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# Growth Responses, Physiological Alterations and Alleviation of Salinity Stress in Sunflower (*Helianthus annuus* L.) Amended with Gypsum and Composted Cow Dung

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**Abstract:** Salt accumulation in soils poses severe challenges for crop production in arid and semi-arid regions. Scarcity of rainfall and a high evaporation rate in these regions are considered major reasons for salt accumulation. It drastically reduces the leaching of excessive salts below the root zone of crops. The toxic effects of salts on plants can be greatly reduced with the use of biological and inorganic amendments. The present study was conducted to investigate the positive influence of gypsum (GP), composted cow dung (CCD) and the combined use of gypsum and composted cow dung (GP+CCD) on the growth, seed yield, and physiological and chemical attributes of sunflowers (*Helianthus annuus*) in salty soil conditions. Saline-sodic soil was prepared using salts that include NaCl, Na<sub>2</sub>SO<sub>4</sub>, MgSO<sub>4</sub>, and CaCl<sub>2</sub>. It contained three levels of electrical conductivity (EC), i.e., 1.8, 6, and 12 dS m<sup>-1</sup>, and had a sodium adsorption ratio (SAR) of 15. We noted significant deleterious effects of excessive salt stress on multiple attributes of the growth, produce, physiology, and chemical factors of sunflowers. However, treatment with GP+CCD improved all these attributes in all these conditions over the control treatment. Treatment with GP+CCD also significantly increased N, P and K contents over the control in the absence of salt stress, i.e., normal conditions. Conversely, treatment with GP+CCD caused an extreme decline in antioxidant enzyme activity (APX, GPX, CAT and SOD) and Na<sup>+</sup>/K<sup>+</sup> ratio in seeds of up to 90, 75, and 71% over control at an EC level of 1.8, 6, and 12 dS m<sup>-1</sup>, respectively. This study suggests the combined application of gypsum and composted cow dung for better production of sunflowers in salt-affected soils, and augmented growth, yield, physiology, biochemistry and nutritional value in the sunflower seeds.

**Keywords:** salt stress; sodicity; compost; nutrient homeostasis; yield

## 1. Introduction

Salt stress is the leading cause of abiotic stress on plants in arid and semi-arid regions, and markedly decreases the normal growth and development of important crops. Currently, about 62 million hectares (26%) of the world's irrigated land is damaged by salt accumulation [1]. The salinization of soils is expanding globally and is estimated to cause 50% land deterioration by the year 2050 [2]. Salt stress has decreased the production of

food in some regions like South Asia and sub-Saharan Africa and aggravated the already serious situation of food security [3,4]. A high level of salts (mainly Na) in these soils alters the soil's physicochemical state, reduces the uptake of essential nutrients to crops, and disturbs various metabolic processes in plants, eventually causing a reduction in crop yields [5–7]. Moreover, the plants growing on saline-sodic soil conditions undergo multiple consequences caused by osmotic stress, leading to hormonal imbalances, nutritive syndrome, and the toxicity of a particular ion [8,9]. Furthermore, severe conditions of salinity cause changes in the osmotic potential of the soil solution, severely affecting the native microbial population involved in the cycling of plant nutrients [10]. In addition, a high accumulation of salts in plants triggers the production of reactive oxygen species that disturb the metabolic machinery of the plants and cause low productivity [11]. Land degradation with its associated crop yield reduction is only one of the adverse consequences of salt-affected soils [12]. On a global scale, the productivity of crops can be reduced up to 20% due to salt stress [13].

Numerous management and amelioration approaches have been adopted to prevent the barrenness of salt-affected soils [14–17]. The improvement of saline-sodic soils, using chemical adjustments, such as gypsum (GP) ( $\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$ ), sulfuric acid ( $\text{H}_2\text{SO}_4$ ), and calcium chloride ( $\text{CaCl}_2$ ), has been verified to successfully rehabilitate saline-sodic soils [18,19]. Among these amendments, GP is the most frequently utilized chemical ameliorant due to its relatively high solubility, ease of handling, and accessibility at low prices [15]. The application of GP may preclude the dispersion of soil due to retaining a high  $\text{Ca}^{2+} : \text{Na}^+$  ratio, hence enhancing the flocculation of clay particles and improving the physical firmness of soils [20]. However, amending soils with GP as a sole ameliorant cannot reclaim already degraded soils, because of its inability to ameliorate those biological and physical properties of soils that are otherwise important for sustaining crop production, especially under saline-sodic conditions [15,21]. This is because the effectiveness of gypsum application is further governed by the site-specific characters of climate, soil, and the concentration of soluble salts in a given soil sample. These factors result in the lower efficacy of gypsum for reclamation of these soils when applied in isolation.

The majority of the agricultural soils of Pakistan are characterized by lower contents of plant nutrients and organic matter [22]. The crop production in such cases solely relies on external fertilization for optimum production. This is specifically true for saline soils, having insufficient plant nutrients to support normal crop production unless properly managed. Numerous organic additives in this regard, for example, animal manure, composts, and municipal solid waste, have been examined for their progressive impacts on the remediation of soils to sustain crop production [23,24]. The usage of organic amendments is prioritized to improve the chemical, biological and physical properties of land damaged by salt stress. In addition, the application of organic amendments enhances the absorption of nutrients (like nitrogen, phosphorus, potassium) by plants, improves organic carbon content, microbial biomass, soil aggregation, and aggregate stability, and hence improves its structure, for better crop growth [23,25]. Moreover, the addition of carbon-rich materials along with GP has been found to be more effective in alleviating salt stress on plants [26]. Previously, Sarwar et al. [15] reported that using GP and composts in combination can modify soil properties, and hence, the fertility status of saline-sodic soils. However, the effects of the combined application of composted cow dung (CCD) and GP on the growth, physiology, and mineral nutrition of plants grown on saline-sodic soils have been relatively little studied. Moreover, improvements in physiological adaptations and water relationships of sunflowers through the combined application of CCD and GP need to be explored under saline-sodic soil conditions.

In this context, it was hypothesized that the combined use of CCD and GP may improve the growth, physiology and antioxidant responses of sunflowers grown under increasing salinity levels; however, their effects on stress amelioration may vary according to the level of salinity.

Therefore, the objectives of the present study were to evaluate the relative impacts of the single and combined use of CCD and GP on the growth, physiology, nutrient accumulation, and water relativity of sunflowers under salt stress.

## 2. Materials and Methods

### 2.1. Preparation of Saline-Sodic Soil

An 8-kg pot trial was carried out in the wire-house of the Institute of Soil and Environmental Sciences (ISES) at the University of Agriculture, Faisalabad (UAF), Pakistan. The soil collected from the agricultural area of the ISES was air-dried, pulverized and passed through a sieve (2 mm diameter). A sub-sample of sieved soil was examined concerning various physicochemical characteristics by using the procedures defined by Estefan et al. [27]. The experimental soil contained 50% sand, 35% silt and 15% clay. Its saturation percentage was 30%, and the electrical conductivity of the extract of the saturated soil was  $1.824 \text{ dS m}^{-1}$ . The soil was basic ( $\text{pH} = 7.5$ ),  $0.84 \text{ mmol}_c \text{ L}^{-1}$  soluble  $\text{CO}_3^{2-}$  and  $0 \text{ mmol}_c \text{ L}^{-1}$  soluble  $\text{HCO}_3^{2-}$ ,  $11.5 \text{ cmol}_c \text{ kg}^{-1}$  CEC and 0.74% organic matter.

