The Impact of Salt Stress on Plant Growth, Mineral Composition, and Antioxidant Activity in *Tetragonia decumbens* Mill.: An Underutilized Edible Halophyte in South Africa

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Abstract: Climate change, expanding soil salinization, and the developing shortages of freshwater have negatively affected crop production around the world. Seawater and salinized lands represent potentially cultivable areas for edible salt-tolerant plants. In the present study, the effect of salinity stress on plant growth, mineral composition (macro-and micro-nutrients), and antioxidant activity in dune spinach (*Tetragonia decumbens*) were evaluated. The treatments consisted of three salt concentrations, 50, 100, and 200 mM, produced by adding NaCl to the nutrient solution. The control treatment had no NaCl but was sustained and irrigated by the nutrient solution. Results revealed a significant increase in total yield, branch production, and ferric reducing antioxidant power in plants irrigated with nutrient solution incorporated with 50 mM NaCl. Conversely, an increased level of salinity (200 mM) caused a decrease in chlorophyll content (SPAD), while the phenolic content, as well as nitrogen, phosphorus, and sodium, increased. The results of this study indicate that there is potential for brackish water cultivation of dune spinach for consumption, especially in provinces experiencing the adverse effect of drought and salinity, where seawater or underground saline water could be diluted and used as irrigation water in the production of this vegetable.

Keywords: dune spinach; NaCl; functional food; salt tolerance; underexploited vegetable

1. Introduction

Global agriculture feeds over 7 billion people and alarmingly, this number is expected to increase by a further 50% by 2050 [1]. The global need for food production has never been greater, especially in developing nations where an increase of 90% of the population growth anticipates that food insecurity will become a greater problem [2,3]. To meet the additional food demand, the world development report has estimated that crop production should increase between 70% and 100% by 2050 [4]. However, increasing crop production has led to a loss of soil fertility and the phenomena of salinization and desertification, which makes soils unsuitable for cultivation [5]. This is caused by the accumulation of soluble salts in the root zone. These salts restrict the absorption of water by the plant roots, which leads to osmotic stress and thus nutritional imbalance due to the high concentration of toxic salts in plant cells [6,7]. In addition, the accumulation of toxic ions inhibits physiological processes such as photosynthesis, respiration, and nitrogen fixation [8]. These effects can result in reduced leaf area, plant biomass production, and yield [9,10]. Moreover, soil salinity and drought stress are known factors to induce oxidative stress in plants through the production of superoxide radicals by the process of the Mehler reaction [11]. These free radicals initiate the chain of reactions that produce more harmful oxygen radicals [12]. These reactive oxygen species (ROS) are continuously generated during normal metabolic processes in mitochondria, peroxisomes, and cytoplasm, which disturb normal metabolism through oxidative damage of lipids, proteins, and nucleic acids when produced in excess [13,14].
To overcome salt-mediated oxidative stress, plants detoxify ROS by up-regulating antioxidative enzymes, which includes the superoxide dismutase (SOD) found in various cell compartments [15]. This enzyme catalyzes a conversion from two $O_2$ radicals to $H_2O_2$ and $O_2$ [16]. In alternative ways, several antioxidant enzymes can also eliminate the $H_2O_2$, such as catalases (CAT) and peroxidases (POX), by converting it to water [17]. During this process, the antioxidant capacity of some species increases when exposed to salinity stress to eliminate or reduce the ROS. Thus, research on oxidative stress is imperative due to the usefulness of these antioxidants against free radicals that predispose humans to sickness and diseases. The author of [18] stated that these antioxidants exert a large spectrum of biological and physiological functions on human health, such as anti-allergic, anti-atherogenic, anti-inflammatory, and anti-microbial activities.

The catastrophic effect of salinity and drought on crop yield call for a creative, sustainable, and sufficient crop production method, given the rising population and increasing demand for plant-based food [19,20]. With this in mind, numerous researchers have pointed out the use of salt-tolerant plant species with possible commercial value as a proposed up-front strategy for saline lands [21,22]. This led to a worldwide interest in edible salt-tolerant plant species in addressing the challenges of food and nutritional deficiency. Currently, underutilized edible halophytes are slowly becoming a viable alternative to popular crops in regions experiencing the adverse effect of drought and salinity [23]. Moreover, edible halophytes have been reported to be rich in nutrients and bioactive compounds [24], which are considered as important mediators of various health effects [25]. The medicinal value of edible halophytes has been documented and proven for prophylaxis against various chronic diseases that afflict modern societies [26].

