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Macro-Mineral Uptake, Relative Water Content, Retention Capability, and Tolerance Index of Sunn Hemp (*Crotalaria juncea* L.) under Salinity Stress at Early Seedling

Gülcan Demiroğlu Topçu [1,*], Hazım Serkan Tenikecier [2] and Ertan Ateş [2]

[1] Field Crops Department, Faculty of Agriculture, Ege University, 35040 Izmir, Türkiye
[2] Field Crops Department, Faculty of Agriculture, Tekirdağ Namık Kemal University, 59030 Tekirdağ, Türkiye; hstenikecier@nku.edu.tr (H.S.T.); ertan_ates@hotmail.com (E.A.)
* Correspondence: gulcan.demiroglu.topcu@ege.edu.tr; Tel.: +90-(232)311-26-79

Abstract: Salt stress exerts adverse effects on yield by inhibiting or delaying seed germination and impeding seedling growth. Additionally, different salt concentrations have adverse effects on plant wet and dry weight and stem and shoot development. *Crotalaria juncea* L., the fastest-growing species within the *Crotalaria* genus, demonstrates a high degree of adaptability to both tropical and subtropical climates. To assess the tolerance of sunn hemp to salinity during the germination and early seedling stages, several indicators were determined at different (0, 50, 100, 150, 200, 250, and 300 mM) salt concentrations. Germination was conspicuously absent at salt concentrations of 250 mM and 300 mM. Notably, seedling characteristics, such as shoot length, root length, root fresh weight, seedling fresh weight, retention capability of the shoot, and the relative water content, experienced adverse effects with escalating salt concentrations. Intriguingly, the apex of seedling and root dry weights manifested at the pinnacle of salt concentration at 200 mM. Despite the discernible influence of heightened salt concentrations during the nascent seedling stage, the tolerance index was quantified at 100 mM, 150 mM, and 200 mM. Analyzing the study results through the lens of macro-minerals revealed an augmentation in Na and Cl content concomitant with increasing salt concentrations.

Keywords: *Crotolaria juncea* L.; sunn hemp; salinity; macro-mineral; salt tolerance

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1. Introduction

In recent decades, the synergistic impacts of climate change have resulted in water scarcity, soil contamination, and elevated levels of soil and water salinity. The depletion of viable arable land and the burgeoning human population pose significant threats to agricultural sustainability [1]. As long as these continue, the stress on plants increases and species extinction accelerates. Stress in the context of plant physiology can be categorized into two main types: biotic and abiotic stress [2]. Elevated soil salinity or high salt concentrations in irrigation water pose significant environmental challenges for agriculture globally, serving as primary abiotic stresses that restrict crop growth [3,4]. Abiotic stress, specifically during the germination phase and early seedling stage, exerts detrimental effects on plant growth and development, particularly when it comes to salt stress [5,6]. Salt stress is a multifaceted phenomenon encompassing osmotic stress, toxic ion imbalances, and perturbations in nutrient homeostasis, and it is a crucial challenge that plants need to address at the morphological, anatomical, and molecular levels [7].

Salinity, as an abiotic stressor, stands out as a prominent factor that restricts plant growth and development [8]. In response to salinity stress, plants undergo various morphological, physiological, and metabolic adjustments that can lead to a substantial reduction in crop yield and quality [9,10]. Thus, salinity is recognized as one of the most pervasive soil-related stress conditions globally, significantly impeding plant growth and productivity [11]. Factors such as climatic conditions, impermeable soil layers, erosion, and improper irrigation methods contribute to soil salinity and degradation. Given the challenges and expenses involved in ameliorating saline soils, it is imperative to identify and cultivate plant species suitable for these conditions [12].

Salinity detrimentally affects various aspects of plant biology, including growth, water uptake, and seed germination [12]. The limiting effects of salinity on plant growth and productivity primarily result from the toxic consequences of sodium (Na^+) and chloride (Cl^-) ions that disrupt ionic balance and induce osmotic and oxidative stress [13]. Salinity reduces the osmotic potential, making it more challenging for plant roots to absorb water [14]. As salt concentrations increase, germination time lengthens, while shoot and root length decrease [15].

Salinity stress exerts detrimental effects on various developmental stages throughout the plant lifecycle, encompassing seed germination, seedling establishment, and subsequent growth [16]. In the context of most grain crops, salt stress exerts adverse effects on yield by inhibiting or delaying seed germination and impeding seedling growth [17–20]. Additionally, researchers have indicated that different salt concentrations have adverse effects on plant wet and dry weight and stem and shoot development [21–24].