The salinity was developed at an EC of 1.8 (normal/control), 6 (saline-sodic) and  $12 \text{ dS m}^{-1}$  (saline-sodic) by using commonly used salts, i.e., NaCl,  $\text{Na}_2\text{SO}_4$ ,  $\text{CaCl}_2$ , and  $\text{MgSO}_4$ , for salinity development by artificial means, and the SAR was static, i.e., 15 in two (high) EC levels.

### 2.2. Procurement of Cow Dung, Composting and Experimental Design

The cow dung was collected from the Dairy and Livestock Section, Direct Farms, University of Agriculture Faisalabad. Prior to its amendment for composting, unwanted materials were removed manually. Air-dried cow dung was spread on the plastic sheets and composting was achieved following the windrow method. Molasses 0.5% (*v/v*) and urea solution 1% (*w/v*) were mixed with the manure heap and manually aerated well. The moisture content was adjusted to 50–55% during the composting process. The material was thoroughly mixed every week, and the composting process was continued for 4 weeks. Then, GP (Latif Stone Supplier Ltd., Taunsa Sharif, Distt. D.G. Khan, Pakistan) was mixed at a 50:50 ratio (*w/w*), and then incubated for one week to enhance the nutritional quality of the finished product. The chemical characteristics of the composted cow dung (CCD), gypsum (GP), and CCD+GP were achieved following the standard procedure given by Estefan et al. [27] (Table 1). The calcium contents of the CCD and CCD+GP were analyzed with an atomic absorption spectrophotometer (AAS) equipped with a Ca-cathode lamp.

**Table 1.** The chemical analyses of CCD, GP, and their mixtures.

Parameters	CCD	GP	GP + CCD (1:1 <i>w/w</i> )
Carbon ( $\text{g kg}^{-1}$ )	$201 \pm 14.3$	-	$103.8 \pm 9.2$
Nitrogen ( $\text{g kg}^{-1}$ )	$2.89 \pm 0.5$	-	$1.75 \pm 0.3$
Total P ( $\text{g kg}^{-1}$ )	$1.49 \pm 0.7$	-	$1.06.0 \pm 0.2$
Olson P ( $\text{mg kg}^{-1}$ )	$241 \pm 12.3$	-	$123 \pm 7.5$
Ca ( $\text{g kg}^{-1}$ )	$0.042 \pm 0.02$	21 (%)	$98.3 \pm 9.4$
Sulfur ( $\text{g kg}^{-1}$ )	$0.74 \pm 0.06$	17 (%)	$78.75 \pm 6.7$
C:N	$18.34 \pm 1.3$	-	$12.05 \pm 0.9$
pH	$6.33 \pm 0.27$	7.78	$6.11 \pm 0.18$

Data are average of three repeats  $\pm$  Standard Error (SE). CCD—composted cow dung; GP—gypsum.

Composted cow dung, gypsum and their combined application (CCD+GP) were applied to pots at  $10 \text{ g kg}^{-1}$  soil. Pots were fertilized using urea, diammonium phosphate (DAP) and sulfate of potash (SoP) to fulfill the nutrient requirement of the plants at 45 kg, N, 35 kg, P, and 15 kg, K, per hectare. Sunflower seeds were collected from the Oilseed Research Institute, Faisalabad, and four seeds were sown in each pot, and, on germination, thinned to two plants in each pot. The arrangement of pots was made according to a completely randomized design (CRD) with three replicates.

### 2.3. Plant Growth and Yield Traits

Standard procedures were followed to determine plant growth parameters after harvesting. The lengths of roots and shoots were measured with a meter rod. The fresh and dried weights (placed in an oven at 65 °C for 72 h) of the roots and shoots were taken using a physical balance (Setra Systems Inc., Boxborough, MA, USA). Seeds were isolated from the shoots after dehydration, and the weight of one thousand seeds and the seed yield per plant (SYP) were measured. The stem diameter (cm) and head diameter (cm) of each plant from each pot were measured with a Vernier caliper before harvesting. Moreover, the leaf area (cm<sup>2</sup>) was also calculated by multiplying the length and width of leaves by a constant factor (0.75).

### 2.4. Physiological Measurements

The physiological attributes of plants were measured, beginning at the fully matured second leaf from the top. The chlorophyll meter (SPAD-502; Minolta, Osaka, Japan) gave us the soil plant analysis development (SPAD) index [28]. The gaseous exchange measurements were taken by an infrared gas analyzer (IRGA). This gave us parameters including the plant photosynthetic rate (A), stomatal conductance (gs), transpiration rate (E), internal CO<sub>2</sub> concentration and evaporation rate.

The relative water content of the plant leaves was measured in a 1-cm<sup>2</sup> piece of a leaf without a midrib. Its fresh, turgid, and dry weights, with 3 repeats, were taken according to Bashir et al. [29]. Leaves were placed in 100% humid conditions in the dark at a temperature of 4 °C for 24 h in order to obtain their turgid weight. All the values were placed in the following formula to obtain the relative water content.

$$\text{RWC (\%)} = \frac{\text{Fresh weight} - \text{Dry weight}}{\text{Turgid weight} - \text{Dry weight}} \times 100$$

Electrolyte leakage from the leaves was measured using the formula of Lutts et al. [30]. One square centimeter area of leaf material was placed in test tubes filled with 10 mL of distilled water. Electrical conductivity (EC1) was measured at room temperature with an EC meter. The contents of the test tubes were autoclaved at 120 °C. Electrical conductivity (EC2) was recorded after autoclaving. Electrolyte leakage (EL) was determined using the following formula:

$$\text{EL (\%)} = \frac{\text{EC1}}{\text{EC2}} \times 100$$

whereas the membrane stability index of the leaf was computed by following the equation of Yang et al. [31], as follows:

$$\text{MSI (\%)} = 1 - \frac{\text{EC1}}{\text{EC2}} \times 100$$

The osmotic potential was calculated using a cryoscopic osmometer (Osmomat 030-Rs, Genote, GmbH, Berlin, Germany) [32].

### 2.5. Antioxidant Assay Measurement

Frozen leaf material was used for the determination of antioxidant enzymes, and homogenized in ice-cold potassium phosphate buffer (0.2 M, pH 7) and EDTA (0.1 mM). Nakano and Asada's method [33] was used for the determination of ascorbate peroxidase (APX) activity. Aebi's method [34] was used for the measurement of glutathione peroxidase (GPX) activity. The method developed by Cakmak and Marschner [35] was used for the calculation of catalase (CAT) activity by thinned enzyme extract in potassium phosphate. Roth and Gilbert's method [36] was used for the calculation of superoxide dismutase (SOD) activity. Analytical-grade reagents and chemicals were used, and were provided by Merck, Darmstadt, Germany, and Sigma-Aldrich, St. Louis, MO, USA.

## 2.6. Elemental Analysis

Oven-dried plant samples were ground down and the wet digestion method was used for the determination of elemental constituents, i.e., N, P, K, and Na, as given by Wolf [37]. Samples after digestion were cooled down and filtered using #42 filter paper, and the filtrate was collected in plastic bottles. The collected samples were then subjected to chemical analysis. The nitrogen content was measured by a micro-Kjeldahl apparatus, the potassium and sodium contents by a flame photometer, and the phosphorus content by spectrophotometer [38]. Afterward, the ratio of sodium to potassium in leaves and seeds was also calculated.