*Tetragonia decumbens* commonly known as ‘dune spinach’ or ‘duinespinasie’ (Afrikaans) is an edible halophyte belonging to the Aizoaceae family and is largely distributed along the coastal regions from southern Namibia to the Eastern Cape [27,28]. It is an endemic sprawling perennial shrub with branches (runners) that can grow up to 1 m long [29]. The leaves and soft stems have a salty taste and can be used like spinach, served raw in green salads, or cooked with other vegetables. They can also be fermented, pickled, and used in stews and soups and are particularly tasty in a stir fry. However, the leaves and soft stems are foraged rather than cultivated and are known only by a small group of local chefs and food enthusiasts [30]. Thus, there is a need for agronomical studies to support its domestication and ensure its sustainable use. The cultivation of this native halophyte for food production in South Africa could be a climate change adaptation strategy, as freshwater continues to become scarce and rain becomes more sporadic particularly in sub-Saharan Africa [31,32]. Moreover, it has also been stated that South Africa is approaching physical water scarcity by 2025, and its agricultural sector has been directly hampered by the recent drought [33]. Hence, it is of utmost importance to cultivate crops that are adapted to harsh conditions within the framework of saline agriculture [34].

This study was therefore undertaken to evaluate the effect of salt stress on plant growth, mineral composition, and antioxidant activity in dune spinach, to lay a potential growing protocol for the use of brackish water or saline soil. Moreover, the dearth of literature on the nutritional value of this halophyte under saline conditions is a contributing factor to its underutilization and consumption among coastal households. Hence, data from this study are expected to serve as a template for future researchers, households, and potential farmers, who may want to exploit this plant for diet diversity and as pharmaceutical precursors.

2. Materials and Methods

2.1. Experimental Location

The experiment was conducted in the greenhouse of the Department of Horticultural Sciences at the Cape Peninsula University of Technology (CPUT), Bellville campus, Cape Town, South Africa, located at $33^\circ 55' 56''$ S, $18^\circ 38' 25''$ E. The greenhouse was equipped with environmental control with temperatures set to range from 21 to 26 $^\circ$C during the
day and 12–18 °C at night, with relative humidity averages of 60%. The average daily photosynthetic photon flux density (PPFD) was 420 µmol/m²/s and the maximum was 1020 µmol/m²/s.

2.2. Plant Preparation, Irrigation and Treatments

Softwood cuttings of *T. decumbens* were harvested on 1 August 2019 from a selected plant population growing along the coast at the Granger Bay campus of CPUT located at 33°53'58.2'' S, 18°24'41.4'' E. Only cuttings taken using homogeneous methods, i.e., stem cuttings with about two-thirds of leaves removed, ±15 cm long with a stem thickness of approx. 8 mm were used for the experiment. One hundred cuttings were made to ensure the minimum number of 60 rooted cuttings required for the experiment was available. The cuttings were then soaked in 0.1% Sporekil™ for precaution against fungal infection and, thereafter, were dipped in a rooting hormone (Dynaroot™ No. 1 with active ingredient 0.1% I.B.A) for two seconds. The cuttings were then placed in trays containing washed and sterilized coarse river sand and peat of equal volume. The trays were then placed in the main greenhouse on heated propagation beds. Once rooted, 80 *T. decumbens* cuttings of uniform size were individually transplanted in 12.5 cm plastic pots containing a mixture of commercial peat and sand (V:V) and placed in a greenhouse to acclimatize. Only cuttings showing the strongest growth were selected and left to grow for two weeks. During this period, rooted cuttings were irrigated with a nutrient solution three times a week. The nutrient solution was formed by adding NUTRIFEED™ (manufactured by STARKE AYRES Pty. Ltd. Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, South Africa, 1619) to municipal water at 10 g per 5L. The nutrient solution contained the following ingredients: N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg), and Zn (240 mg/kg). After 14 days of growth, the established cuttings were watered with clean water for 5 days to wash off any salt residue and, thereafter, were organized into 4 treatments each containing 15 replicates. Salt concentrations were set up on three treatments by adding increasing concentrations of NaCl in the nutrient solution (50, 100, and 200 mM). A total of 300 mL of the nutrient solution was prepared for each plant with and/or without NaCl. The plants were then watered every three days. The control treatment was sustained and irrigated only by the nutritive solutions. In all of the treatments, the pH was maintained at 6.0. Ten weeks after salt treatments, all plants were harvested, and various postharvest measurements were made.

