available on the role of AcC application to saline-calcareous soil in minimizing the adverse effects of soil stress on the quantity and quality of forage yield of *A. nummularia*. This research work hypothesized that the application of AcC on the tested saline-calcareous soil would efficiently enhance soil properties and the growth and productivity of *A. nummularia*. Therefore, the aim of the study was to explore the enhancing influences of saline-calcareous soil treatment with AcC at a rate of 2 kg per m^2 on soil characteristics, *A. nummularia* growth, photosynthetic machinery, membrane integrity, oxidative stress markers, osmotic regulators, antioxidant activities, and nutritional status under the soil adverse conditions.

2. Materials and Methods

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

Standard *Atriplex nummularia* Lindl transplants (75 days old) 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, soil samples were collected at 20-cm depth to analyze soil properties applying the Page et al. [26] and Klute and Dirksen [27] procedures (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	The soil texture class is Loamy Sand
Silt		8.10 ±0.81	
Clay		8.70 ±0.84	
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^+	mg kg^{-1}	24.2 ±2.15	
P		3.24 ±0.24	
N		11.4±1.04	

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

The analysis shows that the soil ECe and $CaCO_3$ content were 8.16 dS m^{-1} and 32.8%, respectively, indicating that this soil is classified as saline calcareous according to Dahnke and Whitney [28] and Soil Survey Division Staff [29], respectively. When plant samples were taken for analysis (90 days after transplantation), the soil samples were taken also at the same time to determine the physicochemical properties of the soil treated with AcC (Table 2).

Table 2. Physico-chemical properties of the experimental saline calcareous soil (from 0–20 cm depth) treated with acidified leguminous compost (+AcC) compared to untreated soil (–AcC). All values are the average of the 2020 and 2021 seasons.

Treatment	pH	ECe	CEC	FC (%)	OM (%)
		dS m^{-1}	meq 100^{-1} g soil	%	
Control	8.19 ±0.70[a]	8.16 ±0.74[a]	4.62±0.38[b]	11.6±1.10[b]	0.52 ±0.02[b]
+AcC	7.29 ±0.66[b]	7.30 ±0.71[b]	9.44±0.78[a]	23.8±2.19[a]	0.61 ±0.02[a]
% of control	−10.99	−10.54	+104.3	+105.2	+17.31
	$CaCO_3$	Na^+	Ca^{2+}	K^+	P
	%	meq L^{-1}		mg kg^{-1}	
Control	32.8 ±2.74[a]	64.3 ±5.48[a]	25.0 ±2.10[a]	24.2 ±2.15[b]	3.24 ±0.24[b]
+AcC	30.2 ±2.55[b]	56.4 ±4.92[b]	23.2 ±2.04[b]	42.4 ±2.30[a]	9.10 ±0.33[a]
% of control	−7.93	−12.29	−7.20	+75.21	+180.9
	N	Fe	Mn	Zn	Cu
	mg kg^{-1}				
Control	11.4±1.04[b]	3.10 ±0.31[b]	2.08 ±0.18[b]	1.42 ±0.10[b]	1.11 ±0.08[b]
+AcC	33.2±1.11[a]	3.44 ±0.29[a]	2.32 ±0.19[a]	1.79 ±0.12[a]	1.29 ±0.09[a]
% of control	+191.2	+10.97	+11.54	+26.10	+16.22

Values are means (±SE). * and ** indicate significant differences at $p \leq 0.05$ and $p \leq 0.01$ probability levels, respectively, and NS= not significant. Means followed by different lowercase letters in each column are significantly different according to the LSD test ($p \leq 0.05$). pH was measured in soil paste at 25°C, ECe= Electrical conductivity in soil paste extract at 25°C, OM= Organic matter, FC= field capacity of soil, CEC= Cation exchange capacity, 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 [25]. The experimental area was divided into 6 plots for three replicates for both control and acidified leguminous compost (AcC) 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 $hill^{-1}$.