## 2.7. Statistical Analysis

Using an analysis of variance (ANOVA), the observed data was analyzed at  $p \leq 0.05$  using Statistix 8.1 computer-based software (Statistix, Tallahassee, FL, USA). Tukey's HSD (honestly significant difference) test was used for comparison among mean values [39]. The standard error (SE) of mean was calculated through MS Excel 2016. All the graphs were prepared with Excel software (Microsoft Excel 2016), while the correlation matrices were plotted using R Studio (R Software® 4.0.2).

## 3. Results

### 3.1. Growth Parameters

Increments in the growth-related attributes of the plants were observed, as they were affected by the combined use of gypsum (GP) and composted cow dung (CCD) under salt-stressed soil, in comparison to the control and separate application of CCD or GP (Tables 2 and 3). The highest fresh weight of shoots was detected under the combined use of CCD and GP for normal and salt-stressed soils, in relation to the control and to separate use of CCD or GP (Table 1). Furthermore, the sole utilization of CCD resulted in an enhanced shoot fresh weight of 70% at an EC level of  $1.8 \text{ dS m}^{-1}$ , 91% at an EC level of  $6 \text{ dS m}^{-1}$ , and 97% at an EC level of  $12 \text{ dS m}^{-1}$  compared to control. The separate use of GP increased shoot fresh weight up to 37% at an EC level of  $1.8 \text{ dS m}^{-1}$ , 54% at an EC level of  $6 \text{ dS m}^{-1}$ , and 58% at an EC level of  $12 \text{ dS m}^{-1}$  compared to control. Likewise, the combined application of CCD and GP caused the maximum increase in shoot dry weight, up to 137, 168, and 189% at EC levels of 1.8, 6 and  $12 \text{ dS m}^{-1}$ , respectively, in relation to the control (Table 1). It was found that sole utilization of CCD and GP increased shoot dry weight by 93, 118, and 127%, and 72, 90, and 92% at EC levels of 1.8, 6, and  $12 \text{ dS m}^{-1}$ , respectively, relative to the control treatment (Table 1).

The combined utilization of CCD and GP increased plant height to 144% at an EC level of  $1.8 \text{ dS m}^{-1}$ , to 174% at an EC level of  $6 \text{ dS m}^{-1}$ , and to 196% at an EC level of  $12 \text{ dS m}^{-1}$  relative to control (Table 2). It was tracked by the separate use of CCD and GP. An increment of 66, 86, and 118% in plant height, relative to the control, was noted with the separate use of CCD at these same EC levels, respectively. In the same way, the separate application of GP increased plant height by 44, 52, and 78%, at these same EC levels, respectively, in comparison to the control treatment.

The use of CCD alone increased root length up to 55% at an EC level of  $1.8 \text{ dS m}^{-1}$ , 67% at an EC level of  $6 \text{ dS m}^{-1}$ , and 94% at an EC level of  $12 \text{ dS m}^{-1}$ , in contrast to the control treatment (Table 2). Root length was enlarged up to 32, 44, and 54% at these same EC levels, respectively, relative to the control when GP was applied on its own. However, the CCD and GP together instigated the greatest increment in root length by 119, 156, and 179% at these same EC levels, respectively, in relation to the control. Equally, the combined application of CCD and GP enhanced root fresh weight up to 108% at an EC level of  $1.8 \text{ dS m}^{-1}$ , 136% at an EC level of  $6 \text{ dS m}^{-1}$ , and 174% at  $12 \text{ dS m}^{-1}$ , respectively, in comparison to the control treatment (Table 2). CCD on its own increased the root fresh weight up to 57, 74, and 91% at these same EC levels, respectively, compared with the control. A similar trend was detected in root dry weight.

**Table 2.** Effect of CCD and GP on growth attributes of sunflowers (*H. annuus*) under saline-sodic soil conditions.

Treatments	Plant Height (cm)			Shoot Fresh Weight (g)			Shoot Dry Weight (g)		
	1.8 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	12 dS m <sup>-1</sup>	1.8 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	12 dS m <sup>-1</sup>	1.8 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	12 dS m <sup>-1</sup>
Control	40.9 ± 0.8g	30.0 ± 0.4i	20.7 ± 0.9j	31.4 ± 0.9g	23.4 ± 1.4h	15.2 ± 0.9i	9.9 ± 0.4g	7.4 ± 0.7h	5.1 ± 0.5i
CCD	68.0 ± 0.5c	55.8 ± 1.1e	45.3 ± 0.3f	53.3 ± 1.6c	44.6 ± 0.9d	30.0 ± 1.3g	19.0 ± 0.4b	16.1 ± 0.4cd	11.7 ± 0.3f
GP	59.1 ± 0.8de	45.7 ± 1.4f	36.9 ± 2.2h	43.1 ± 0.8de	36.0 ± 0.8f	24.1 ± 0.4h	17.0 ± 0.9c	14.1 ± 0.3e	9.9 ± 0.2g
CCD + GP	100.3 ± 1.2a	82.4 ± 1.5b	61.4 ± 0.6d	70.4 ± 1.2a	59.9 ± 1.3b	40.6 ± 1.3e	23.4 ± 0.9a	19.9 ± 0.7b	14.9 ± 0.2de
	Root Length (cm)			Root Fresh Weight Plant <sup>-1</sup> (g)			Root Dry Weight (g)		
Control	9.2 ± 0.4f	6.7 ± 0.2h	4.7 ± 0.15i	6.0 ± 0.2g	4.7 ± 0.3h	3.4 ± 0.2i	5.3 ± 0.2e	4.2 ± 0.14fg	3.5 ± 0.3g
CCD	14.2 ± 0.7c	11.3 ± 0.5de	9.1 ± 0.20fg	9.5 ± 0.3c	8.1 ± 0.3de	6.4 ± 0.2fg	7.4 ± 0.4b	6.1 ± 0.1d	4.9 ± 0.2ef
GP	12.1 ± 0.5d	9.7 ± 0.3ef	7.2 ± 0.2gh	8.9 ± 0.3cd	7.3 ± 0.2ef	5.8 ± 0.3gh	6.4 ± 0.5cd	5.2 ± 0.2e	4.4 ± 0.3f
CCD + GP	20.1 ± 0.3a	17.2 ± 0.3b	13.0 ± 0.4cd	12.5 ± 0.2a	11.0 ± 0.2b	9.2 ± 0.3cd	8.9 ± 0.2a	7.2 ± 0.2bc	6.4 ± 0.2d

Quantities sharing similar letters in each parameter do not differ significantly at  $p \leq 0.05$ . The data is the average of three replicates  $\pm$  SE. CCD—composted cow dung, GP—gypsum.

**Table 3.** Effect of CCD and GP on the growth and yield attributes of sunflowers (*H. annuus*) under saline-sodic soil conditions.

