



Article

Seaweed-Derived Bio-Stimulant (Kelpak[®]) Enhanced the Morphophysiological, Biochemical, and Nutritional Quality of Salt-Stressed Spinach (*Spinacia oleracea* L.)

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Abstract: Biostimulants such as seaweed extracts are emerging as crop management products that can enhance crop productivity and nutritional quality under abiotic stress conditions. Therefore, this study aimed to assess the effectiveness of a seaweed-derived biostimulant (Kelpak[®]) in alleviating salinity stress in spinach. A greenhouse experiment which consisted of five treatments (T₁ = Control plants (no NaCl or seaweed extract (SWE)), T₂ = plants subjected to 300 mM NaCl without SWE, T₃ = 300 mM NaCl + 1% dilution of SWE, T₄ = 300 mM NaCl + 2.5% dilution of SWE, and T₅ = 300 mM NaCl + 5% dilution of SWE) was conducted. The results showed that salinity without the addition of SWE reduced crop growth, relative water content, chlorophyll, and nutritional quality. Similarly, salinity induced severe oxidative stress, indicated by excessive amounts of superoxide radicals, malondialdehyde and the upregulation of catalase, peroxidase, polyphenols, and flavonoids. Interestingly, plants treated with 5% SWE displayed a substantial enhancement in crop performance, reduction in oxidative stress, and improved nutritional quality, characterised by considerable amounts of minerals, proximate constituents, and vitamins. These results support the use of seaweed extract (Kelpak[®]) as a biostimulant in enhancing growth and nutritional quality of spinach under saline cultivation.

Keywords: abiotic stress; antioxidant enzymes; biostimulants; nutritional value; *Spinacia oleracea* L.



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1. Introduction

Salinity is one of the major factors limiting the growth and yield of vegetable crops worldwide [1,2]. While exact measures remain elusive, the prevalence of salinity-affected soil is on the rise, particularly in irrigated areas [3]. This has been attributed to the overuse of low-quality water and extensive irrigation coupled with intensive farming and inadequate drainage systems, resulting in the build-up of soluble salt ions in the soil [4]. These salt ions impede the ability of the plant roots to absorb water, causing osmotic stress and nutritional deficiencies that disrupt essential physiological processes such as photosynthesis and tissue hydration, and may also trigger oxidative stress and membrane damage, leading to diminished plant biomass [5].

To curb the devastating effects of salinity on food crops, farmers have been using synthetic chemical amendments for the amelioration of saline and sodic soils [6]. However, this process has become expensive, especially for small-scale farmers in developing countries, due to the competing demand from industry [7,8]. Thus, it is crucial to identify cost-effective and environmentally friendly techniques such as the use of naturally derived plant biostimulants to favourably modify physiological processes and enhance plant productivity under saline soils [9]. Recent studies have shown that the exogenous application

of biostimulants from seaweed extracts could improve water retention, nutrient availability, and plant defence systems under abiotic stress [10–12]. Additionally, the positive effect of seaweed extract under saline conditions was reported in maize (*Zea mays*), tomato (*Solanum lycopersicum* L.), and in bell pepper (*Capsicum annuum* L.), where its application optimised photosynthetic performance, antioxidant defence systems, leaf relative water content, leaf greenness, and the accumulation of potassium and phosphorous, resulting in higher yield [13–15]. Hence, seaweed extracts have gained popularity as commercial biostimulants that positively influence agronomic and physio-biochemical responses in plants under saline conditions.

Spinacia oleracea (Fordhook giant Swiss chard), a widely consumed vegetable in South Africa, is recognised for its nutritional value due to the abundance of antioxidant compounds, minerals, vitamins, and dietary fibres [16]. However, its production has been negatively impacted by increasing soil salinity in South African vegetable farms, especially in semi-arid regions [17], which necessitates the application of cost-effective and environmentally friendly farming techniques to alleviate salinity. Despite the findings of El-Nakhel et al. [18], no comprehensive studies have been conducted on the use of seaweed-derived biostimulants to assess salt tolerance and increase the yield and nutritional profile of spinach under saline conditions. Thus, this study was conducted to assess the effectiveness of a South African seaweed-derived biostimulant (Kelpak®) in alleviating salinity stress in spinach. The results from this study are hoped to provide a sustainable and cost-effective technique to small-scale vegetable farmers in semi-arid regions affected by soil salinity.

2. Material and Methods

2.1. Greenhouse Cultivation

2.1.1. Growth Conditions

This study was conducted in the research greenhouse facility of the Cape Peninsula University of Technology during October and November 2023. The temperature within the greenhouse was configured to be 21–26 °C during the daytime and 12–17 °C at night, with 60% relative humidity. Under natural light conditions, the daily average photosynthetic photon flux density (PPFD) was 420 $\mu\text{mol}/\text{m}^{-2}\text{s}^{-1}$, with the intensity peaking at 1020 $\mu\text{mol}/\text{m}^{-2}\text{s}^{-1}$. The photoperiod corresponded to the prevalent conditions of early spring to summer.