The leguminous compost (LC) was prepared according to the detailed procedures of Abdelfattah et al. [14] and Bamagoos et al. [13] with a slight modification (the whole faba bean plants were utilized instead of plant shoots) made by Alghamdi et al. [25]. Whole green faba bean plants were collected after harvesting the green pods, and the roots were cleaned well with tap water while preserving the root nodules as much as possible. 25.0 kg of faba bean plants (50.0% of the mixture) were mixed well with 500 g of bulking agents (0.5 %), 12.0 kg of cattle manures (24.0 %), 500 g of potassium humate (0.5 %), and 12.0 kg of shoots of Egyptian alfalfa (24.0 %). After that, composting was done (started on September 15th and ended on March 15th) in a factory applying the turning-pile system (the dimensions of the pile mixture were 5.0 × 20.0 × 7.5 m; height × length × wide, respectively). The pile was turned over once every two weeks, during the bio-oxidation phase, keeping the moisture level at 40–60% and noting the pile temperature inside the pile. Then, the produced LC was mixed well with humic acid at a rate of 2% and sulfuric acid (20%) at a rate of 2 mL per kg of LC to produce the acidified leguminous compost (AcC). The AcC was prepared in two seasons separately by the same method and under the same controlled conditions resulting in the production of AcC with approximately the same ingredients and proportions. The LC and AcC were analyzed for their chemical characteristics (Table 3). After the application of the AcC, the soil was left two weeks before the *A. nummularia* transplantation with regular watering.

Table 3. Chemical analysis of both normal and acidified leguminous composts

Compost type	pH	EC	OM	K	P	N	Fe	Mn	Zn	Cu
		dS m^{-1}	%	g kg^{-1}						
Normal LC	7.54	2.13	19.4	151	32.8	112	8.10	6.64	3.12	2.82
Acidified LC (AcC)	6.58	1.84	21.8	174	38.1	144	11.3	8.14	4.20	3.12

LC= Leguminous compost, AcC= Acidified leguminous compost, EC= Electrical conductivity, and OM= Organic matter.

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. Plant 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.

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. [30] 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 [31] was applied to evaluate Fv/Fm. The procedure of Clark et al. [32] 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 Osman and Rady [33] 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 [34]. 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 [35], and contents of two oxidative stress markers [$O_2^{\bullet-}$ [36] and H_2O_2 [37]] 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 [38]. 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. [39], 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 [40] 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 [41](Huang et al., 2005). The procedures of Yu et al. [42], which supported with a minor modification [43] 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 [44]. 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.

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 [45]. 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}) [46]. Activity of APX was assessed applying the procedures of Nakano and Asada [47], by noting oxidation of AsA that identified as a decrease in absorbance readings at 290 nm ($\varepsilon=2.8\times10^{-3}$ M^{-1} cm^{-1}). The Foster and Hess [48] 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 [49]. The blue-color-method detailed in Jackson [50] 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 [26]. Ca^{2+}, Zn, Mn, and Fe contents were assessed using an Atomic Absorption Spectrophotometer [51].

2.10. Analysis of data

After testing for error variance homogeneity [52], 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 [53] 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 saline-calcareous soil treatment with acidified leguminous compost (AcC) on soil characteristics and stressed *A. nummularia* seedlings.

3.1. Soil chatacteristics

The data in Table 3 show that, compared with the control, the pH, ECe, and the soil contents of $CaCO_3$, Na^+, and Ca^{2+} were reduced by 11.0, 10.5, 7.93, 12.3, and 7.20%, respectively, while the soil CEC, FC, and OM content were increased by 104.3, 105.2, and 17.3%, respectively. The soil contents of

K^+, P, N, Fe, Mn, Zn, and Cu were also increased by 75.2, 180.9, 191.2, 11.0, 11.5, 26.1, and 16.2%, respectively.

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

Supplementing the tested saline-calcareous soil with AcC at a rate of 2 kg per m^2 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) of *A. nummularia* seedlings compared to the control. The increases caused by AcC are shown in Tables 4 and 5.