Climate plays a substantial role in influencing the salt tolerance of plants. Generally, salinity has a more detrimental impact under hot and arid conditions compared to cooler and more humid environments [25]. In addition to salinity tolerance, plants must also exhibit tolerance to heat and drought stress, since a significant portion of salinized areas worldwide are concentrated in hot and arid regions [26]. In regions characterized by high salinity levels, the selection of high-tolerance plant varieties is crucial for achieving optimal yields [27].

The genus *Crotalaria* encompasses approximately 600 species and belongs to the tribe *Genisteae* [28]. The distribution of *Crotalaria* species spans primarily tropical and subtropical regions, with limited representation in temperate climates. They thrive in regions at altitudes below 600 m in temperate locales. *Crotalaria juncea* L., a member of the *Fabaceae* family, is a monocarpic plant with diverse utility and global cultivation. *Crotalaria juncea* L. species have been historically cultivated across various countries, particularly the United States, for both soil conservation and enhancement, as well as fodder production [29]. *Crotalaria juncea* L., commonly known as sunn hemp or Indian hemp, is regarded as indigenous to India and serves multiple purposes, including soil amelioration, fiber production, and fodder-crop utilization. In tropical regions, notably Indonesia, Malaysia, Taiwan, Thailand, and China, it is extensively cultivated as a green manure crop. Within South Asian nations, *Crotalaria juncea* L. has stood as a pivotal fiber crop for centuries, contributing to rope and paper manufacture [30]. Sunn hemp is native to India and cultivated in different parts of the world. India, Brazil, Bangladesh, Pakistan, China, and Korea are the major producers of sunn hemp [31] and as a cover crop and green manure in regions such as Hawaii, Brazil, and South Africa [32]. Additionally, *Crotalaria* seeds can be utilized in the production of biodiesel through transesterification processes [33].

Crotalaria juncea L., the fastest-growing species within the *Crotalaria* genus, demonstrates a high degree of adaptability to both tropical and subtropical climates [14,34]. It was said that sunn hemp is more productive in high humidity, is well adapted to humid areas, is not winter hardy, and has low to moderate tolerance to saline soils [35].

Salt tolerance is particularly critical during germination, as elevated soil salinity in the vicinity of the soil surface can impede growth [36,37]. The decline in germination is attributed to the elevation in the osmotic pressure of the soil solution, leading to delayed imbibition and constraining the water absorption necessary for metabolic processes [38]. Saline soil often harbors elevated concentrations of Na^+, Cl^-, and SO_4^{2-}, which can adversely impact plant growth by inducing osmotic stress and ionic toxicity. These conditions disrupt plant nutrient uptake and metabolism, leading to imbalances within the plant [4]. The objective of this research was to assess the macro-mineral uptake, relative water content, retention capability, and tolerance index of sunn hemp under salinity during germination and in an early stage.

2. Materials and Methods

2.1. Experimental Design

The experiment was carried out in a growth chamber (Mikrotest, −20 to +70 °C) using petri dishes with four replicates, following a completely randomized design to investigate the impact of salinity on sunn hemp seeds during germination and in an early stage.

2.2. Germination Tests

Crotalaria juncea L. seeds (cv. Tillage Sun) were used as the material to determine the macro-mineral uptake, relative water content, retention capability, and tolerance index under salinity stress at the early seedling stage.

The seeds were soaked in hot water for 30 min [39]. Subsequently, the seeds were sterilized with a 1.5% sodium hypochlorite solution for 15 min [40,41] and rinsed with sterilized distilled water three times. Fifteen seeds were placed between Whatman No. 1 filter paper in 9 mm diameter petri dishes. The papers were soaked with Hoagland nutrient solution, and 20 mL of sterilized distilled water were added at seven different concentrations of NaCl, namely 0 (control), 50, 100, 150, 200, 250, and 300 mM. The petri dishes were placed in a growth chamber at 25 °C with a 16 h light and 8 h dark photoperiod for germination assessment at 4 days and early shoot-stage evaluation at 10 days [42]. Seed germination was defined as the emergence of a radicle exceeding 1 mm, and germinated seeds were counted to calculate the germination rate and mean germination time [43].

2.3. Determination of Tolerance Indices

Subsequently, after ten days of seedling emergence, ten seedlings were selected, and their roots and shoots were separated [44]. Measurements were taken from the root crown to the apex of the root to determine the root length and from the root crown to the apex of the shoot to determine the shoot length [45]. Roots and shoots were weighed to determine the root fresh weight and shoot fresh weight; root and shoot samples were oven-dried at 55 °C for 48 h to determine root dry weight and shoot dry weight [46].