2.1.2. Preparation of Plant Material

Seeds of *Spinacia oleracea* were acquired from a commercial garden store (Stodels™ Eversdal Rd, Bellville, Cape Town, South Africa) and germinated in seedling trays following the procedure outlined by Yavuz et al. [19]. Evenly established *S. oleracea* seedlings were transplanted individually in black plastic pots filled with a combination of peat moss and silica sand (1:1) and stationed in the hardening off area to acclimatise. The plants were then watered daily with a full Nutrifeed™ solution produced by STARKE AYRES Pty. Ltd., Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, South Africa. After sixteen days of plant development, plants were irrigated with distilled water for six days to eliminate any salt residue before being subjected to salinity and seaweed extract treatments.

2.1.3. Seaweed Extract Preparation

Seaweed extract (Kelpak®) was sourced from Kelp Products (Pty) Ltd., Capricorn Business Park, 2 Link, Capricorn, Simon's Town, South Africa and the treatments were prepared by diluting the liquid concentrate with distilled water to obtain three dilutions at 1%, 2.5%, and 5% (*v/v*) as described by Aremu et al. [20]. The seaweed extract was then applied weekly via soil drenching using 50 mL per potted plant. The chemical composition of Kelpak® is shown in Supplementary Tables S1 and S2.

2.1.4. Seaweed Extract and Saline Treatment

Fifty healthy plants were arranged into five treatments each containing ten replicates: T1—plants irrigated with nutrient solution (control); T2—plants irrigated with nutrient solution spiked with 300 mM of NaCl; T3—plants irrigated with nutrient solution spiked with 300 mM and treated with 1% seaweed extract; T4—plants irrigated with nutrient solution spiked with 300 mM and treated with 2.5% seaweed extract; and T5—plants irrigated with nutrient solution spiked with 300 mM and treated with 5% seaweed extract. To avoid osmotic shock, NaCl was gradually elevated by 50 mM daily until the maximum concentration (300 mM) was attained. Thereafter, the plants were irrigated every two days with 300 mL of nutrient solution with or without NaCl.

2.2. Biomass Evaluation

After 60 days of plant development under the tested treatments, plants were thoroughly watered with distilled water to loosen up the soil and were harvested cautiously to prevent root and plant damage. They were then rinsed multiple times with distilled water and dried with tissue paper. Thereafter, the shoots and roots were split, and the fresh samples were weighed using a precision weighing scale, followed by oven-drying at 30 °C to complete dryness, and the dry samples were also weighed.

2.3. Physiological Attributes

Chlorophyll *a* and *b* content was estimated spectrophotometrically as demonstrated by Wang et al. [21]. Fresh leaf material (100 mg) was extracted with 5 mL of 99.5% dimethyl sulfoxide (DMSO; Sigma-Aldrich, Gauteng, South Africa). The samples were then incubated at 35 °C for three hours in the dark before being centrifuged at 13,300 rpm for 10 min at 4 °C. The absorbance of the supernatants was measured at 649 and 665 nm. The contents of Chl *a* and Chl *b* were subsequently determined using the formula outlined by Lichtenthaler and Wellburn [22].

The variability in leaf relative water content (RWC) was evaluated using the procedure previously described by Bistgani et al. [23]. Briefly, three leaves were excised from three distinct plants in each treatment and weighed (FW). Subsequently, they were immersed in distilled water and kept in darkness for 4 h, following which the turgid weight (TW) was determined. Finally, the leaves were air-dried in an oven at 56 °C for 24 h to determine the dry weight (DW). The RWC percentage was calculated using the equation below:

$$\text{RWC}\% = \frac{(\text{FW} - \text{DW})}{(\text{TW} - \text{DW})} \times 100$$

2.4. Oxidative Stress Markers

The superoxide radical content was measured following the technique reported by Gokul et al. [24]. Three 1 cm² leaf sections were excised from three distinct plants in each treatment. These leaf sections were then immersed in 800 µL of potassium phosphate buffer (pH 7.0) [50 mM potassium phosphate buffer (pH 7.0) containing 10 mM potassium cyanide, 10 mM hydrogen peroxide, 2% sodium dodecyl sulphate and 80 µM NBT]. Leaf materials were then incubated in this buffer for 20 min at 21 °C before being crushed within the solution with a small pestle. The samples were centrifuged at 13,000 rpm for 5 min, and the supernatant was transferred to a clean 2 mL Eppendorf tube. A spectrophotometer was used to read the sample at 600 nm after loading 200 µL of it into a microtiter plate. The extinction coefficient of 12.8 mM cm^{−1} was used to calculate the superoxide radical content, expressed as nanomole (nmol) g^{−1} FW.