Table 4. Growth traits and root activity of *Atriplex nummularia* Lindl seedlings under saline calcareous soil conditions treated with acidified compost. 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±5.2^b	3.56±0.29^b	1.27±0.11^b	78.4±2.2^b
+AcC	70.3±6.2^a	3.91±0.34^a	1.39±0.12^a	85.2±2.5^a
% of control	+10.5	+9.8	+9.4	+8.7

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 AcC= Acidified compost applied at a level of 2 kg per m^2 of soil. The samples were taken 75 days after transplantation of 75-day-old transplants.

Table 5. Efficiency of photosynthetic machinery and leaf integrity of *Atriplex nummularia* Lindl seedlings under saline calcareous soil conditions treated with acidified compost. 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±2.0^b	0.78±0.02^b	16.4±0.32^b	77.4±4.0^b	65.2±3.4^b
+AcC	57.2±2.2^a	0.82±0.02^a	18.4±0.34^a	83.7±4.2^a	70.6±3.6^a
% of control	+12.6	+5.1	+12.2	+8.1	+8.3

Values are means (±SE). Means followed by different lowercase letters in each column are significantly different according to the LSD test ($p \leq 0.05$). 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 AcC= Acidified compost applied at a level of 2 kg per m^2 of soil. The samples were taken 75 days after transplantation of 75-day-old transplants.

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

Soil (saline-calcareous) supplementation with AcC at a rate of 2 kg per m^2 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^+ in *A. nummularia* seedlings compared to the control. The reductions induced by AcC are shown in Table 6.

Table 6. Levels of oxidative stress biomarkers, oxidative damage, phenolics, and Na^+ of *Atriplex nummularia* Lindl seedlings under saline calcareous soil conditions treated with acidified compost. 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±0.6^a	1.32±0.03^a	4.16±0.06^a	2.52±0.04^a	10.44±0.18^a	41.2±1.8^a
+AcC	18.8±0.5^b	0.92±0.02^b	2.78±0.04^b	1.94±0.03^b	7.82±0.12^b	28.4±1.1^b
% of control	−19.0	−30.3	−33.2	−23.0	−25.1	−31.1

Values are means (±SE). Means followed by different lowercase letters in each column are significantly different according to the LSD test ($p \leq 0.05$). EL= Electrolyte leakage, MDA= malondialdehyde, H_2O_2= hydrogen peroxide, $O_2^{\bullet-}$= superoxide, and AcC= Acidified compost applied at a level of 2 kg per m^2 of soil. The samples were taken 75 days after transplantation of 75-day-old transplants.

3.4. Osmoregulators and non-enzymatic antioxidants

Supplementation of the tested saline-calcareous soil with AcC considerably increased the contents of soluble protein, soluble sugars, free proline, ascorbate, and glutathione of *A. nummularia* seedlings compared to the control. The increases stimulated by AcC are shown in Table 7.

Table 7. Osmoregulators and non-enzymatic antioxidant levels of *Atriplex nummularia* Lindl seedlings under saline calcareous soil conditions treated with acidified compost. All values are the average of the 2020 and 2021 seasons.

Treatment	Parameters				
	Soluble protein	TS sugars	Free proline	AsA	GSH
	g kg^{-1} DW		µM g^{-1} DW	µM g^{-1} FW	
Control	101.6±4.5^{b}	15.2±0.2^{b}	122.2±2.2^{b}	1.38±0.02^{b}	0.74±0.01^{b}
+AcC	178.2±5.9^{a}	23.8±0.3^{a}	170.8±2.6^{a}	1.87±0.04^{a}	0.94±0.02^{a}
% of control	+75.4	+56.6	+39.8	+35.5	+27.0

Values are means (±SE). Means followed by different lowercase letters in each column are significantly different according to the LSD test ($p \leq 0.05$). TPh= Total phenolic compounds, TS sugars= total soluble sugars, AsA= Ascorbate, GSH= Glutathione, Sim= Silymarin, and AcC= Acidified compost applied at a level of 2 kg per m^2 of soil. The samples were taken 75 days after transplantation of 75-day-old transplants.

3.5. Enzymatic antioxidants

Providing the tested saline-calcareous soil with AcC significantly increased the activities of superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX), and glutathione reductase (GR) in *A. nummularia* seedlings compared to the control. The increases of enzyme activities stimulated by AcC are shown in Table 8.