The retention capability of the shoot (RCS) (mg) was determined according to [47] by using the formula:

$$\text{RCS (mg)} = (\text{SFM} - \text{SDM})/\text{SDM} \quad (1)$$

SFM: shoot fresh matter, SDM: shoot dry matter.

The relative water content (RWC) (%) was calculated according to [48] by using to formula:

$$\text{RWC (\%)} = (\text{SFM} - \text{SDM}/\text{SFM}) \times 100 \quad (2)$$

The tolerans index (TI) was determined according to [49] by using the formula:

$$\text{TI} = (\text{SDM in salinity stress}/\text{SDM in control}) \times 100 \quad (3)$$

2.4. Chemical Analyses

The oven-dried samples were ground into small (≤1 mm) pieces. The nitrogen (N) content of the sunn hemp's shoots was determined with the micro-Kjeldahl method according to [50]. The Na^+ concentrations were determined using a flame photometer (Shanghai Precision and Scientific Instrument Co., Ltd., Shanghai, China, 6400 A type) following the methods described by [46,48]. The samples were wet-fired with nitric-perchloric acid, and the phosphorus (P) content (%) was determined spectrophotometrically, while the potassium (K, %), calcium (Ca, %), magnesium (Mg, %), copper (Cu, ppm), zinc (Zn, ppm), manganese (Mn, ppm) and iron (Fe, ppm) contents were obtained using an atomic absorption spectrophotometer (ICP-OES, inductively coupled plasma–optical emission spectrometer, PerkinElmer Inc., Optima 5300 DV, Markham, ON, Canada) [51,52]. K^+/Na^+ ratios of shoots were calculated. All samples were analyzed in duplicate.

2.5. Statistical Analyses

Statistical analysis was performed using the TARIST (V 4.0) [53] statistical software package, and Fisher's least significant difference (LSD) test was used for post hoc comparisons [54]. The principal component analysis (PCA) was carried out using R Statistical Environment (V 4.3.1) with the FactoMiner library [55].

3. Results

This study investigated the impact of different salt concentrations on germination, macro-mineral uptake, and various physiological attributes of sunn hemp seeds. Given that germination failed at 250 mM and 300 mM salt concentrations, analyses were conducted at 0 mM, 50 mM, 100 mM, 150 mM, and 200 mM salt concentrations.

The germination rates varied between 93.33 and 100.00%, and the highest was determined at a 100 mM concentration (Table 1). The mean germination times of the sunn hemp seeds were affected by the salt concentrations. The fastest mean germination times were observed at 100 mM and 150 mM (1.92 and 1.93 days). The slowest germination was determined at the highest concentrations of the experiment (150 mM and 200 mM) (Table 1). The shoot length, root length, and root fresh weight were influenced negatively by the increasing salt concentrations. The shoot lengths, root length, and root fresh weights of the sun-hemp shoots were varied between 1.94–5.56 cm, 0.65–4.24 cm, and 14.67–54.94 mg respectively. The highest shoot length, root length, and root fresh weight were determined at 0 mM, the second highest at 50 mM (4.34 cm, 3.03 cm, and 44.46 mg), and the lowest at 200 mM (Table 1). The highest dry weights were obtained from 0 mM (3.14 mg), 100 mM (3.03 mg), and 150 mM (2.93 mg) (Table 1).

Table 1. Some germination and seedling characteristics, retention capability of the shoot, relative water content, and tolerance index of sunn hemp. (means with different letters (in the rows) are significant at $p < 0.01$).

Characteristics	Salinity Level (mM NaCl)						
	0	**50**	**100**	**150**	**200**	**Mean**	**$LSD_{0.01}$**
Germination rate (%)	95.00 bc	96.66 b	100.00 a	93.33 c	96.66 b	96.33	2.97
Mean germination time (day)	2.00 b	1.92 c	1.98 bc	2.04 ab	2.07 a	2.00	0.07
Shoot length (cm)	5.56 a	4.34 b	4.08 b	3.26 c	1.94 d	3.84	0.39
Root length (cm)	4.24 a	3.03 b	1.75 c	0.81 d	0.65 d	2.10	0.17
Root fresh weight (mg)	54.94 a	44.46 b	39.93 b	28.03 c	14.67 d	30.34	5.39
Root dry weight (mg)	3.14 a	2.49 b	3.03 a	2.93 a	1.77 c	2.67	0.31
Shoot fresh weight (mg)	283.93 a	217.71 d	265.07 b	240.13 c	177.15 e	236.80	14.03
Shoot dry weight (mg)	20.47 b	21.13 b	25.80 a	23.67 ab	25.37 a	23.29	3.48
Retention Capability of Shoot (RCS) (mg)	12.90 a	9.42 b	9.30 b	9.15 b	6.02 c	9.36	1.76
Relative Water Content (RWC) (%)	92.79 a	90.30 b	90.25 b	90.14 b	85.61 c	89.82	2.02
Tolerance Index (TI)	100.00 c	103.65 bc	126.41 a	115.91 abc	124.15 ab	114.02	20.72