To quantify the malondialdehyde content within the tested samples, the procedure detailed by González-Orenga et al. [25] was followed. Leaf extracts were mixed with 0.5% thiobarbituric acid (TBA) prepared in 20% trichloroacetic acid (TCA) and with 20% TCA without TBA for the control and then incubated at 95 °C for 20 min before being cooled on ice and centrifuged at 13,300 rpm for 10 min at 4 °C. The absorbance of the supernatants

was measured at 532 nm. The non-specific absorbance at 600 and 440 nm was subtracted, and the MDA concentration was calculated and expressed as nanomole (nmol) g^{-1} FW.

2.5. Antioxidative Enzymes

The method outlined by Ali and Ludidi [26] was utilised to extract protein from the leaf samples. Briefly, 200 mg of plant tissue from each treatment was homogenised in 400 μL of protein extraction solution, consisting of 40 mM phosphate buffer at pH 7.4, 1 mM ethylenediaminetetraacetic acid (EDTA), and 5% (*w/v*) polyvinylpolypyrrolidone (PVPP). The homogenate was centrifuged at 13,000 rpm for 20 min at 4 °C, and the supernatant was then used for various enzymatic assays. The protein concentration in the extracts was measured using the Bio-Rad reagent and bovine serum albumin (BSA) as standard.

The activity of superoxide dismutase (SOD) was measured at 560 nm spectrophotometrically by measuring the suppression and photoreduction of nitro blue tetrazolium (NBT); the reaction solution contained riboflavin as a determinant of superoxide radicals. Briefly, 1 mL of the reaction solution was prepared in 50 mM potassium phosphate buffer using 2 μM riboflavin, 75 μM Nitrotetrazolium blue (NBT), 100 μM EDTA, 13 mM DL-methionine, and 50 μL of enzyme extract. A unit SOD was labelled as the quantity of enzyme required to block NBT photoreduction by 50% in experimental conditions as outlined in the methodology of Kaur et al. [27] and expressed as U g^{-1} FW.

Catalase (CAT) activity was measured by observing a decrease in absorbance at 240 nm following the consumption of H_2O_2 in a 1 mL reactive mixture consisting of 10 mM H_2O_2 and 20 μL of enzyme extract in 50 mM of potassium phosphate buffer (pH = 7) [28]. One CAT unit (U) was defined as the quantity of enzyme degrading one millimole (mmol) of H_2O_2 at 25 °C per minute and expressed as U g^{-1} FW. Meanwhile, the enzyme activity of peroxidase (POD) was determined using the techniques and principles described by Omran [29] with slight modifications. A 1 mL reactive mixture consisting of 50 mM of potassium phosphate buffer (pH = 7), 3.5 guaiacol, 10 mM H_2O_2 , and 20 μL of enzyme extract. The rate change in absorbance at 436 nm was measured and POD was expressed as U g^{-1} FW.

2.6. Metabolites and Antioxidant Activity

Crude extracts were obtained by mixing approximately 100 mg of finely pulverised dry plant materials into 25 mL of 70% (*v/v*) ethanol (EtOH) (Merck, Modderfontein, South Africa) for 1 h. The mixture was centrifuged at 4000 rpm for 5 min, and the supernatants were utilised for phytochemical and antioxidant assays.

The Folin–Ciocalteu method, as elucidated by Jasson et al. [30] was employed to assess the total phenolic content of the analysed samples. A 7.5% solution of sodium carbonate (Sigma-Aldrich, Gauteng, South Africa) and diluted Folin and Ciocalteu's phenol reagent (2 N, Sigma, Gauteng, South Africa) were made. A 25 μL volume of the crude extract, 125 μL of Folin and Ciocalteu's phenol reagent, and 100 μL of Na_2CO_3 were mixed in a 96-well plate at room temperature for 30 min. A Multiskan Spectrum plate reader was then used to measure the absorbance at 765 nm. The values were calculated using a gallic acid (Sigma-Aldrich, Gauteng, South Africa) standard curve with concentration varying between 0 and 500 mg/L and the results were expressed as mg gallic acid equivalents (GAE) per g dry weight (mg GAE/g DW).

The total flavonol content of the extracts was estimated from the quercetin standard curve established from concentration ranges of 0, 5, 10, 20, 40, and 80 mg/L of quercetin dissolved in 95% ethanol. A volume of 12.5 μL crude extracts was combined with 12.5 μL of 0.1% HCl (Merck, Modderfontein, South Africa) in 95% ethanol and 225 μL of 2% HCl for each sample. The mixture was then incubated at room temperature for 30 min before obtaining absorbance at 360 nm. The results were represented as milligrams of quercetin equivalent (mg QE/g DW) per gram of dry weight [31].

The radical scavenging activity was determined using the DPPH free radical scavenging assay following the methods described by Ngxabi et al. [32]. A solution of 0.135 mM

DPPH was used to produce DPPH radicals. About 300 µL of the DPPH solution was mixed with 25 µL of the crude extract and graded concentrations (0 and 500 µM) of Trolox standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid). After incubating for 30 min, the absorbance at 517 nm was measured and expressed as µM/Trolox equivalent per gram of dry weight (µM TE/g DW).