2.3. Determination of Plant Growth

2.3.1. Plant Weight

The weight of the plants was measured using a standard laboratory scale (RADWAG® Model PS 750.R2) before planting out to ensure homogeneity within the samples. Post-harvest, shoots, stems, and roots were separated, and the fresh/wet weights of the individual samples were recorded. The plant material was then oven-dried at 55 °C in a LABTECH™ model LDO 150F (Daian Labtech India. Pty. Ltd. 3269 Ranjit Nagar, New Delhi, India) to a constant weight and recorded. The difference between the fresh and dry weight was compared with the amount of water held within the plants’ tissues [35,36].

2.3.2. Shoot Length and Branch Number

The shoot length and branch number were used as a variable to determine new growth. Shoot length was measured every two weeks with a metal tape measure from the substrate level to the tip of the tallest shoot, while branch number was counted [37].

2.4. Mineral Analysis

To determine the mineral composition of each set of replicates in the experiment, three plants (shoots/leaves) were randomly selected from each treatment at the end of
the experiment. The vegetative material was then removed, labelled, and sent to Bemlab Laboratory, located at 16 van der Berg Crescent, Gant’s Centre, Strand, Cape Town for mineral analysis. The methodology to determine macronutrients (N, K, P, Ca, Mg, and Na) and micronutrients (Cu, Zn, Mn, Fe, Al, and B) was conducted by ashing 1 g ground sample of plant material in a porcelain crucible at 500 °C overnight. This was followed by dissolving the ash in 5 mL of HCl and placing it in an oven at 50 °C for 30 min. Thirty-five milliliters of deionized water was then added and the extract filtered through Whatman No. 1 filter paper. Nutrient concentrations in plant extracts were determined using an inductively coupled plasma (ICP) emission spectrophotometer (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, MA, USA) [38,39].

2.5. Chlorophyll Readings

The chlorophyll content was measured every two weeks using a Soil Plant Analysis Development (SPAD-502) meter supplied by Konica Minolta. The readings of two fully formed leaves were taken from each plant, and the figures were averaged out by the SPAD-502 meter to produce a final number. The readings were taken between 11 a.m. and midday from week 4 to 10 of the experiment [40].

2.6. The Antioxidant Analysis

2.6.1. Sample Preparation

Harvested shoot materials were immediately dried in a fan-drying laboratory oven (Oxidative Stress Research Centre, Faculty of Health and Wellness Sciences at CPUT, Bellville) at 40 °C for 7–14 days. The dried plants were ground into a fine powder using a Junkel and Kunkel model A 10 mill. Shoot material was extracted by mixing 100 mg of the dried powdered material with 25 mL of 70% (v/v) ethanol (EtOH) (Merck, Modderfontein, South Africa) for 1 h. It was centrifuged at 4000 rpm for 5 min, and the supernatants were used for all analyses.

2.6.2. Determination of Antioxidant Capacity and Content

Antioxidant activity and accumulation of secondary metabolites within the leaves were assessed using assays for total polyphenols, ABTS, and ferric reducing antioxidant power (FRAP).

2.6.3. Polyphenol Assay

The total polyphenols assay (Folin assay) was performed as described by [41]. Folin and Ciocalteu’s phenol reagent (2 N, Sigma, Gauteng, South Africa) were diluted 10 times with distilled water, and a 7.5% sodium carbonate (Sigma-Aldrich, Gauteng, South Africa) solution was prepared. In a 96-well plate, 25 µL of the crude extract was mixed with 125 µL of Folin and Ciocalteu’s phenol reagent and 100 µL of sodium carbonate. The plate was incubated for 2 h at room temperature. The absorbance was then measured at 765 nm in a Multiskan Spectrum plate reader (Thermo Electron Corporation, USA). The samples’ polyphenol values were calculated using a gallic acid (Sigma-Aldrich, Gauteng, South Africa) standard curve with concentration varying between 0 and 500 mg/L. The results were expressed as mg gallic acid equivalents (GAE) per g dry weight (mg GAE/g DW).