Treatments	Stem Diameter (cm)			Head Diameter (cm)			No. of Leaves Plant <sup>-1</sup>		
	1.8 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	12 dS m <sup>-1</sup>	1.8 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	12 dS m <sup>-1</sup>	1.8 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	12 dS m <sup>-1</sup>
Control	3.2 ± 0.1ef	2.5 ± 0.3gh	2.0 ± 0.1h	12.7 ± 0.2ef	10.1 ± 0.4h	7.7 ± 0.3i	14.7 ± 0.9de	11.6 ± 0.3fg	8.6 ± 0.8h
CCD	4.9 ± 0.3bc	4.3 ± 0.1d	3.6 ± 0.2e	15.5 ± 0.5bc	13.0 ± 0.4e	12.0 ± 0.7ef	21.3 ± 0.9b	16.0 ± 1.2cd	13.0 ± 0.6efg
GP	4.3 ± 0.2d	3.5 ± 0.2e	2.9 ± 0.1fg	14.2 ± 0.2d	11.6 ± 0.6fg	10.6 ± 0.6gh	18.3 ± 1.2c	14.0 ± 0.6def	10.6 ± 0.9gh
CCD + GP	6.6 ± 0.2a	5.3 ± 0.2b	4.5 ± 0.3cd	18.1 ± 0.3a	16.0 ± 0.4b	14.8 ± 0.1cd	26.7 ± 0.7a	21.0 ± 0.6b	18.0 ± 0.6c
	Leaf Area (cm <sup>2</sup> )			1000-Seeds Weight (g)			Seed Yield per Plant (g)		
Control	42.2 ± 0.9g	31.2 ± 0.7i	22.5 ± 1.3j	32.9 ± 1.3e	22.4 ± 0.7g	12.8 ± 0.4h	45.2 ± 0.3f	35.4 ± 0.7gh	26.6 ± 0.6i
CCD	67.5 ± 1.1c	51.0 ± 0.6e	45.0 ± 0.6fg	57.4 ± 0.9b	41.1 ± 1.8d	29.1 ± 0.8f	65.2 ± 1.4cd	54.9 ± 1.1e	46.4 ± 1.6g
GP	61.0 ± 1.1d	45.7 ± 1.9f	34.6 ± 0.3h	45.8 ± 0.9c	30.8 ± 1.2ef	24.5 ± 2.4g	62.5 ± 0.9d	50.2 ± 1.6f	41.2 ± 1.1h
CCD + GP	104.6 ± 1.2a	82.4 ± 0.6b	62.4 ± 1.9d	79.7 ± 0.9a	60.1 ± 1.3b	38.0 ± 1.4d	82.3 ± 0.7a	73.1 ± 1.5b	66.3 ± 0.9c

Quantities carrying different letters in each parameter differ significantly at  $p \leq 0.05$ . The data represent the average of three replicates  $\pm$  SE. CCD—composted cow dung, GP—gypsum.

Similarly, the separate application of CCD or GP demonstrated a minor increment in stem diameter at 1.8, 6, and 12 dS m<sup>-1</sup> relative to the control (Table 3). The extreme increment in the diameter of stems was detected regarding the combined application of CCD and GP, i.e., 104% at an EC level of 1.8 dS m<sup>-1</sup>, 110% at an EC level of 6 dS m<sup>-1</sup>, and 127% at an EC level of 12 dS m<sup>-1</sup>, when compared with control. Treatment with GP+CCD demonstrated an extreme increase in head diameter by 42, 59, and 93% at these same EC levels, respectively, relative to the control (Table 2). Moreover, this treatment also caused the greatest increase in the number of leaves per plant, by 81, 80, and 108% at these same EC levels, respectively, as compared to the control (Table 2). It also caused an immense increase in leaf area, by 148% at an EC level of 1.8 dS m<sup>-1</sup>, by 164% at an EC level of 6 dS m<sup>-1</sup>, and by 177% at an EC level of 12 dS m<sup>-1</sup>, respectively, relative to the control (Table 2).

### 3.2. Yield Parameters

Regarding the growth parameters, yield parameters like 1000-seed weight and seed yield were considerably reduced under salt-stressed soil conditions without any amendment (Table 3). A statistically significant increment was recorded in treatment with GP+CCD in both normal and salt-stress plants. The combined application of CCD and GP increased 1000-seed weight by 142% at an EC level of 1.8 dS m<sup>-1</sup>, by 167% at an EC level of 6 dS m<sup>-1</sup>, and by 196% at an EC level of 12 dS m<sup>-1</sup>, relative to the control. A non-significant increment in 1000-seed weight was noted with the separate use of GP and CCD at all EC levels. In the case of seed yield per plant, the individual application of CCD slightly increased this up to 44, 55, and 74% at 1.8, 6, and 12 dS m<sup>-1</sup>, respectively, compared with the control. The individual application of GP slightly increased the seed yield per plant, up to 38, 42, and 55% at 1.8, 6, and 12 dS m<sup>-1</sup>, respectively, relative to the control. However, treatment with GP+CCD produced a maximum seed yield which was 82% higher than the control at an EC level of 1.8 dS m<sup>-1</sup>, 106% higher than the control at an EC level of 6 dS m<sup>-1</sup>, and 138% higher than the control at an EC level of 12 dS m<sup>-1</sup>.

### 3.3. Physiological Parameters and Water Relativity

Data related to physiological attributes indicated that the separate application of CCD instigated a statistically non-significant improvement in photosynthetic rate by 59% at an EC level of 1.8 dS m<sup>-1</sup>, 72% at an EC level of 6 dS m<sup>-1</sup>, and 89% at an EC level of 12 dS m<sup>-1</sup>, relative to the control (Table 4). Moreover, the combined utilization of CCD and GP caused the highest increase in the rate of photosynthesis, by 114, 139, and 183% at these same EC levels, respectively, relative to the control. Similarly, a higher impact on stomatal conductance was recorded with the combined application of CCD and GP, i.e., 111, 135, and 193% at these same EC levels, respectively, relative to the control (Table 4). The combined application of CCD and GP caused a maximum improvement in the transpiration rate of up to 96, 105, and 169% at these same EC levels, respectively, compared with the control. Alike drift was detected in the case of the evaporation rate. Treatment with GP+CCD produced an extreme increment in internal CO<sub>2</sub> concentration, i.e., 73, 91, and 107% at 1.8, 6, and 12 dS m<sup>-1</sup>, respectively, compared with the control.

**Table 4.** Effect of CCD and GP on the physiological attributes of sunflowers (*H. annuus*) under saline-sodic soil conditions.

Treatments	Photosynthetic Rate ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ )			Stomatal Conductance ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )			Evaporation Rate ( $\mu\text{mol H}_2\text{O m}^{-2} \text{ s}^{-1}$ )		
	1.8 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	12 dS m <sup>-1</sup>	1.8 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	12 dS m <sup>-1</sup>	1.8 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	12 dS m <sup>-1</sup>
Control	16.8 ± 0.4f	12.6 ± 0.3g	8.8 ± 0.2h	0.35 ± 0.1fg	0.19 ± 0.1g	0.10 ± 0.0h	4.1 ± 0.1h	3.6 ± 0.4i	2.4 ± 0.1k
CCD	26.8 ± 0.6c	21.8 ± 0.6e	16.7 ± 0.3f	0.39 ± 0.1cd	0.32 ± 0.1de	0.21 ± 0.1fg	6.1 ± 0.2c	5.1 ± 0.4f	4.1 ± 0.2h
GP	22.5 ± 0.3de	18.0 ± 0.5f	13.8 ± 0.6g	0.34 ± 0.1cde	0.28 ± 0.1ef	0.17 ± 0.0fg	5.7 ± 0.3e	4.7 ± 0.2g	3.3 ± 0.1j
CCD + GP	36.0 ± 0.6a	30.3 ± 0.9b	25.0 ± 0.6cd	0.53 ± 0.1a	0.45 ± 0.3b	0.29 ± 0.0bc	8.2 ± 0.4a	7.0 ± 0.1b	5.9 ± 0.2d
	Transpiration Rate ( $\mu\text{mol m}^{-2} \text{ s}^{-1}$ )			Internal CO <sub>2</sub> ( $\mu\text{mol CO}_2 \text{ mol}^{-1}$ )			SPAD Value		
Control	7.6 ± 0.3efg	6.3 ± 0.7g	4.3 ± 0.3h	225 ± 1.4g	183 ± 2.0i	145 ± 0.8j	27.9 ± 0.9g	20.9 ± 0.8h	13.8 ± 1.0i
CCD	11.3 ± 0.3bc	9.3 ± 0.6de	8.3 ± 0.3def	323 ± 1.5c	281 ± 2.9e	235 ± 1.5g	45.1 ± 0.7c	38.2 ± 0.6e	27.3 ± 1.0g
GP	10.0 ± 0.6cd	8.7 ± 0.8de	6.7 ± 0.8fg	300 ± 1.4d	254 ± 2.3f	207 ± 1.2h	40.3 ± 0.6de	32.1 ± 1.0f	21.8 ± 0.6h
CCD + GP	15.0 ± 0.6a	13.0 ± 0.6b	11.7 ± 1.2bc	389 ± 2.3a	350 ± 3.5b	300 ± 2.3d	60.3 ± 0.5a	51.3 ± 0.7b	39.3 ± 0.4d