The shoot fresh weights of the sunn hemp at the different salt concentrations varied between 177.15 and 283.93 mg. In the environment where there was no salt effect (0 mM), the highest shoot fresh weight was determined, while at the highest concentration of 200 mM, the lowest shoot fresh weight was observed (Table 1). The retention capability of the shoot and the relative water content responded negatively to increasing salt concentrations. The highest retention capability of the shoot and the relative water content were obtained from 0 mM (12.90 mg and 92.79%) and the lowest were determined at the highest salt concentration (200 mM) (9.36 mg and 89.82%) (Table 1). The tolerance index was the highest at 100 mM (126.41) and the lowest at 0 mM (100.00) (Table 1).

Nitrogen means were found statistically significant at $p < 0.05$. All other minerals' means exhibited statistically significant variations ($p < 0.01$) in response to different salt concentrations. The nitrogen contents fluctuated at increasing salt concentrations. However, the highest nitrogen contents were determined at 50 mM (5.47%) and the lowest was at

0 mM (5.35%) concentration (Figure 1). The P and Ca content of sunn hemp shoots were not significantly affected by the salt concentrations (Figures 1 and 2).

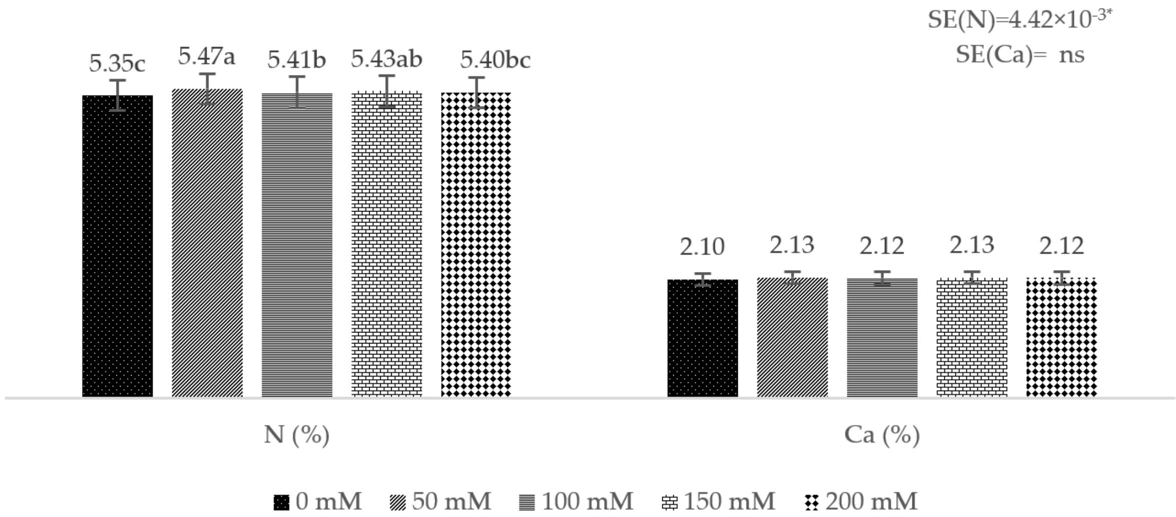

Figure 1. Nitrogen and calcium content of sunn hemp shoots. (*: Different letters indicate significant differences ($p < 0.05$) between the means associated with salt concentrations, ns: non-significant).