2.7. Nutritional Constituents

The mineral constituents (macro- and micronutrients) of the tested samples were analysed using the Inductively Coupled Plasma-Optical Emission Spectrometer (ICP-OES; Varian 710-ES series, SMM Instruments, Cape Town, South Africa) as previously explained by Bulawa et al. [33]. The proximate composition (moisture, ash, crude fat, crude protein, non-fibre carbohydrate and neutral detergent fibre) was determined using the accredited in-house standard Association of Official Analytical Chemists (AOAC) procedures reported by Tshayingwe et al. [34]. The composition of vitamin C (ascorbic acid) and vitamin E (α -tocopherol) in the leaf samples was also evaluated using the AOAC methods described by Nemzer et al. [35].

2.8. Statistical Analysis

The experimental data were analysed with Minitab 17 statistical software and a multi-variate analysis of variance was employed to discern significant differences across treatments followed by Tukey's (LSD) test at $p \leq 0.05$. The data were subsequently presented as mean $\pm$ standard error.

3. Results

3.1. Biomass Evaluation

Salinity and the application of seaweed extracts (SWE) had a considerable impact on the biomass yield of spinach, as seen in Table 1. Plants irrigated with salinity without seaweed extract had the lowest biomass yield when compared to other treatments. The highest yield in shoot fresh weight was noted in control plants, and this was comparable to the yield obtained in plants treated with 5% SWE under saline irrigation. The same trend was also noted for shoot dry weight, root fresh weight, and total fresh weight. On the contrary, both the root dry weight and total dry weight were substantially higher in control plants as compared to plants subjected to either salinity or its combination with various SWE dilutions.

Table 1. Effects of salinity and seaweed extracts (SWE) on biomass yield of spinach.

Treatments	SFW (g)	SDW (g)	RFW (g)	RDW (g)	TFW (g)	TDW (g)
Control	237 $\pm$ 1.7 a	22.2 $\pm$ 0.3 a	22.4 $\pm$ 1.1 a	9.7 $\pm$ 0.7 a	259.2 $\pm$ 1.8 a	32.1 $\pm$ 0.6 a
300 mM NaCl	92.5 $\pm$ 1.1 d	8.7 $\pm$ 1.6 d	10 $\pm$ 0.8 c	1.9 $\pm$ 0.9 e	101.2 $\pm$ 1.6 d	11.9 $\pm$ 1 e
300 mM + 1% SWE	144.9 $\pm$ 1.6 c	12.2 $\pm$ 0.4 c	17.7 $\pm$ 0.3 b	3.5 $\pm$ 0.4 d	157.1 $\pm$ 0.8 c	21.2 $\pm$ 0.7 d
300 mM + 2.5% SWE	191.4 $\pm$ 2.1 b	16.1 $\pm$ 0.9 b	21.7 $\pm$ 1.2 a	4.4 $\pm$ 0.1 c	207.5 $\pm$ 1.2 b	26.1 $\pm$ 0.9 c
300 mM + 5% SWE	214.3 $\pm$ 1.1 a	21.3 $\pm$ 1.5 a	21.5 $\pm$ 0.9 a	7.8 $\pm$ 0.7 b	235.6 $\pm$ 1.1 a	29.4 $\pm$ 0.2 b

Means with different letters in the same column are significantly different (Tukey LSD, $p \leq 0.05$). Note: SFW = shoot fresh weight; SDW = shoot dry weight; RFW = root fresh weight; RDW = root dry weight; TFW = total fresh weight and TDW = total dry weight.

3.2. Physiological Attributes

Salinity and seaweed extracts induced physiological changes in spinach leaves, as shown in Table 2. The highest photosynthetic pigments (Chl *a* and *b*) were noted in control plants, but these values were comparable to those recorded in plants subjected to salinity and treated with 5% SWE. On the contrary, salinity without SWE caused a substantial reduction in photosynthetic pigments as anticipated. When assessing the relative water content within treatments, the same trend was noted where the control had the highest relative water content, but this value was again comparable to plants treated with 5% SWE.