Table 8. Activities of antioxidant enzymes of *Atriplex nummularia* Lindl seedlings under saline calcareous soil conditions treated with acidified compost. 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±0.5^{b}	18.5±0.4^{b}	21.6±0.4^{b}	15.4±0.2^{b}
+AcC	46.5±0.7^{a}	24.3±0.5^{a}	30.6±0.6^{a}	19.1±0.2^{a}
% of control	+31.4	+31.4	+41.7	+24.0

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 AcC= Acidified compost applied at a level of 2 kg per m^2 of soil. The samples were taken 75 days after transplantation of 75-day-old transplants.

3.6. Nutritional status

Supplementation of the tested saline-calcareous soil AcC noticeably increased the contents of macro- and micronutrients (N, P, K^+, Fe, Mn, Zn, and Ca^{2+}) of *A. nummularia* seedlings compared to the control. The increments caused by AcC are shown in Table 9.

Table 9. Nutritional status of *Atriplex nummularia* Lindl seedlings under saline calcareous soil conditions treated with acidified compost. 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±0.6^{b}	1.98±0.08^{b}	19.4±0.7^{b}	792±16^{b}	494±10^{b}	291±6^{b}	4.42±0.36^{b}
+AcC	22.9±0.8^{a}	2.42±0.11^{a}	25.6±0.9^{a}	882±18^{a}	553±10^{a}	342±8^{a}	5.62±0.48^{a}
% of control	+25.8	+22.2	+32.0	+11.4	+11.9	+17.5	+27.1

Values are means (±SE). Means followed by different lowercase letters in each column are significantly different according to the LSD test ($p \leq 0.05$). DW= Dry weight, N= Nitrogen, P= Phosphorus, K= Potassium, Fe= Iron, Mn= Manganese, Zn= Zinc, Ca^{2+}= Calcium, Na^+= Sodium, and AcC= Acidified compost applied at a level of 2 kg per m^2 of soil. The samples were taken 75 days after transplantation of 75-day-old transplants.

4. Discussion

To maintain an adequate amount of fresh water and essential nutrients as essential growth factors a requirement for crop growth, the soil, and exploited resources should be effectively managed. In this study, *Atriplex nummularia* seedlings were grown in defective (saline calcareous) soil and faced many stresses on such soil. Among these stresses, 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 or unproductive. Accordingly, to get suitable and satisfactory crop productivity from this tested saline-calcareous soil, a salt-tolerant plant such as *A. nummularia* should be used along with treating this soil with effective organic manure or compost.

The AcC, used in this study, is a leguminous compost; LC [14] supplemented with acidic substances (humic acid + sulfuric acid). As it has the potential to reclaim the defective soil tested, the AcC has 6.58, 1.84 dS m^{-1}, and 21.8% for pH, EC, and OM, respectively. It is also rich in various nutrients (e.g., 174, 38.1, 144, 11.3, 8.14, 4.20, and 3.12 g kg^{-1} for K, P, N, Fe, Mn, Zn, and Cu, respectively) (Table 3). Compared with the addition of 2 kg of LC per m^2 of the tested soil, the addition of 2 kg of AcC per m^2 was better and made the soil productive (Table 3). After the addition of AcC to the tested soil, the crucial mechanism responsible for lowering the soil pH to 6.58 is attributed to humic and sulfuric acids, causing the accumulation of active organic acids in the soil. As earlier stated [12], this finding helps increase cation exchange caacity (CEC) and field capacity (FC) due to CEC of humate. In addition, the reduction in soil ECe is possibly due to the presence of charged groups (i.e., COO-), which enable humate to retain cations by chelating them into inactive forms as another mechanism [12,17]. Another mechanism, the lower EC of the AcC (1.84 dS m^{-1}) could contribute to the lower ECe of the soil. Also, sulfuric acid in AcC was beneficial for the tested defective (alkaline) soil to decrease soil pH and $CaCO_3$ and Na^+ ion contents. It provides the plants with SO_4, and make nutrients more available [54,55], aiding in the reclamation of the soil under study. The findings of this study also show that soil OM content was increased considerably (from 0.52 to 0.61%) after adding AcC, which contributed to the increase of different available soil nutrients (Table 3). These positive findings are probably due to the richness of the AcC in various nutrients, the acids found in the AcC that could reduce soil pH, the medium and useful micropores creation between simple packing soil particles, and thus the increase in soil nutrients, capillary potential, all of which could be attributed to the increased FC and available water content [12,55]. The increase in available soil nutrients possibly due to the release of AcC nutrients into the soil by microbial degradation [12,56]. In this study, all of the enhancing influences of the AcC on soil properties led, at least in part, to the reclamation of the soil under study and the maintenance of a suitable growth medium as possible in favor of the productivity and immune system of *A. nummularia*. Therefore, this study documented that treating the defective soil tested with AcC offers many crucial mechanisms that lead to a positive modification of soil properties in favor of *A. nummularia* growth and productivity.