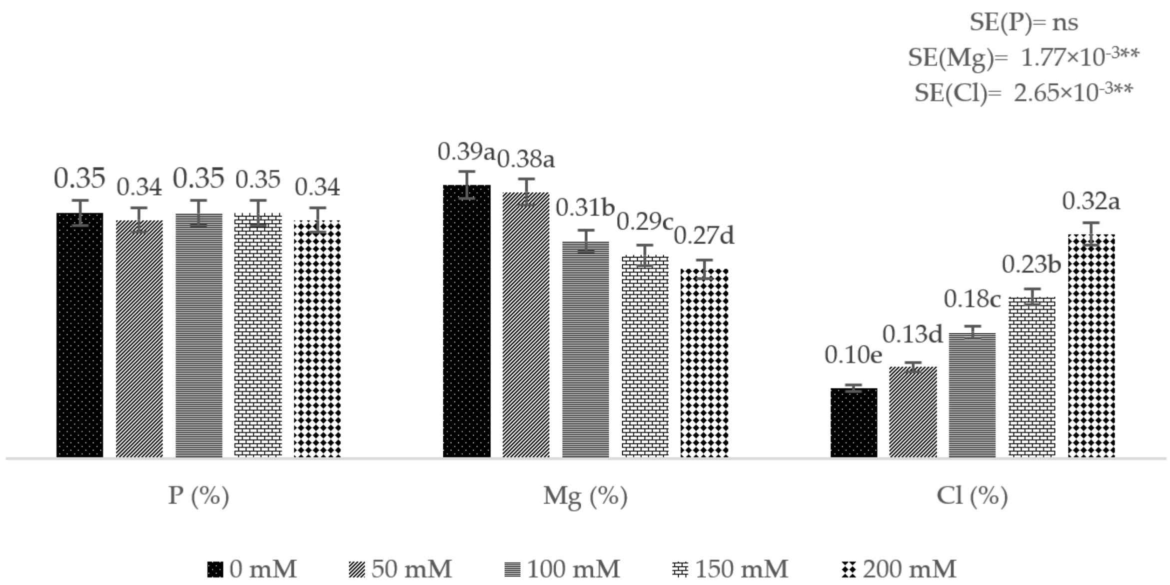

Figure 2. Phosphorus, magnesium, and chlorine content of sunn hemp shoots. (**: Different letters indicate significant differences ($p < 0.01$) between the means associated with salt concentrations).

The K, Mg, Na, and Cl concentrations varied between 1.07–1.36%, 0.27–0.39%, 0.13–0.73%, and 0.10–0.32%, respectively (Figures 2 and 3). The highest Mg contents were determined at 0 mM and 50 mM salt concentrations. The Cl contents raised with the salt concentrations, as expected. The lowest Cl concentration was determined at 0 mM (0.10%), and the highest was at 200 mM (0.32%). (Figure 2). The highest K contents were determined at 0 mM, 50 mM, and 100 mM (1.36%, 1.35%, and 1.34%) respectively. The lowest K content was determined at the highest salt concentration (200 mM, 1.07%) (Figure 3). The K^+/Na^+ ratio was determined to be the highest at 0 mM (10.85) and the lowest at 200 mM (1.48) (Figure 4).

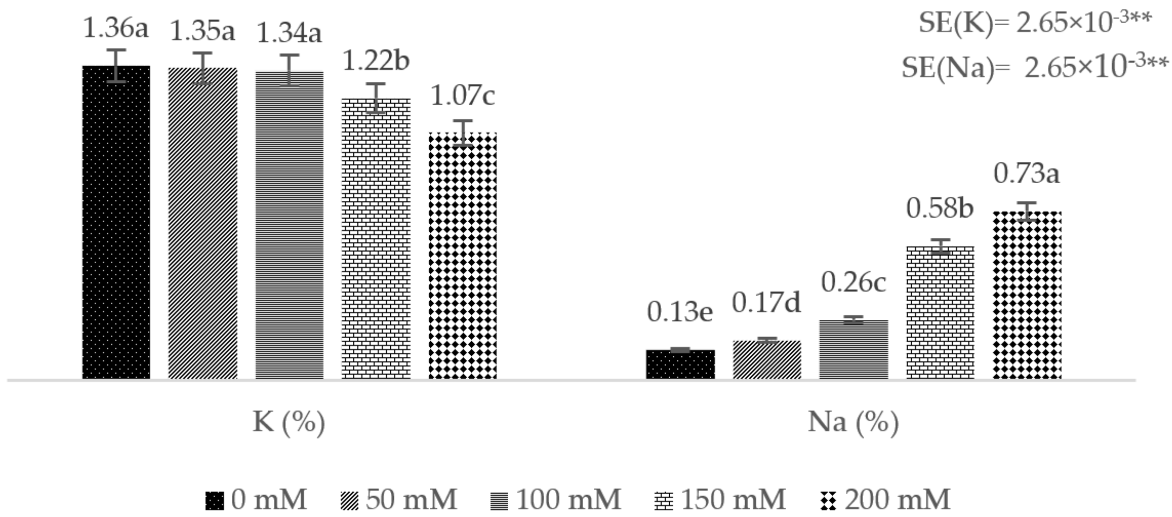

Figure 3. Potassium and sodium content of sunn hemp shoots. (**: Different letters indicate significant differences ($p < 0.01$) between the means associated with salt concentrations).