2.6.4. ABTS Assay

The ABTS assay was performed following the method of [42]. The stock solutions included a 7 mM ABTS and 140 mM potassium–peroxodisulphate (K$_2$S$_2$O$_8$) (Merck, Modderfontein, South Africa) solution. The working solution was then prepared by adding 88 µL of K$_2$S$_2$O$_8$ to 5 mL of ABTS solution. The two solutions were mixed well and allowed to react for 24 h at room temperature in the dark. Trolox (6-Hydrox-2,5,7,8-tetramethylchroman-2-carboxylic acid) was used as the standard with concentrations ranging between 0 and 500 µM. Crude sample extracts (25 µL) were allowed to react with 300 µL of ABTS in the dark at room temperature for 30 min before the absorbance was read.
at 734 nm at 25 °C in a plate reader. The results were expressed as µM/Trolox equivalent per g dry weight (µM TE/g DW).

2.6.5. Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed using the method of [43]. The FRAP reagent was prepared by mixing 30 mL of acetate buffer (0.3 M, pH 3.6) (Merck, Modderfontein, South Africa) with 3 mL of 2,4,6- tripyridyl-s-triazine (10 mM in 0.1 M hydrochloric acid) (Sigma-Aldrich, Gauteng, South Africa), 3 mL of iron (III) chloride hexahydrate (FeCl₃·6H₂O) (Sigma-Aldrich, Gauteng, South Africa) and 6 mL of distilled water. In a 96-well plate, 10 µL of the crude sample extract was mixed with 300 µL of the FRAP reagent and incubated for 30 min at room temperature. The absorbance was then measured at 593 nm in a Multiskan Spectrum plate reader (Thermo Electron Corporation, USA). The samples’ FRAP values were calculated using an L-Ascorbic acid (Sigma-Aldrich, Gauteng, South Africa) standard curve with concentrations varying between 0 and 1000 µM. The results were expressed as µM ascorbic acid equivalents (AAE) per g dry weight (µM AAE/g DW) [41,44].

2.7. Statistical Analysis

For minerals, three samples were analyzed for each treatment, while all the assays were carried out in triplicate. The results were expressed as mean values and standard error (SE) and analyzed using one-way analysis of variance (ANOVA) followed by Fisher’s least significant test at \( p \leq 0.05 \) significance level. This analysis was carried out using the STATISTICA version 13.5.0.17 program [45].

3. Results

3.1. Effects of Salt Stress on Plant Growth

3.1.1. Shoot Length and Lateral Branch Number

The results showed that T. decumbens growth response to NaCl was variable (Table 1). The shoot length and lateral branch number were significantly affected by salinity concentrations at \( p \leq 0.05 \). The control had the highest shoot length, and this was significantly higher than 100 mM NaCl and 200 NaCl mM concentrations, respectively, but did not differ significantly from the 50 mM NaCl concentration. However, this was not the case with the lateral branch number, where the 50 mM NaCl concentration had the highest number of branches compared to the control.

Table 1. Effects of salt stress on growth parameters of T. decumbens.

| Treatments | SL (cm) | BN (n) | FWS (g) | DWS (g) | FWSR (g) | DWSR (g) | TFW (g) | TDW (g) |
|------------|--------|-------|--------|--------|---------|---------|--------|--------|
| Control    | 101.1 ± 3.8 ^a  | 3.4 ± 0.2 ^c  | 136.8 ± 6.7 ^c  | 23.1 ± 1.2 ^c  | 61.4 ± 5.4 ^b  | 14.5 ± 1 ^b  | 198.3 ± 8.3 ^c  | 37.6 ± 1.1 ^c  |
| 50 mM      | 96.5 ± 3.1 ^ab  | 5.4 ± 0.4 ^a  | 210.8 ± 10.4 ^a  | 32.1 ± 1.3 ^a  | 89.3 ± 6.7 ^a  | 18.6 ± 1 ^a  | 300.2 ± 12.8 ^a  | 50.6 ± 1.6 ^a  |
| 100 mM     | 90.7 ± 2.2 ^b  | 4.7 ± 0.2 ^ab  | 186.4 ± 6.8 ^b  | 26.9 ± 0.7 ^b  | 78.0 ± 3.8 ^a  | 17.3 ± 0.8 ^ab  | 264.4 ± 6.6 ^b  | 44.1 ± 0.9 ^b  |
| 200 mM     | 73.7 ± 3.1 ^c  | 3.8 ± 0.3 ^bc  | 119.7 ± 6.3 ^c  | 15.6 ± 0.8 ^d  | 79.5 ± 6.2 ^a  | 15.3 ± 1.1 ^b  | 199.2 ± 7.8 ^c  | 30.9 ± 1.2 ^d  |
| E-statistic| 14.6 ^a  | 7.6 ^a  | 29.8 ^a  | 45 ^a  | 4.2 ^a  | 3.2 ^a  | 30 ^a  | 45 ^a  |