Quantities carrying different letters in each parameter differ significantly at  $p \leq 0.05$ . The data represent the average of three replicates ± SE. CCD—composted cow dung, GP—gypsum, SPAD—soil plant analysis development.

A higher increase in the chlorophyll content (SPAD index) was assessed with the combined use of CCD and GP, showing a 116, 145, and 184% increase in chlorophyll contents at 1.8, 6, and 12 dS m<sup>-1</sup>, respectively, relative to the control (Table 4). Treatment with GP+CCD caused the highest increase in RWC by 100, 137, and 185% at 1.8, 6, and 12 dS m<sup>-1</sup>, respectively, in relation to the control. Likewise, the greatest increase in the membrane stability index was detected with the combined application of CCD and GP, as it increased to 114% at an EC level of 1.8 dS m<sup>-1</sup>, 146% at an EC level of 6 dS m<sup>-1</sup>, and 187% at an EC level of 12 dS m<sup>-1</sup>, relative to the control (Table 5). A similar trend was observed with the combined application of CCD and GP in the case of osmotic potential under normal conditions and salt stress. However, the combined application of CCD and GP considerably decreased electrolyte leakage by 132, 152, and 170% at 1.8, 6, and 12 dS m<sup>-1</sup>, respectively, in contrast to the control treatment (Table 5).

**Table 5.** Effect of CCD and GP on the water relativity of sunflowers (*H. annuus*) under saline-sodic soil conditions.

Treatments	Relative Water Contents (%)			Membrane Stability Index (%)		
	1.8 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	12 dS m <sup>-1</sup>	1.8 dS m <sup>-1</sup>	6 dS m <sup>-1</sup>	12 dS m <sup>-1</sup>
Control	44.4 ± 0.4g	32.0 ± 1.3h	22.2 ± 1.1i	32.4 ± 0.9f	24.0 ± 0.9g	13.7 ± 1.5h
CCD	70.7 ± 1.0c	58.3 ± 1.1e	50.3 ± 0.5f	56.6 ± 1.2b	44.7 ± 0.6d	29.9 ± 0.7f
GP	65.4 ± 0.7d	51.4 ± 0.7f	43.9 ± 0.7g	50.0 ± 0.3c	37.2 ± 0.6e	25.0 ± 0.4g
CCD + GP	88.9 ± 0.8a	75.7 ± 0.4b	63.2 ± 1.0d	69.2 ± 1.0a	59.1 ± 0.7b	39.2 ± 0.5e
	Electrolyte Leakage (%)			Osmotic Potential (osmol kg <sup>-1</sup> )		
Control	38.6 ± 1.7d	52.0 ± 1.1b	68.7 ± 1.3a	0.25 ± 0.02fg	0.20 ± 0.02h	0.12 ± 0.01i
CCD	25.0 ± 0.8f	33.0 ± 1.4e	43.1 ± 1.3c	0.45 ± 0.01c	0.32 ± 0.01e	0.26 ± 0.01f
GP	32.4 ± 0.8e	41.2 ± 1.4cd	52.2 ± 0.9b	0.39 ± 0.01d	0.25 ± 0.02fgh	0.20 ± 0.02gh
CCD + GP	16.7 ± 0.6h	20.7 ± 0.8g	25.4 ± 0.8f	0.64 ± 0.02a	0.54 ± 0.02b	0.47 ± 0.02c

Quantities carrying different letters in each parameter differ significantly at  $p \leq 0.05$ . The data represent the average of three replicates ± SE. CCD—composted cow dung, GP—gypsum.

### 3.4. Antioxidant Enzymes Analysis

Similarly, a remarkable improvement in antioxidant enzymes, including GPX, APX, SOD and CAT were observed in the control, particularly at the 6 and 12 dS m<sup>-1</sup> salinity levels (Figure 1). The application of CCD considerably lowered the APX activity by up to 35% at an EC level of 1.8 dS m<sup>-1</sup>, 43% at an EC level of 6 dS m<sup>-1</sup>, and 49% at an EC level of 12 dS m<sup>-1</sup> over the control. The combined application of CCD and GP lowered the APX the most, up to 57% at an EC level of 1.8 dS m<sup>-1</sup>, 64% at an EC level of 6 dS m<sup>-1</sup> and 67% at an EC level of 12 dS m<sup>-1</sup> over the control. Likewise, a significant reduction in GPX activity was observed with CCD application of up to 34% at an EC level of 1.8 dS m<sup>-1</sup>, 47% at an EC level of 6 dS m<sup>-1</sup>, and 52% at an EC level of 12 dS m<sup>-1</sup>, respectively, compared with the control. The combined utilization of CCD and GP caused the greatest decrease in GPX activity, by 61% at an EC level of 1.8 dS m<sup>-1</sup>, 65% at an EC level of 6 dS m<sup>-1</sup>, and 68% at an EC level of 12 dS m<sup>-1</sup>, in relation to the control treatment. Correspondingly, GP application decreased the CAT activity up to 27% at an EC level of 1.8 dS m<sup>-1</sup>, 35% at an EC level of 6 dS m<sup>-1</sup> and 42% at an EC level of 12 dS m<sup>-1</sup> over the control treatment, whereas a 64, 68 and 70% decline in CAT activity was found at these same EC levels, respectively, with the combined application of CCD and GP, in contrast to the control. In the same way, a reduction in SOD activity was observed of up to 61, 64 and 67% by the combined application of CCD and GP at these same EC levels, consistently, relative to the control.