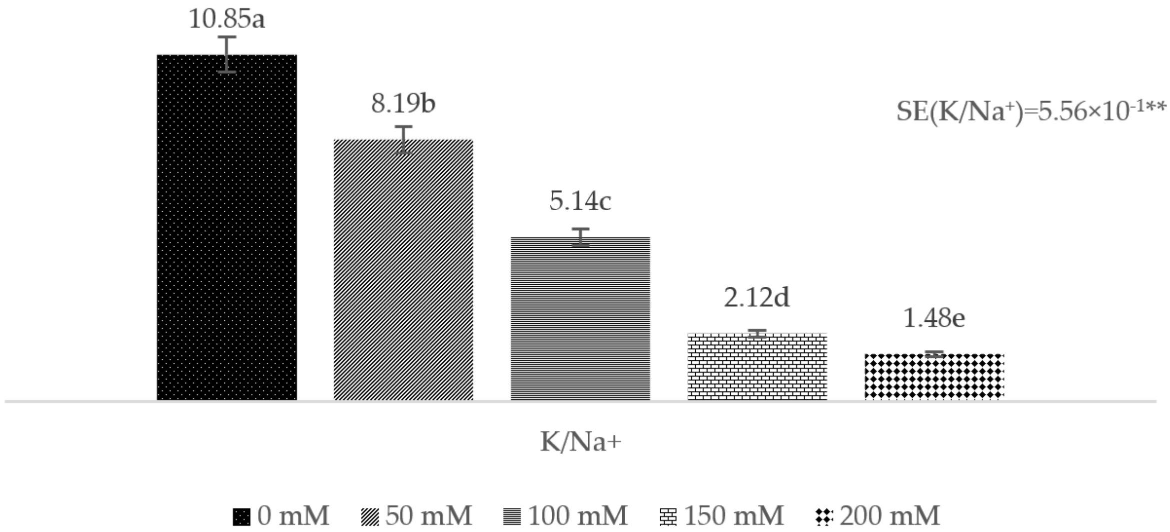

Figure 4. K/Na^+ of sunn hemp shoots. (**: Different letters indicate significant differences ($p < 0.01$) between the means associated with salt concentrations).

The correlation between germination, seedling characteristics, macro-minerals, and the tolerance indices of sunn hemp shoots was ascertained. The entire experimental data were subjected to a principal component analysis based on the clustering method (Figure 5). The PCA loading plot revealed that Dim1 and Dim2 accounted for 93.00%, 82,30%, 95.30% 87.10%, and 91.40% of the total variation among the studied parameters at 0 mM, 50 mM, 100 mM, 150 mM, and 200 mM, respectively. At 0 mM, GR, MGT, Cl, P, K, N, Ca, and K/Na were positively correlated. At 50 mM, Mg, Na, Ca, Cl, N, P, and K were positively correlated. At 100 mM, Na, P, Ca, K, N, Cl, and RFW were positively correlated. At 150 mM, RWC, RCS, RL, RFW, and RDW were positively correlated. AT 200 mM, RCS, RWC, SL, SFW, GR, and RDW were positively correlated.