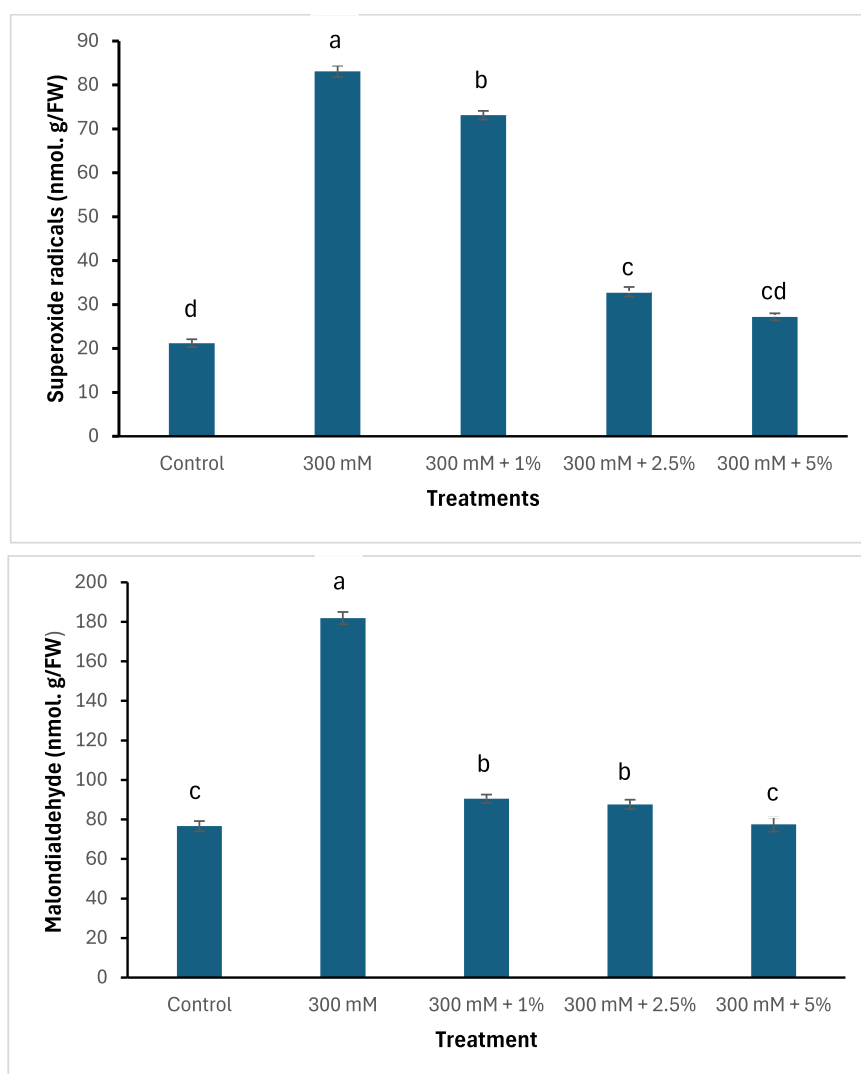
Table 2. Effects of salinity and seaweed extracts (SWE) on the photosynthetic pigments and relative water content (RWC) of spinach leaves.

Treatments	Chl a (mg/g FW)	Chl b (mg/g FW)	Chl a + b (mg/g FW)	RWC %
Control	6.7 ± 0.5 a	4.1 ± 0.2 a	10.8 ± 0.3 a	90.5 ± 1.3 a
300 mM NaCl	2.7 ± 0.1 c	0.8 ± 0.6 d	3.5 ± 0.8 d	65 ± 1 d
300 mM + 1% SWE	4.7 ± 0.3 b	2.1 ± 0.5 c	6.8 ± 0.6 c	68 ± 1.4 c
300 mM + 2.5% SWE	5 ± 0.2 b	3.1 ± 0.9 b	8.1 ± 0.3 b	79.9 ± 1 b
300 mM + 5% SWE	6.5 ± 0.4 a	3.9 ± 0.3 a	10.4 ± 0.3 a	88.8 ± 1.2 a

Means with different letters in the same column are significantly different (Tukey LSD, $p \leq 0.05$).

3.3. Oxidative Stress Markers

Salinity and seaweed extracts had an influence on the accumulation of superoxide radicals and malondialdehyde (MDA) within the leaves of spinach, as displayed in Figure 1. Salinity without the addition of SWE upregulated the accumulation of oxidative stress markers, which are depicted by excessive amounts of superoxide and MDA. Interestingly, plants treated with 5% SWE had lower contents of superoxide radicals and MDA, which were comparable to the control plants.

**Figure 1.** Effects of salinity and seaweed extracts on oxidative stress markers in spinach. Means with different letters are significantly different (Tukey LSD, $p \leq 0.05$).

3.4. Antioxidant Defence Mechanisms

Salinity and seaweed extract stimulated the activation of antioxidative defence system in spinach, as seen in Table 3. The contents of antioxidative enzymes (CAT and POD) were all significantly enhanced in plants subjected to salinity without SWE. Superoxide dismutase (SOD), on the other hand, was substantially upregulated in plants treated with 5% SWE. When assessing the phytochemicals and antioxidant activity of the leaf samples, the highest polyphenol, flavonol, and DPPH contents were all noted in plants irrigated with salinity with no SWE treatment.

Table 3. Effects of salinity and seaweed extracts (SWE) on the antioxidant defence attributes of spinach.