### 3.5. Elemental Analysis

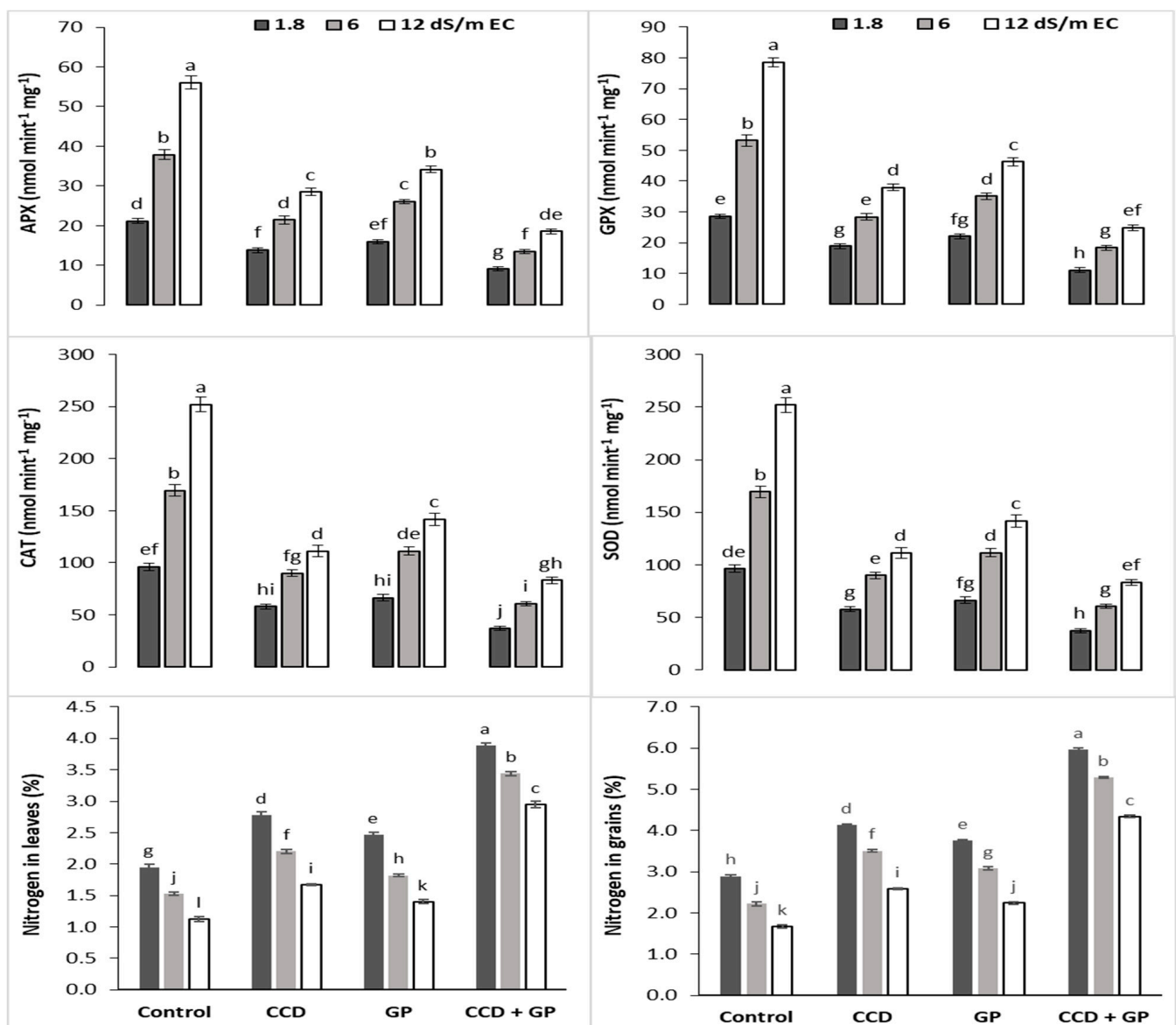
A statistically noteworthy influence as regards NPK concentration was noted in treatment with GP+CCD in both normal and salt-stress soil conditions. The maximum nitrogen concentration in leaves was detected with the combined application of CCD and GP, i.e., 99% at an EC level of 1.8 dS m<sup>-1</sup>, 125% at an EC level of 6 dS m<sup>-1</sup>, and 164% at an EC level of 12 dS m<sup>-1</sup> relative to the control (Figure 1). Similar drift was detected with the combined application of CCD and GP in the case of nitrogen concentration in seeds (Figure 1). The combined application of CCD and GP also caused a significant increase in phosphorus concentration in leaves, by 128, 166, and 180% at these same EC levels, respectively, relative to the control. A similar drift was detected with the combined application of CCD and GP in the case of phosphorus concentration in seeds (Figure 1). Likewise, treatment with GP+CCD markedly increased potassium concentration in leaves, up to 108, 128, and 176% at these same EC levels, respectively, compared with the control (Figure 1). Similar effects were observed in the treatment with GP+CCD in the case of potassium concentration in seeds (Figure 2).

In contrast, the Na<sup>+</sup>/K<sup>+</sup> ratio of leaves was considerably reduced by up to 67% at an EC level of 1.8 dS m<sup>-1</sup>, 71% at an EC level of 6 dS m<sup>-1</sup>, and 73% at an EC level of 12 dS m<sup>-1</sup> with the combined use of GP and CCD, in contrast to the control. Likewise, the maximum reduction in the Na<sup>+</sup>/K<sup>+</sup> ratio in seeds was recorded with the combined application of CCD and GP as a 65, 69, and 71% reduction in the Na<sup>+</sup>/K<sup>+</sup> ratio in seeds at these same EC levels, respectively, relative to the control (Figure 2).

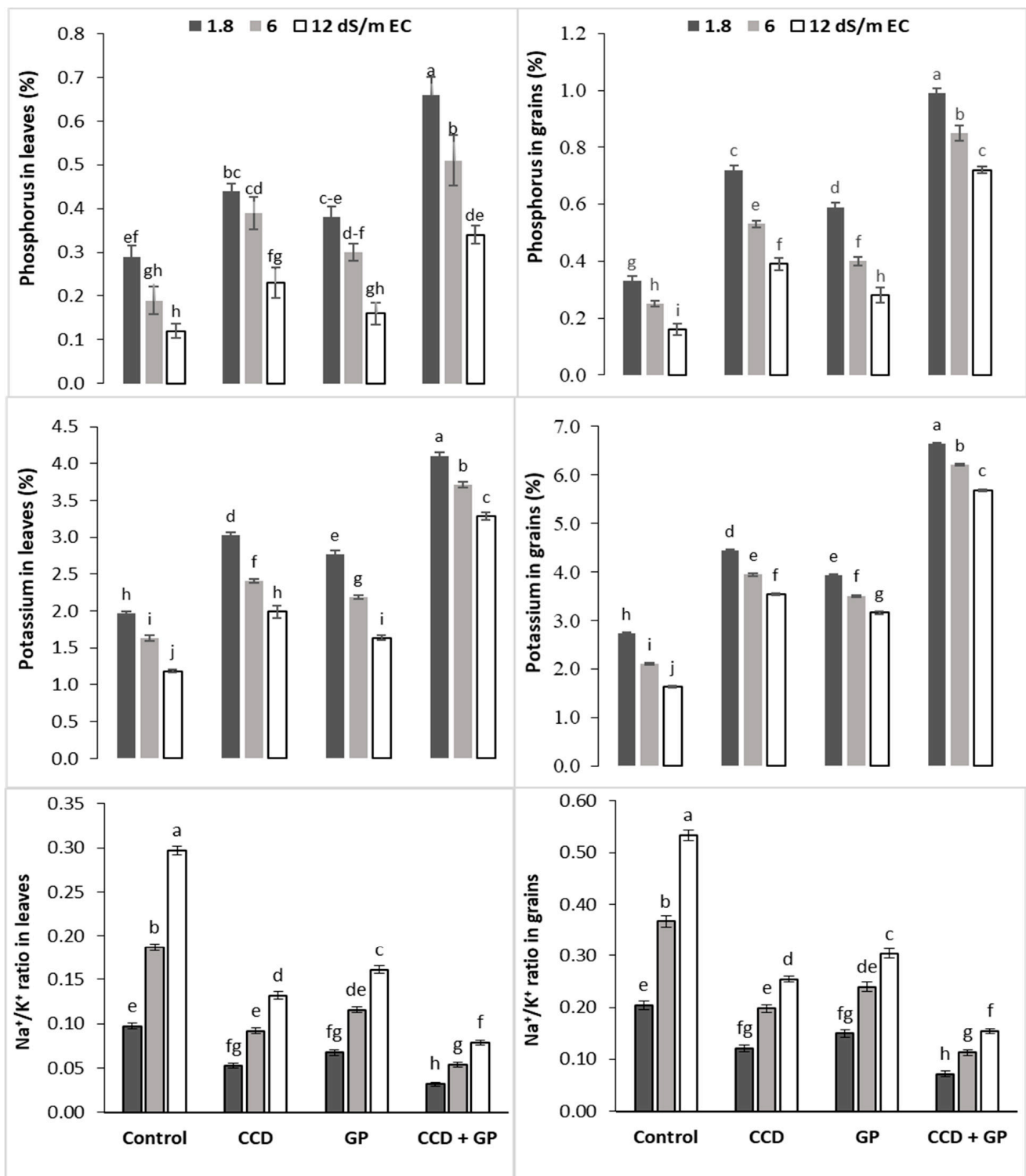
### 3.6. Results from the Correlation Matrix