Note. SL: shoot length; BN: branch number; FWS: fresh weight of shoots; DWS: dry weight of shoots; FWSR: fresh weight of stem and roots; DWSR: dry weight of stem and roots; TFW: total fresh weight; TDW: total dry weight. The values (mean ± SE) followed by dissimilar letters in each column are significantly different at \( p \leq 0.05 \) (*).
3.1.3. Fresh and Dry Weight of Stem and Roots

NaCl concentrations positively influenced the fresh and dry weights of the stem and roots. The highest fresh weight of the stem and roots was obtained at 50 mM NaCl concentration. This was significantly higher than the control but did not differ significantly to 100 mM and 200 mM NaCl concentrations, respectively. The highest dry weight of the stem and roots was again recorded at 50 mM concentration. This was significantly higher than the control and 200 mM NaCl but did not differ significantly from the 100 mM NaCl concentration.

3.1.4. Total Fresh and Dry Weight

Experimental results also showed that NaCl concentrations significantly \((p \leq 0.05)\) affected the fresh and dry weight of dune spinach (Table 1). Plants exposed to different salt concentrations had fresh weights that were higher than the control. The highest measurement of fresh weight was obtained at 50 mM NaCl concentration, and this was significantly higher than all treatments, including the control. Although plants exposed to the 200 mM NaCl concentration had a higher fresh weight than the control, they did not differ significantly from each other. Conversely, this was not the case in total dry weight, where the 200 mM NaCl concentration recorded the lowest dry weight compared to all treatments, including the control. The highest total dry weight was again obtained at 50 mM NaCl concentration, and this was significantly higher than all other treatments, including the control.

3.2. Effect of Salt Stress on the Mineral Content of Dried Leaves of T. decumbens

3.2.1. Macronutrients

Salinity stress significantly increased macronutrients (N, P, and Na) in the leaves of dune spinach. The highest N, P, and Na were all obtained from the highest salt concentration (200 mM). Conversely, the accumulation of K, Ca, and Mg significantly lowered in comparison to the control (Table 2).

### Table 2. Effect of salt stress on the concentration of macronutrients in the leaves of T. decumbens.

| Treatments | N (g/kg) | P (g/kg) | K (g/kg) | Ca (g/kg) | Mg (g/kg) | Na (g/kg) |
|------------|----------|----------|----------|-----------|-----------|-----------|
| Control    | \(23.5 \pm 0.4\) \(^{a}\) | \(4.7 \pm 0.4\) \(^{a}\) | \(44.1 \pm 1.1\) \(^{a}\) | \(6.9 \pm 0.7\) \(^{a}\) | \(6.9 \pm 0.5\) \(^{a}\) | \(6.9 \pm 0.4\) \(^{b}\) |
| 50 mM      | \(20.8 \pm 0.1\) \(^{b}\) | \(3.3 \pm 0.3\) \(^{a}\) | \(26.8 \pm 1.1\) \(^{b}\) | \(3.6 \pm 0.2\) \(^{b}\) | \(3.3 \pm 0.1\) \(^{b}\) | \(35.8 \pm 1.3\) \(^{a}\) |
| 100 mM     | \(20.9 \pm 0.3\) \(^{b}\) | \(3.4 \pm 0.4\) \(^{a}\) | \(22.6 \pm 1\) \(^{c}\) | \(3.7 \pm 0.5\) \(^{b}\) | \(3.2 \pm 0.2\) \(^{b}\) | \(54.7 \pm 1.3\) \(^{c}\) |
| 200 mM     | \(24.4 \pm 0.3\) \(^{a}\) | \(4.8 \pm 0.4\) \(^{a}\) | \(21.9 \pm 0.7\) \(^{c}\) | \(2.5 \pm 0.1\) \(^{b}\) | \(2.8 \pm 0.1\) \(^{b}\) | \(59 \pm 0.9\) \(^{c}\) |
| F-statistic| \(14.9 \ast\) | 1.9 ns   | 81.3 \ast\) | 18.6 \ast\) | 18.6 \ast\) | 58.8 \ast\) |

Values (mean ± SE) followed by dissimilar letters in each column are significantly different at \(p \leq 0.05\) (*); ns = not significant.