Treatments	SOD Activity (U/g ⁻¹ Protein)	CAT Activity (U/g ⁻¹ Protein)	POD Activity (U/g ⁻¹ Protein)	Polyphenols (mg GAE/g)	Flavonols (mg QE/g)	DPPH (μmol TE/g)
Control	25.9 ± 0.3 d	9.3 ± 0.4 d	11.8 ± 1.6 d	5.1 ± 0.1 c	0.7 ± 0.1 c	27.3 ± 1.3 d
300 mM NaCl	70.6 ± 0.4 c	22.6 ± 0.3 a	24.7 ± 1.8 a	7.4 ± 0.7 a	1.7 ± 0.02 a	51.5 ± 1 a
300 mM + 1% SWE	70.5 ± 1.3 c	19 ± 0.5 b	18.4 ± 1.4 b	6.6 ± 0.5 b	1.1 ± 0.01 b	39.6 ± 1.4 b
300 mM + 2.5% SWE	73.6 ± 0.8 b	12.1 ± 0.1 c	15.1 ± 0.8 c	6.4 ± 0.2 b	1 ± 0.01 bc	31.9 ± 1.9 c
300 mM + 5% SWE	79.9 ± 0.5 a	10.2 ± 0.7 cd	12.6 ± 1.3 d	5.1 ± 0.9 c	0.9 ± 0.04 bc	28.5 ± 1.1 d

Means with different letters in the same column are significantly different (Tukey LSD, $p \leq 0.05$).

3.5. Nutritional Evaluation

3.5.1. Minerals and Vitamin Constituents

Salinity and the application of SWE significantly influenced the accumulation of minerals and vitamins in spinach leaves (Tables 4 and 5). Macronutrients such as nitrogen, potassium, magnesium, and calcium were all noted in enhanced quantities in the control plants. However, the contents of magnesium and calcium were comparable to the values recorded in plants treated with 5% SWE, while a high amount of sodium was recorded in plants subjected to saline irrigation only. When assessing the accumulation of trace elements and vitamins, we observed that plants treated with 5% SWE had an enhanced quantity of zinc, manganese, and iron as compared to other treatments, including the control. On the contrary, vitamin C and E contents were more pronounced in control plants, but these values were comparable to plants treated with 5% SWE.

Table 4. Effects of salinity and seaweed extracts (SWE) on the macronutrient contents of spinach leaves.

Treatments	N (mg/100 g)	P (mg/100 g)	K (mg/100 g)	Mg (mg/100 g)	Na (mg/100 g)	Ca (mg/100 g)
Control	4204 ± 4 a	612 ± 12.5 b	6837 ± 16 a	773 ± 12 a	3625 ± 4.2 d	528 ± 11 a
300 mM NaCl	3107 ± 7 e	407 ± 9.3 d	4122 ± 25 e	507 ± 11 d	7812 ± 12.5 a	242 ± 9 d
300 mM + 1% SWE	3521 ± 11.3 d	505 ± 5 c	5065 ± 5 d	702 ± 14 c	4662 ± 2.5 b	342 ± 14 c
300 mM + 2.5% SWE	3651 ± 6.5 c	522 ± 18 c	6554 ± 4 c	722 ± 15 b	3826 ± 13 c	407 ± 16 b
300 mM + 5% SWE	3728 ± 19.1 b	662 ± 14 a	6645 ± 15 b	767 ± 11 a	3629 ± 16 d	514 ± 12 a

Means with different letters in the same column are significantly different (Tukey LSD, $p \leq 0.05$).

Table 5. Effects of salinity and seaweed extracts (SWE) on the micronutrient and vitamin contents of spinach leaves.

Treatments	Zn (mg/100 g)	Mn (mg/100 g)	Cu (mg/100 g)	Fe (mg/100 g)	Vitamin C (mg/100 g)	Vitamin E (μg/100 g)
Control	8.6 ± 0.1 b	11.2 ± 0.4 e	0.3 ± 0.02 a	44.2 ± 0.1 e	51.3 ± 0.5 a	15.3 ± 0.08 a
300 mM NaCl	5.5 ± 0.05 d	12 ± 0.02 d	0 ± 0.0 d	72.7 ± 0.2 d	20.7 ± 0.2 d	4.3 ± 0.1 d
300 mM + 1% SWE	7.6 ± 0.01 c	16.6 ± 0.5 c	0 ± 0.0 d	91.7 ± 0.8 c	34.9 ± 0.4 c	8.3 ± 0.9 c
300 mM + 2.5% SWE	8 ± 0.1 b	18.6 ± 0.8 b	0.1 ± 0.0 c	121.1 ± 0.1 b	40.6 ± 0.5 b	10.2 ± 0.1 b
300 mM + 5% SWE	10.1 ± 0.3 a	23.1 ± 1.9 a	0.3 ± 0.0 b	173 ± 0.8 a	51.1 ± 1.3 a	15.2 ± 1.1 a

Means with different letters in the same column are significantly different (Tukey LSD, $p \leq 0.05$).