Significant positive and negative correlations were noted among the different growth, physiological, biochemical and nutritional parameters of sunflowers in saline-sodic soil conditions, treated with composted cow dung and gypsum (Figure 3).

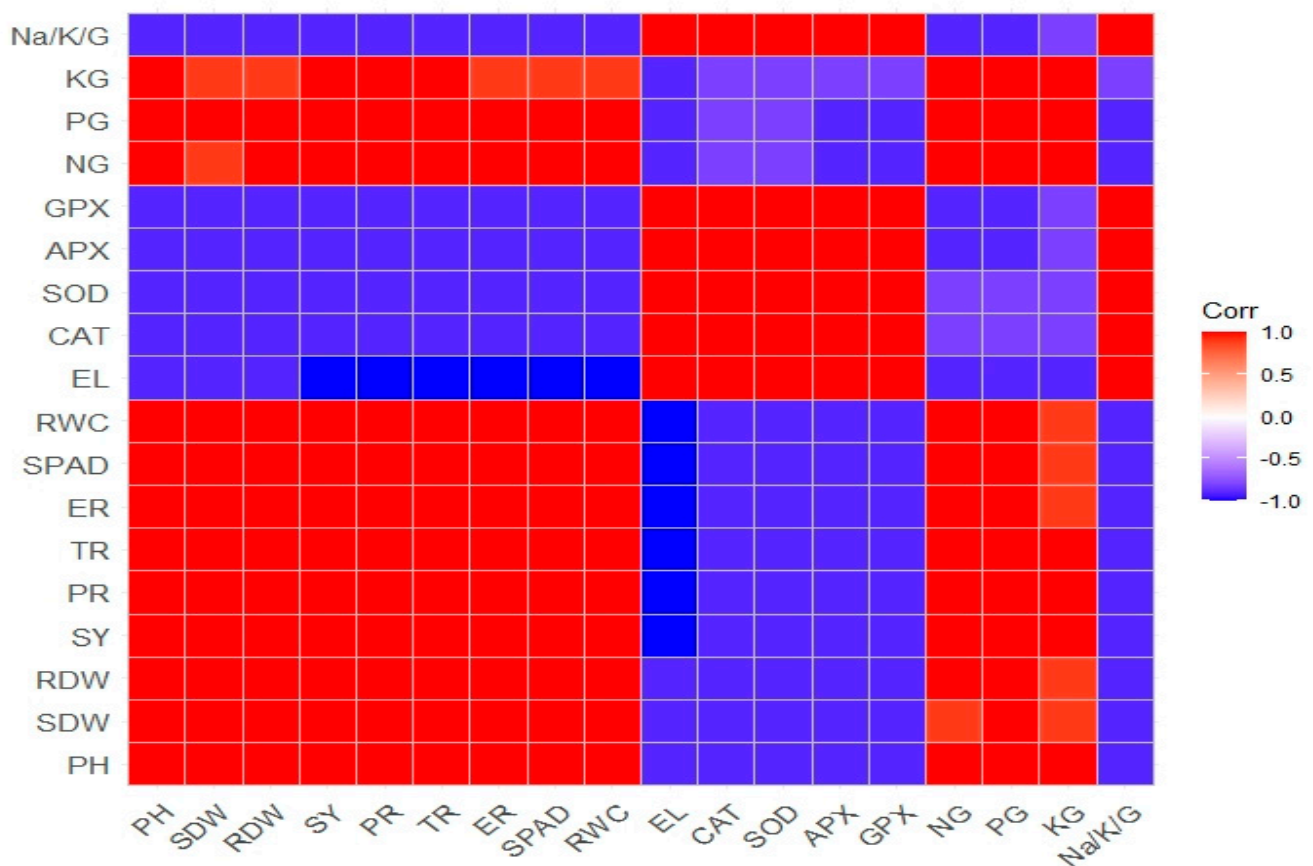




**Figure 1.** Effect of CCD and GP on APX, GPX, CAT, SOD activity and the nitrogen concentrations in the leaves and seeds of sunflowers (*H. annuus*) under saline-sodic soil conditions. Treatments sharing similar lowercase letters do not differ significantly from each other at  $p \leq 0.05$ ; the data represent the average of three replicates  $\pm$  SE; CCD—composted cow dung; GP—gypsum.



**Figure 2.** Effect of CCD and GP on the concentration of P and K, and the Na<sup>+</sup>/K<sup>+</sup> ratio in the seeds and leaves of sunflowers (*H. annuus*) in saline-sodic soil conditions. Different lowercase letters represent significant differences among treatments at  $p \leq 0.05$ ; the data represent the average of three replicates  $\pm$  SE; CCD—composted cow dung; GP—gypsum.



**Figure 3.** Correlation matrix among different attributes of sunflowers by the ameliorative effects of composted cow dung and gypsum in saline-sodic soils. The abbreviations are plant height (PH), shoot dry weight (SDW), root dry weight (RDW), seed yield (SY), chlorophyll contents (SPAD), photosynthetic rate (PR), transpiration rate (TR), evaporation rate (ER), relative water content (RWC), electrolyte leakage (EL), catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX), glutathione peroxidase (GPX), grain N (NG), grain P (PG), grain K (KG), Na/K of grain (Na/K/G).

#### 4. Discussion

Salt accumulation in soils is associated with high electrical conductivity and the low uptake of nutrients, such as nitrogen [40]. For the improvement of salt-stressed soils, the replacement of ions causing salt stress, either chemically or by adding organic material, is considered an effective approach. Several scientists have observed that the application of chemical additives, e.g., gypsum (GP), can replace sodium from interchangeable sites and eventually ameliorate salt-stressed soils. The current study was carried out using the combined application of composted cow dung (CCD) and gypsum (GP).