3.2.2. Micronutrients

Salt stress positively influenced the micronutrients (Mn, Fe, and Cu) in the leaves of dune spinach. The highest Mn, Fe, and Cu were all obtained from the moderate salt concentration (100 mM). However, the highest mean values in Mn and Cu were not significantly \((p \leq 0.05)\) different from all other treatments, including the control. However, the opposite was true for Fe. Conversely, salt stress negatively influenced Zn and B accumulation in the leaves. The control had the highest mean values in both Zn and B, and these were significantly different from all salt treatments (Table 3).
Table 3. Effect of salt stress on the concentration of micronutrients in the leaves of *T. decumbens*.

| Treatments | Mn (mg/kg) | Fe (mg/kg) | Cu (mg/kg) | Zn (mg/kg) | B (mg/kg) |
|------------|------------|------------|------------|------------|------------|
| Control    | 74.3 ± 1.3 a | 66.4 ± 2.2 b | 1.7 ± 0.3 a | 56.7 ± 1.8 a | 35.1 ± 1.5 a |
| 50 mM      | 67.5 ± 1.9 a | 61.3 ± 0.83 bc | 1.7 ± 0.2 a | 35.4 ± 0.5 b | 20.7 ± 0.7 b |
| 100 mM     | 81.3 ± 1.9 a | 89.2 ± 2.7 a | 4.2 ± 1.9 a | 51.6 ± 1.4 a | 24.7 ± 2.4 b |
| 200 mM     | 64.4 ± 1.2 a | 57.9 ± 2.2 c | 2.3 ± 0.5 a | 36 ± 0.3 b | 22.1 ± 0.9 b |
| F-statistic| 0.4 ns      | 44.9 *      | 1.2 ns      | 17.9 *      | 5.2 *      |

Values (mean ± SE) followed by dissimilar letters in a column are significantly different at \( p \leq 0.05 \) (*); ns = not significant.

3.3. Effect of Salt Stress on Chlorophyll Content

As shown in Figure 1, the total chlorophyll contents were negatively affected by 50 mM, 100 mM, and 200 mM salinity concentrations during the fourth week of growth. However, plants exposed to lower salinity (50 mM NaCl) had the highest SPAD-502 values, which was significantly higher than the other treatments, including the control. During the sixth week, salinity concentrations positively affected the chlorophyll values and were significantly different from one another at \( p \leq 0.05 \). The highest SPAD-502 values were obtained at 200 mM NaCl concentration followed by 100 mM, 50 mM, and the control. During the eighth week, chlorophyll values were negatively affected by salinity as all treatments, including the control, had lower chlorophyll values when compared to week 6. However, a higher concentration (200 mM NaCl) had the highest SPAD-502 value, but it was not significantly different from the other treatments except for the control at \( p \leq 0.05 \). During the 10th week, salinity stress further reduced the chlorophyll values of all treatments except the control. The control had the highest mean value, which was significantly higher than all salt treatments.

![Figure 1](image_url)
3.4. Effects of Salt Stress on Phenolic Content and Antioxidant Capacity

3.4.1. Polyphenol Content

The polyphenol content in the leaves of *T. decumbens* varied significantly at \( p \leq 0.05 \) when different NaCl concentrations were compared with each other and with the control (Table 4). Plants exposed to the higher NaCl concentration (200 mM) had the highest polyphenol content (2.6 GAE/g DW) compared to all treatments, including the control. This was significantly different from the control and the 50 mM NaCl concentration but did not differ significantly from the moderate NaCl concentration (100 mM).