3.5.2. Proximate Composition

The concentration of proximate constituents within the leaves of spinach was significantly affected by saline irrigation and the application of seaweed extracts (Table 6). The ash and neutral detergent fibre were found in abundance in plants subjected to salinity without SWE addition. These values were substantially higher than all treatments, including the control. On the contrary, crude fat was found in higher quantities in plants treated with 5% SWE, while protein, moisture, and non-fibre carbohydrate contents were enhanced in control plants. Nevertheless, it must be noted that the protein content recorded in control plants was comparable to the value attained in plants treated with 5% SWE.

Table 6. Effects of salinity and seaweed extracts (SWE) on the proximate constituents of spinach leaves.

Treatments	Ash%	Crude fat%	Protein%	Moisture%	NFC%	NDF%
Control	33.9 ± 2.1 e	1.5 ± 0.05 c	26.4 ± 1.6 a	7.1 ± 0.5 a	32.1 ± 1.2 a	27.8 ± 0.2 d
300 mM NaCl	54.5 ± 1.3 a	1.1 ± 0.1 d	18.1 ± 0.8 d	4.1 ± 0.9 e	16.8 ± 0.3 e	42.2 ± 1 a
300 mM + 1% SWE	41.2 ± 1.1 b	1.5 ± 0.3 c	20.2 ± 0.4 c	5.4 ± 0.2 d	19.3 ± 0.9 d	35.3 ± 0.4 b
300 mM + 2.5% SWE	38.5 ± 1.4 c	2.2 ± 0.8 b	21.8 ± 0.8 b	5.9 ± 0.3 c	22.4 ± 1 c	33.8 ± 0.9 c
300 mM + 5% SWE	36.8 ± 1.5 d	2.7 ± 0.05 a	26 ± 0.3 a	6.2 ± 0.8 b	30 ± 1.1 b	27.5 ± 1.1 d

Means with different letters in the same column are significantly different (Tukey LSD, $p \leq 0.05$). Note: NFC = non fibre carbohydrate; NDF = neutral detergent fibre.

4. Discussion

Salinity is one of the key factors limiting the development and production of vegetable crops [36]. This is primarily due to the high concentration of sodium ions either in the soil or in irrigation water [37]. These ions disrupt essential physiological activities such as stomatal conductance, photosynthesis, and tissue hydration and trigger oxidative stress and membrane damage, leading to diminished plant biomass [5]. To curb the devastating effects of salinity on food crops, biostimulants such as seaweed extracts are used as crop management products to enhance crop productivity under abiotic stress conditions [10].

In the present study, the effectiveness of a South African seaweed-derived biostimulant (Kelpak®) in alleviating salinity stress in spinach was assessed. The results revealed a substantial improvement in crop development and yield in plants treated with seaweed extracts (SWE) under saline irrigation. Notably, the 5% SWE concentration displayed the greatest improvement in crop yield, whereas the 2.5% concentration also showed good benefits, albeit to a lesser extent when compared to the control. Moreover, the application of SWE also enhanced root development in salt-stressed spinach. These findings suggest that SWE upregulated enzymes' activity and increased the level of endogenous hormones, which accelerated cell division and elongation processes to promote root growth, which enabled efficient water and mineral nutrient uptake, thus leading to improved plant biomass [38]. Radwan et al. [39] and Rouphael et al. [40] also reported similar findings on seed watermelon (*Citrullus lanatus*) and zucchini squash (*Cucurbita pepo*) under saline conditions, where SWE enhanced the root hydraulic conductivity and root activity, enabling efficient water and mineral absorption from the rhizosphere, in turn leading to a higher plant biomass. Additionally, the enhancement of growth parameters in salt-stressed spinach treated with SWE could be attributed to macro and micronutrients as well as growth regulators such as amino acids, auxins, abscisic acid, and gibberellins that have been reported in the SWE (Kelpak®) used in this study [41].

Salinity has also been reported to cause severe impact on various physiological traits in crops such as photosynthetic pigments and relative water content (RWC) [42]. Thus, the assessment of chlorophyll content is frequently used as an index for salt tolerance in many plant species, whilst relative water content is regarded as an appropriate indicator of plant hydration levels [43]. In this study, salt-stressed spinach without the application of SWE exhibited reduced chlorophyll content and RWC. This is mostly due to the impairment of the photosynthetic machinery caused by the excessive build-up of sodium ions in the chloroplasts which disturbs the carbon metabolism and photophosphorylation, resulting

in reduced photosynthesis [44]. Interestingly, salt-stressed spinach treated with 5% SWE exhibited an improved chlorophyll content and an RWC which was comparable to the control plants. The improvement in these two physiological traits could be attributed to growth regulators found in the SWE, which are known to promote the absorption of water and nutrients and enhance the endogenous synthesis of phytohormones, particularly cytokinin, which plays a crucial role in protecting chlorophyll from photodamage, resulting in enhanced chlorophyll content [11,12]. Krid et al. [45] also reported similar findings for tomato grown under saline conditions, where an increase in chlorophyll was noted in plants treated with SWE. This was attributed to the presence of various organic substances within the SWE that played a role in sustaining the functions of photosynthetic enzymes, chlorophyll composition, photosynthetic apparatus, and the ultrastructure of thylakoid membranes.