By treating the tested saline-calcareous soil with efficient compost (e.g., acidified leguminous compost; AcC), economic productivity (biomass as a forage yield) could be obtained under the adverse conditions of this soil. No information is available on the treatment of saline-calcareous soil with AcC to mitigate the harmful impacts of the soil 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 the soil is treated with compost or AcC under stress [12,14,17,19]. These works signalize the noteworthiness of composts, especially the AcC [14]. However, this study contains impressive findings, including a noticeable increase in *A. nummularia* performance (a forage yield) and defensive systems along with nutritional balance due to the application of soil with AcC. 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 defective 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 [16,57–63]. Consistent with Abdelfattah et al. [14] findings, our study indicated the efficacy of AcC in alleviating the saline-calcareous-stress effects in *A. nummularia* plant due to its richness in organic matter (OM) and many essential nutrients such as N, P, K, Fe, Mn, Zn, and Cu. Besides, the AcC's low salt content (1.84 dS m^{-1}) and low pH (6.58). These distinct characteristics of AcC could contribute to preventing, totally or partially, ROS production and oxidative stress damage and thus decreasing lipid peroxidation in cell membranes, increasing cell turgor and integrity, and elevating membrane stability in *A. nummularia* cells under the stress tested [14]. 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.

The noticeable enhanced growth of the *A. nummularia* seedling (length and dry weight of shoots) under soil multi-stress by treating the soil with AcC 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 [64] 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. They 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 [9,58,60,61,65,66].

The noticeable reinforcement of photosynthesis indices (e.g., SPAD, PI, and Fv/Fm) by AcC added more photosynthetic substances (e.g., sugars, amino acids including proline, etc.) 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 soil treatment with AcC due to its contribution to 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 [14,57].

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. [65] and Desoky et al. [67] 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 soil treatment with AcC contributed to enhancing photosynthetic pigments to stimulate carbohydrates metabolism, which creates new strong relationships between sources and elevating accumulation of dry matter to mitigate stress conditions [13,68–70]. 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 [71]. This investigation signalizes a vigorous relationship between the accumulation of the shoot biomass and the leaf tissue RWC under multi-stress due to soil treatment with AcC.

The oxidative stress markers (H_2O_2 and $O_2^{\bullet-}$ levels) and their consequences (MDA level and EL) were noticeably decreased due to the soil treatment with AcC 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 soil treatment with AcC. The high levels of AsA, GSH, and TPh compounds under multi-stress conditions help the *A. nummularia* seedlings 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* seedlings grown in the tested saline-calcareous soil treated with AcC under stress conditions. The reinforced activities of different antioxidants conferred the plant more antioxidative capacity 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 [72–75]. Abou-Sreea et al. [58] and Alharby et al. [61] confirm the findings we reached by soil treatment with AcC.

As a pivotal mechanism, the noticeable increase in the *A. nummularia* root activity under multi-stress by the soil treatment with AcC 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 soil treatment with acidified leguminous compost increased *Atriplex nummularia* seedling tolerance, suppressed saline-calcareous-stress influences, and enhanced seedling productivity. The findings indicate that the application of acidified leguminous compost, as a promising nutrient-source, 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 acidified composts, such as acidified leguminous compost 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 Contribution
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, IAA, and SMAA. 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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