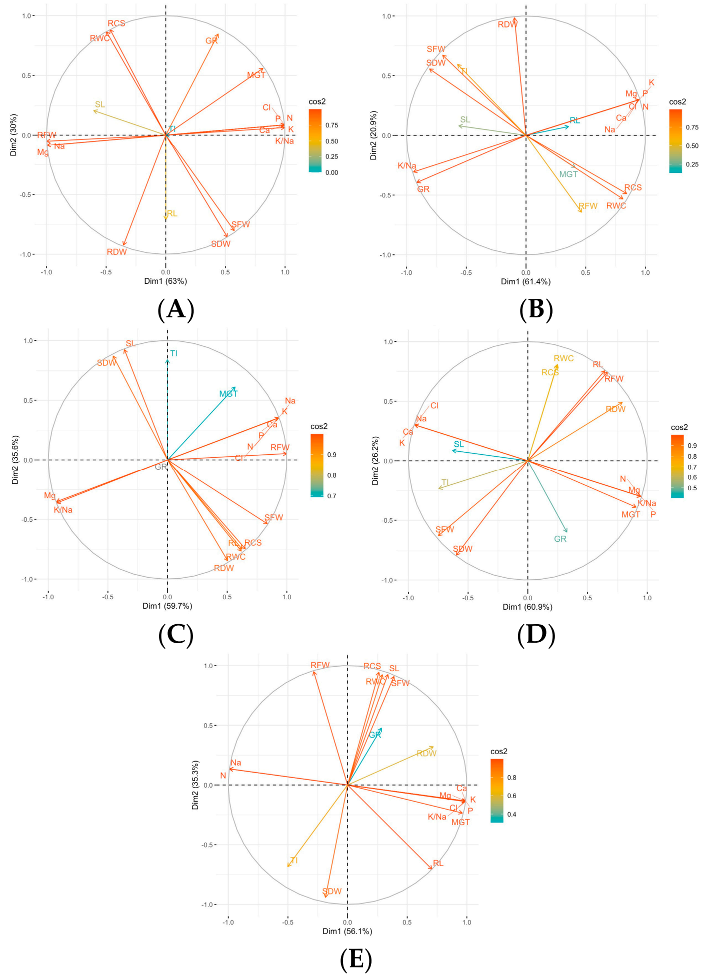

Figure 5. Principal component analysis of sunn hemp seedlings under five salt concentrations 0 mM (**A**), 50 mM (**B**), 100 mM (**C**), 150 mM (**D**), and 200 mM (**E**). The lines starting from the central point of the biplots display negative or positive associations of different variables, and their proximity specifies the degree of correlation with a specific treatment. GR: germination rate, MGT: mean germination time, SL: shoot length, RL: root length, RFW: root fresh weight, RDW: root dry weight, SFR: shoot fresh weight, SDW: shoot dry weight, RCS: retention capability of shoot, RWC: relative water content, TI: tolerance index, N: nitrogen, P: phosphorus, K: potassium, Ca: calcium, Mg: magnesium, Na: sodium, Cl: chlorine and K/Na: K^+/Na^+ rate.

4. Discussion

Pavli et al. [56] declared that the increasing salt concentrations severely affected the germination and seedling growth of soybean. They determined the germination rate on day seven as 42.22%, 33.98%, 27.04%, and 15.56% in 10 genotypes of soybean at 0 mM, 50 mM, 100 mM, and 200 mM salt concentrations, respectively. Those researchers also found the water content to be 72.7%, 66.4%, 64.35%, and 62.73% at the same concentrations on day seven. Furthermore, they announced that the length of roots decreased proportionally to the stress level, and the growth of shoots was strongly inhibited at increasing salt concentrations. Okcu [57] in the study investigating the effects of different salt concentrations on germination and seedling development in forage cowpeas at doses of 0–270 mM, stated that the germination rate varied between 30.20 and 98.60%, and the highest value was determined in the control group, while the highest average germination time varied between 2.00 and 3.36 days, and reported that germination times were determined at concentrations of 210, 240, and 270 mM. Nóbrega et al. [58] have found a shoot length of 7.46 cm and a root length between 2.68 and 3.14 cm without any implementation. Ates and Tekeli [48] determined the germination rate on day seven at 100,00%, 100.00%, and 7.17% at 0 mM, 100 mM, and 150 mM in three different Persian clover lines, respectively. They reported shoot lengths of 21.05 cm, 20.20, and 10.10 cm, and root lengths of 10.47 cm, 10.67 cm, and 4.52 cm at the same concentrations. Certain morphological alterations induced by salinity stress include a reduction in both root and shoot length, accompanied by restricted rooting [59–61]. The researchers determined the shoot fresh weight to be 12.20 mg, 12.20 mg, 16.27 mg, 15.40 mg, 8.50 mg, and 5.26 mg, the shoot dry weight 4.08 mg, 4,02 mg, 4,19 mg, 3,98 mg, 2,56 mg, and 1.41 mg, the relative water content of the shoot 66.67%, 67.00%, 74.00%, 74.00%, 70.00%, and 72.00%, the Na^+/K^+ rate of the shoot 0.37, 0.40, 0.40, 0.45, 0.46, and 0.55, the root fresh weight 4.44 mg, 4.12 mg, 3.82 mg, 2.57 mg, 1.86 mg, and 1.1 mg, and the root dry weight 1.47 mg, 1.39 mg, 1.26 mg, 0.83 mg and 0.41 mg, at 0 mM, 1 mM, 10 mM, 50 mM, 100 mM, and 150 mM concentrations, respectively. They also obtained the tolerance index of Persian clovers at 100 mM between 0.97 and 1.03 and 150 mM between 0.40 and 0.48. The results on soybean, cowpea, and Persian clover are similar to the research results. These findings showed that the increasing salt concentration has negative effects on different plant species at germination and early stages.