Moreover, salinity triggers oxidative stress in crops by producing superoxide radicals through the Mehler reaction [46–48]. The overproduction of these free radicals interferes with normal metabolic functions in the cytoplasm, mitochondria, and peroxisomes by inducing oxidative damage to proteins and lipids [49–51]. This leads to cellular malfunction, which further causes cell death. Several biochemical indicators, such as cell death viability, superoxide radicals, and malondialdehyde, are routinely utilised to determine the extent of oxidative stress in plants exposed to harsh conditions [4,52]. Therefore, this study found that plants subjected to salinity without SWE treatment had higher amounts of superoxide radicals and malondialdehyde than those treated with SWE. This has been reported in numerous studies conducted on salt sensitive species such as spinach [19,53–55]. Intriguingly, salt-stressed spinach treated with 5% SWE had reduced oxidative stress and the amounts were comparable to the control plants. This was followed by the upregulation of superoxide dismutase, an antioxidative enzyme known to be the first line of defence against the excessive production of superoxide radicals [56,57]. This suggests that the SWE triggered this enzyme to dismutate these superoxide radicals into H_2O_2 and O_2 and mitigated salinity stress in spinach.

Nutritional imbalances in vegetable crops due to salinity have been documented in the literature, typically resulting from the disruption of ion transport and accumulation induced by competing Na^+ and Cl^- ions, which restrict the absorption of vital nutrients necessary for optimal growth [58]. This was the case in this study, where the accumulation of Na^+ in spinach without SWE resulted in a substantial reduction in the content of essential nutrients, such as phosphorus, potassium, calcium, magnesium, and vitamins C and E. Nonetheless, spinach plants treated with 5% SWE had an improved magnesium and calcium content which was comparable to the control plants. Sufficient potassium (K), calcium (Ca), and magnesium (Mg) contents are required to meet the basic metabolic processes such as intracellular K homeostasis, which is essential for the optimal functioning of the photosynthetic machinery and the maintenance of stomatal opening [55]. These results imply that the application of SWE upregulated the absorption of K, Ca, and Mg by new shoots under saline irrigation and maintained a suitable ratio needed for normal metabolism, thus the improvement in plant growth. These results are in line with those reported by Yarşı [59] on pepper (*Capsicum annuum* L.) seedlings subjected to saline irrigation, where the application of SWE improved the contents of K, Ca, and Mg. Furthermore, salt-stressed spinach treated with 5% SWE exhibited an increase in Zn, Mn, and Fe content. This enhancement in micronutrient contents might have also influenced nutrient homeostasis in salt-stressed spinach, which is demonstrated by enhanced crop productivity. This is supported by numerous studies which indicated that the supplementation of zinc (Zn) and manganese (Mn) improves plant performance under saline conditions by facilitating plant growth through the protection of photosynthesis and the mitigation of oxidative stress [58,60,61].

When assessing the proximate constituents of the tested samples, ash and neutral detergent fibre (NDF) were found in abundance in plants subjected to salinity without SWE. It is commonly asserted that, with increasing salinity, plants absorb significant amounts

of sodium ions and subsequently translocate them to the shoots, resulting in elevated ash content [62]. Furthermore, elevated salinity augments polysaccharide contents in the cell walls while diminishing soluble carbohydrate contents, resulting in an increase in the content of insoluble fibres such as NDF [63]. Interestingly, salt-stressed spinach treated with 2.5 and 5% SWE had lower contents of ash and NDF, whereas the non-fibre carbohydrate content was enhanced. This shows that the addition of SWE inhibited the formation of polysaccharides in the cell wall, therefore increasing the soluble carbohydrate contents in spinach. Protein content was also increased in salt-stressed spinach treated with 5% SWE, and this could be attributed to the increase in proteolysis as well as amino acid synthesis found in this SWE and the renaturation of enzymes involved in protein and amino acid synthesis [64].

5. Conclusions

This study reveals that the addition of seaweed extract (Kelpak®) by soil drenching at 5% dilution alleviated salinity stress in spinach by enhancing the morphophysiological, biochemical, and nutritional quality of these plants. Therefore, these findings support the use of seaweed extract (Kelpak®) as a biostimulant in vegetable farming systems in regions affected by salinity. Nonetheless, field studies in salinity-affected soils are needed to broaden the mechanism of action and to ascertain the salt alleviation potential of this South African seaweed extract.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/horticulturae10121340/s1>, Table S1: The chemical composition of Kelpak seaweed extract. Adapted from Lötze and Hoffman. Table S2: Amino acid composition of kelpak expressed in mg.100 g⁻¹.

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