Salinity impedes crop growth through osmotic stress, the toxicity of a particular ion, and nutritional inequity [5,41]. In the current study, all the observed growth attributes were considerably decreased under salt-stressed soil conditions. Niamat et al. [24] reported decreased growth and yield of maize grown with increasing salinity levels. Their findings are coherent with another study by Shahzad et al. [42]. Here, the combined application of CCD and GP promoted plant growth under both normal and salt-stress soil conditions. Moreover, the plants' morphological and yield traits were also enhanced by the combined application of GP and CCD at all salinity levels relative to the control treatment. The same observations were reported by using acidified slurry in various crops, including wheat, barley, and maize [43,44]. The reason behind this increment might be the increased amount of accessible nitrogen and phosphorous [45,46]. Mahmood et al. [47] reported that the application of GP as a source of calcium and sulfur instigated a considerable enhancement in crop growth under salt-stressed soil conditions. An appraisal of the physicochemical

attributes of soil via the addition of organic materials could be the explanation for the better physiology of plants and higher growth and yield presented in this study [48,49]. In addition, the applied CCD might have undergone decomposition, resulting in a net release of essential plant nutrients that have improved the observed growth and yield attributes of sorghum.

The current study reports an increase in physiological parameters after treatment with GP+CCD in both normal and salt-stress soil conditions. Chlorophyll, one of the main constituents of chloroplasts for photosynthesis, is a sign of the quality of agricultural produce [50,51]. Chlorophyll content has an affirmative association with the rate of photosynthesis. The decline in total chlorophyll content (SPAD index) under salt stress is considered a characteristic indication of oxidative stress. In oxidative stress caused by salinity, plant cells undergo a shortage of water, decreased transpiration rate, and a reduction in stomatal activity and hence in photosynthesis [52]. Oxidative stress also prompts the generation of reactive oxygen species, causing reduced crop performance by altering the hormonal balance of plants and reducing enzymatic activity and the metabolic efficiency of plants [53–55]. Moreover, in plants under stress conditions, premature leaf senescence occurs, which may cause a reduction in photosynthesis and the leaf area of plants [9].

Relative water content (RWC), the membrane stability index (MSI) and electrolyte leakage (EL) are among the most sensitive indicators of plants under stress [56–58]. The early indicator of water deficiency is associated with a growth reduction caused by the reduced turgor of cells [59]. The suppression of plant growth under salinity is caused by cell shrinkage, damaged membrane integrity, and insufficient nutrition [60]. We found decreased relative water contents, osmotic potential, and increased electrolyte leakage with increasing salinity levels. However, these attributes were significantly improved when CCD and GP were applied in combination. A number of previous reports advocate that the current findings of salinity induced retardation in the water relativity of crops [9,42,61]. Recently, Niamat et al. [24] found decreased water relativity in the form of decreased RWC, osmotic potential, and increased EL in maize grown under increasing salinity levels.

The scale of activity of antioxidant enzymes, i.e., APX, CAT, SOD and GPX, varied in normal and saline stress conditions. Indeed, increases in enzymatic activity were observed in non-amended soil, which might be associated with the increased salinity, indicating that the plants were under stress. As discussed earlier, the accumulation of unnecessary reactive oxygen species (ROS) may have caused oxidative stress in the plants. As a result, the cost of the plant's life synthesis is the antioxidant enzymes to cope with the toxicity of ROS [42,52]. Application of GP provided relief in the decrease in antioxidant enzymes, which might have ameliorated the negative effects of ROS [47]. Similarly, CCD application provided better results in lowering the antioxidant enzyme activity, which might have absorbed a fraction of the exchangeable sodium, reduced its accumulation in plant tissues, and improved nutrient uptake, and thus ameliorated the saline stress [2,24,43].

High concentrations of sodium in soil impede the uptake of essential plant nutrients, especially potassium, by interfering with transportation mechanisms such as the potassium channels [62,63]. Furthermore, plants tend to attain a higher concentration in  $K^+$  than in  $Na^+$  under stress [9]. We found increased  $K^+$  and decreased  $Na^+$  uptake by plants amended with both GP and CCD. This might be due to the increment of CEC in the soil amended with CCD, which might have restricted the entry of  $Na^+$  and boosted the uptake of  $K^+$  in plant tissues [64,65]. The current study reports maximum potassium concentration in leaves and seeds with the combined use of GP+CCD in both normal and salt-stressed soil conditions. Application of organic sources, like compost, farmyard manure, etc., might be an efficient strategy for enhancing crop growth, as it recovers the physical, chemical, nutritional, and biotic attributes of salt-exaggerated soils [66–68]. Additionally, the use of carbon-based sources, such as cow dung, upturns the cation exchange capacity (CEC) of soil, thus restraining sodium to the interchangeable position and eventually enhancing the contents of both soluble and interchangeable potassium [64,65,69].

Previously, it was reported that the use of composts and Ca-containing amendments reverted the negative impacts of  $\text{Na}^+$  ions in plants grown in saline soils [70]. This is specifically true regarding composted municipal solid waste, applied together with GP, which have been found to replace  $\text{Na}^+$  ions with  $\text{Ca}^+$  and hence alleviate salinity stress in plants [48]. In the present study, the applied CCD and GP might have replaced  $\text{Na}^+$  from exchange sites by  $\text{Ca}^+$  released during composting.  $\text{Na}^+$  was found to be at a minimum in the control (normal soil) and thereafter increased with increasing salinity levels. This is due to the high salt concentration in the soil solution, which increases the concentration of  $\text{Na}^+$  in the root zone. This causes cellular membrane damage, resulting in decreased efficiency of the plant's ion exclusion mechanisms, leading to poor growth and development [71,72]. It was clarified previously that the application of GP in the soil at a higher rate eliminates sodium ions ( $\text{Na}^+$ ) from soil columns and leads to a considerable decline in the EC and SAR of soil [73].

Under saline-sodic soil conditions, nutrient imbalances occur due to the toxicity of particular ions (mainly of  $\text{Na}^+$  and  $\text{Cl}^-$ ) and the scarcity of nitrogen, phosphorus, potassium, calcium, sulfur, manganese, and zinc in many crops [74,75]. The use of GP as a cradle for calcium is a common method to reclaim saline-sodic and sodic soils, and to improve water infiltration in soil for managing nutrients in salt-exaggerated soils. In previous studies, it was observed that a combination of GP and farmyard manure has enhanced the nutrient contents of crops [75–77]. In the current study, a concentration of plant nutrients (N, P, and K) considerably increased with the combined use of CCD and GP in normal and salt-stressed soils. This is in accordance with the previous studies [78,79].

The current study reports a lower  $\text{Na}^+/\text{K}^+$  ratio in leaves and seeds with the combined use of GP+CCD in both normal and salt-stress soil conditions. This is obviously because GP can react quickly with sodium chloride to produce sodium sulfate, which can efficiently leach from soils [80,81], whereas organic sources like straw compost can decrease the excess of interchangeable sodium out of the root zone via increasing the water penetration and discharge of salts [82,83]. Furthermore, the efficacy of GP in improving the absorption of calcium in plants has been verified [84–86]. In recent times, it has been proved that the collective application of leaf litter, sedge turf, and furfural (1:1:1 as per volume basis) considerably reduced sodium concentration, and increased CEC and the accessibility of nutrients, especially nitrogen, phosphorus, and potassium [65].

## 5. Conclusions

The present research work draws the conclusion that the combined use of gypsum and composted cow dung (CCD) considerably enhanced the growth, yield, physiology, and nutritional content of sunflowers. This treatment decreased the antioxidant assays and the  $\text{Na}^+/\text{K}^+$  ratio of the leaves and seeds of sunflowers in both normal and salt-stressed soil conditions, as compared to the control treatment and the separate use of CCD and GP. The research revealed the potential of composted cow dung as an effective ameliorant against salinity tolerance in sunflowers, which could be a possible environmentally friendly choice for farmers growing crops in marginal land.

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