The 14 inorganic elements necessary for plants to complete a full lifecycle are referred to as essential plant nutrients. These nutrients are categorized into macronutrients and micronutrients based on their concentration in plant dry matter. The macronutrients include nitrogen (N), phosphorus (P), sulfur (S), potassium (K), calcium (Ca), and magnesium (Mg) [62]. These nutrients also serve diverse functions as ions or constituents of inorganic compounds within plant physiology [63]. Soil salinity commonly suppresses plant growth and reproduction through an initial phase of osmotic stress, followed by ionic toxicity resulting from the accumulation of Na^+ and Cl^- ions within the cell cytosol. This ultimately leads to oxidative stress and nutritional deprivation [64,65]. Oxidative stress perturbs the equilibrium between the production of reactive oxygen species and their scavenging, resulting in damage to cell membranes and leakage of ions [66,67]. Sodium ions (Na^+) are non-essential for plant growth, and excessive concentrations of these ions are toxic to most plant species. The heightened presence of Na+ in salt-stressed plant tissues often hampers the uptake of other essential nutrients, such as K^+, Ca^{2+}, and Mg^{2+}, thereby leading to nutrient deficiencies [68,69]. Mineral deficiencies hinder plant growth by restricting the biosynthesis or expression of vital components involved in energy capture and metabolism. As mineral deficiencies impair both plant growth and metabolism, their most notable consequence in the context of agronomically significant crop plants is a decrease in harvest yields or, in certain instances, the complete loss of the crop [70]. Salt (NaCl) uptake in high concentrations competes with the other nutrient ions' uptake, especially potassium, leading to potassium deficiency. The increasing NaCl treatment induces an increase in Na^+ and Cl^- and a decrease in Mg^{2+}, K^+, and Ca^{2+} levels in several plants [71–73]. Salinity enhances the content of Na^+, Ca^{2+}, and Cl^- in *Vicia faba* L., and the ratio of K^+/Na^+ decreases [74]. A positive correlation is observed between Na^+ and Cl^- concentrations, while a negative correlation is evident between Na^+ and K^+ concentrations in both roots and leaves. The concentration of Mg^{2+} remains unaltered in both leaves and roots, irrespective of changes in the Na^+ concentration. Similarly, the concentration of Ca^{2+} exhibits no variation with the Na^{2+} concentration in leaves; however, it demonstrates an inverse relationship in the roots. [75]. Salt stress directly damages plants by inducing ionic stress and disrupting ionic homeostasis. The accumulation of Na^+ in plants under salt stress perturbs metabolic processes, particularly in environments characterized by low Na^+ and high K^+ and Ca^{2+} concentrations [76]. Kadam Pratima [77] declared that root and shoot length, leaf area, number of leaves, fresh and dry weight, leaf succulence, leaf thickness, relative water content (RWC), and 100 seed weight were affected by salinity and revealed that higher salt concentrations lead to the reduction in plant height in *Crotalaria* species. The shoot length was increased at 100 mM NaCl salinity. The results of the study and the results of different species show that the early development of plants is important in creating strong seedlings. Mbarki et al. [78] stated germination and early growth stages represent the most sensitive phases to be affected by salinity. Although sprouting alone may not suffice to identify salt-stress-tolerant genotypes, varieties exhibiting tolerance to salinity during germination typically maintain resistance in subsequent growth stages.

5. Conclusions

Sunn hemp is a species that grows in tropical and subtropical climates, with moderate tolerance to saline soils. The research findings indicate that saline conditions have a detrimental impact on the germination and seedling phases of *Crotalaria juncea* L. Germination was conspicuously absent at salt concentrations of 250 mM and 300 mM. Notably, seedling characteristics, such as shoot length, root length, root fresh weight, shoot fresh weight, retention capability of the shoot, and relative water content experienced adverse effects with escalating salt concentrations. The tolerance index was quantified at 100 mM, 150 mM, and 200 mM. Analyzing the study results through the lens of macro-minerals revealed an augmentation in Na and Cl content concomitant with increasing salt concentrations. Considering the increasing effects of climate change and the growing environment of sunn hemp, it is important to understand the plant's response to salinity early in its development. Along with the study results, the mineral uptake under saline conditions has been elucidated. The study results have significant implications for conducting further research under field conditions. It may be advisable to prefer cultivation in saline areas with concentrations of 100 mM and below for robust early-stage plant development.

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