| Treatments  | Total Polyphenols (mg GAE/g DW\(^{-1}\)) | ABTS (\(\mu M\) TE/g DW\(^{-1}\)) | FRAP (\(\mu M\) AAE/g DW\(^{-1}\)) |
|-------------|-----------------------------------------|-----------------------------------|----------------------------------|
| Control     | 1.3 ± 0.2 \(b\)                        | 82.5 ± 8 \(a\)                    | 10.9 ± 0.1 \(b\)                 |
| 50 mM       | 1.6 ± 0.3 \(b\)                        | 78.4 ± 6 \(a\)                   | 14.3 ± 0.4 \(a\)                |
| 100 mM      | 1.7 ± 0.4 \(ab\)                      | 70.4 ± 4 \(a\)                   | 12.1 ± 1.1 \(ab\)              |
| 200 mM      | 2.6 ± 0.2 \(a\)                        | 77.8 ± 4.4 \(a\)                 | 11.8 ± 0.7 \(b\)               |
| F-statistic | 3.3 *                                   | 0.41 ns                           | 3.6 *                           |

Values (mean ± SE) followed by dissimilar letters in a column are significantly different at \( p \leq 0.05 \) (*); ns = not significant.

3.4.2. ABTS Capacity

Salt stress had a negative influence on ABTS capacity in the leaves of *T. decumbens*. The control had the highest ABTS capacity; however, this was not significantly different from all the treatments (Table 4).

3.4.3. FRAP Capacity

The total FRAP capacity in the leaves of *T. decumbens* was significantly influenced by the NaCl concentrations at \( p \leq 0.05 \). The lower NaCl concentration (50 mM) had the highest FRAP capacity (14 \(\mu M\) AAE/g DW) compared to the other treatments, including the control. This was significantly higher than the control and 200 mM NaCl concentration but did not differ significantly from the 100 mM NaCl concentration (Table 4).

4. Discussion

There is extensive literature on the reduction of growth caused by salt stress in many plants, which are facilitated by homeostatic transport of Na\(^+\) across intra- and inter-cellular cell boundaries predominated by NaCl [46,47]. Nevertheless, the effect of salt stress varies among plants. In the present study, increasing NaCl concentrations led to a significant decrease in plant height. Height reduction as a result of salinity stress has been reported in several plant species and has been mainly associated with the osmotic stress and ion toxicity that causes a reduction in plant growth [48–50]. However, when comparing the number of branches among the treatments, all plants irrigated with NaCl had more branches compared to the control. This might be caused by the natural adaptation of the species to saline environments, which enhances the ability of the species to remediate saline soil and stabilize the coastal dunes. The increase in branch numbers resulted in a higher total fresh weight. These findings agree with the findings of [51], where the halophyte *Ammophila arenaria* showed increased plant biomass in lower to moderate soil salinity. However, the results contradict those reported by [52], where the authors observed that after 12 weeks of cultivating some halophytes, such as *Inula crithmoides* L., *Plantago crassifolia* Forssk. and *Medicago marina* L., the plants whose irrigation water had not been spiked with salt showed better productivity and growth rates. The ability of dune spinach to withstand these varying salt concentrations could be attributed to osmotic, ion, and tissue tolerance. At high salt concentrations, the growth of dune spinach reduced drastically. This has been reported in numerous studies conducted on halophytes, where increasing salinity negatively affected plant growth performance, causing a reduction in biomass, leaf number, and plant height [47,53,54]. Reference [55] also reported that longer salt exposure in the
root zone restricts the flow of water and nutrients into the plant, causing direct injury to plant cells through the accumulation of toxic ions causing a decline in plant growth.

Salt stress has been reported as one of the major environmental factors affecting the nutritional value of many edible plants. In the present study, salt stress increased the uptake of N, P, and Na in the leaves, while K, Ca, and Mg were reduced drastically. The reduction of these elements may be directly linked to excessive Na$^+$ absorption by the roots as reported by [56]. However, sufficient K, Ca, and Mg are required to meet basic metabolic processes such as intracellular K homeostasis, which is essential for optimal functioning of the photosynthetic machinery and maintenance of stomatal opening [57]. These results suggest that dune spinach can transport K, Ca, and Mg to new shoots and leaves under salt stress and maintain a suitable ratio needed for normal metabolism; hence, the chlorophyll content was not affected for 8 weeks. This could be attributed to the water use efficiency and carbon fixing capacity of this species, which uses Crassulacean acid metabolism (CAM) to adapt to harsh conditions. Our results agree with those conducted by [58,59] on Chenopodium quinoa (genotype A7) and Cichorium spinosum in saline conditions, respectively, where it was reported that higher transport of K and Ca into new shoots and leaves contributed to mitigating ion toxicity in leaf cells.

Moreover, salinity stress also increased the Mn, Fe, and Cu contents in the leaves, while Zn and B were negatively affected. Similar findings were reported on the edible halophyte Salicornia ramosissima by [60]. These results indicate that salt stress caused Zn and B deficiency in the leaves of dune spinach, but since they are required in small quantities, visual symptoms of nutrient deficiency did not occur.

It has been reported in the literature that salinity stress damages nutrition and promotes senescence mechanisms in plants, thereby causing a reduction of chlorophyll content in the leaves [49,50]. However, the extent of reduction depends on the salt tolerance of the plant species [61]. Reference [62] reported that salt-tolerant species, such as Thellungiella halophila, indicated more or unchanged chlorophyll content when exposed to 0–500 mM NaCl, while salt-sensitive species (glycophyte), such as Arabidopsis thaliana, had lower chlorophyll content. In the present study, chlorophyll content was used as a biochemical marker to screen the salt tolerance of dune spinach. SPAD values (chlorophyll content) varied among treatments during the growing weeks, with salt treatments having higher SPAD values on week 6 and 8 when compared to the control (Figure 1). The findings of this study are in agreement with the results obtained by [63] in M-81E sweet sorghum (salt-tolerant genotype), where the chlorophyll content was not affected by 50 mM NaCl. In another study conducted by [64], spinach cultivar Raccoon treated with saline irrigation water maintained SPAD chlorophyll levels but had a reduced photosynthetic rate, stomatal conductance, and transpiration rate.

Under salinity stress, the balance between reactive oxygen species production and activities of an antioxidative enzyme determines whether oxidative damage will occur [65]. To reduce the effects of oxidative stress, plants accumulate metabolites, such as phenolic compounds, which act as reducing agents, hydrogen donors, and singlet oxygen quenchers [49,66]. Moreover, phenolic compounds are of great interest due to the relevant role they play in the taste and flavor of food products, as well as their health-promoting properties [67,68]. In the present study, the total phenolic content was significantly increased by salinity levels, with more prominent content in plants irrigated with the highest NaCl concentration (200 mM). These findings validate that of [69], where an increase in the total phenolic content, antioxidant activity, and cyanidin-3-glucoside content was found in Khamdoisaket and KDML 105 Thai rice cultivars subjected to salinity stress. A similar trend was also reported by [70] on the effects of salinity on biochemical characteristics of the stock plant (Matthiola incana L.), where the phenolic content in severe salt-stressed plants of both cultivars was higher than the control. Reference [71] stated that phenolic compounds may be affected by salinity, but this critically depends on the salt sensitivity of a considered species. The results of the study prove that dune spinach can grow under severe salinity
concentrations and could be considered as an alternative source of nutritional antioxidant in areas with higher and problematic saline soils.

Contrary to the increase in phenolic content, the antioxidant activity (ABTS) in the leaves of *T. decumbens* exposed to various salinity concentrations showed a much weaker antioxidant capacity compared to the control. This contradicts the findings of [16] on edible flowers, where three antioxidant assays (FRAP, DPPH, and ABTS) increased with the application of salinity, with a more pronounced impact at a salinity of 100 mM NaCl. Furthermore, these results also substantiate that of [72] on a traditional Chinese herb, where a much weaker antioxidant capacity was found with increasing NaCl concentrations at 50 mM and 100 mM, respectively, compared to the control. Conversely, the FRAP capacity was positively influenced by salt stress with the strongest antioxidant capacity obtained at 50 mM NaCl. A similar trend was reported earlier by [16] in edible flowers, where the application of salinity enhanced antioxidant activities.

5. Conclusions

The results of this study showed that there is a significant advantage of using nutrient solution incorporated with NaCl to increase yield, mineral composition, and antioxidant activity in dune spinach. Plants irrigated with nutrient solution incorporated with 50 mM NaCl revealed a significant increase in growth parameters and FRAP capacity. As the plant grew beyond week 6, an increased level of salinity caused a decrease in chlorophyll content (SPAD), while the phenolic content, as well as nitrogen, phosphorus, and sodium, increased. Based on this study, it is evident that there is potential for brackish water cultivation of dune spinach for consumption as a leafy vegetable and as a natural source of nutritional antioxidants. This could be a water-saving option in provinces experiencing the adverse effect of drought and salinity, where seawater or underground saline water could be diluted and used as irrigation water in the production of this vegetable.

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