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Growth Characteristics, Phytochemical Contents, and Antioxidant Capacity of *Trachyandra ciliata* (L.f) Kunth Grown in Hydroponics under Varying Degrees of Salinity

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Abstract: This study evaluated the effect of salinity and soilless media on the vegetative growth, phytochemicals, and antioxidant capacity of *Trachyandra ciliata* (wild cabbage) to develop its growth protocol and explore its potential as a natural source of secondary metabolites. Treatments consisted of different concentrations of sodium chloride (NaCl), control- 0 mM, 100 mM, 200 mM, 400 mM, while different in vitro assays were used for phytochemical and antioxidant screenings. Findings from the study showed that low salinity (100 mM) significantly increased chlorophyll content, plant height, leaf number, plant fresh weight, and production of inflorescence, particularly in Peat-Perlite-Vermiculite (PPV) medium. In contrast, the control was the most productive treatment in plant dry weight except for the inflorescence. The highest antioxidant activity was observed in 200 mM of NaCl treatment in combination with PPV medium, which also produced the highest mean values for polyphenols, while 100 mM was the best for flavonols. Therefore, *T. ciliata* proved to be more productive vegetatively under low salinity in combination with PPV soilless media. A combination of 200 mM + PPV treatment was also recommended for maximum production of antioxidants for *T. ciliata*.

Keywords: Asphodelaceae; halophytes; polyphenols; salinity stress; sodium chloride; wild cabbage

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1. Introduction

Agricultural production in Southern Africa is greatly limited by drought. In the agricultural context, drought is a lengthened period of rain deficiency, which leads to an adverse impact on plant growth and yield [1]. Abiotic stress such as drought leads to a reduction in vegetative growth, photosynthetic rates, transpiration, and respiration [2]. Southern Africa is the third most water-scarce prone region after the Middle East and North Africa [3]. Increasing agricultural production, therefore, becomes a challenge to keep up with the increasing population and sustain food and nutrition security. This is partly due to water scarcity and, therefore, an inability to supplement summer rains [4]. Demands for water have tripled since the 1950s, while freshwater supplies have been declining consistently [5]. The Western Cape Province of South Africa has been the most affected by water scarcity. It was predicted that in a few years, this province would be unable to supply water for its agricultural needs [6].

Accumulation of salt in agricultural lands results in salt toxicity, which often reduces the efficiency of stomatal conductance, photosynthesis, transpiration, and respiration [7]. A significant build-up of salts in the root zone results in reduced water holding capacity, leading to reduced yield [8]. Plants respond differently to salinity as affected by different salt levels, including growth reduction resulting from nutritional differences [9]. It has been reported that environmental factors such as salinity, high or low pH, drought, and high temperature may lead to increased production of reactive oxygen species, which

often results in oxidative stress when available in high concentrations [10]. This oxidative stress induced by these environmental factors leads to increased production of secondary metabolites, otherwise known as phytochemicals, to protect the plant from the adverse effect of stress [11]. During oxidative stress, plants release antioxidants that neutralize free radicals, and those antioxidants are very important to humans as they prevent sickness and diseases when consumed in the form of fruits and vegetables [12,13].

Reactive oxygen species (ROS) are derivatives produced during aerobic metabolism and are active in numerous essential signaling pathways within plants [13]. Reactive oxygen species are valuable to plants because they support cellular proliferation, biological functions, and sustainability. Therefore, maintaining a basic level of ROS in all cells is important for life [14]. Although ROS are beneficial to life, extreme concentration in the cell can lead to damage of lipids, proteins, DNA, and nucleic acids [14].

Phenolic compounds are secondary metabolites with antioxidant ability capable of trapping reactive oxygen species. They occur in both edible and non-edible plants deriving from pentose phosphate, shikimate, and phenylpropanoid pathways in plants [15]. Phenolic compounds are one of the most occurring groups of all the phytochemicals and are considered to be very significant for plants as they protect against diseases and grazing [16,17]. Antioxidants are very useful for humans and plants as they can safely react with free radicals, and thus protecting cells and the health of organisms [18]. Oxidation is regarded as the process in which electrons are transferred from one atom to the other, and that is an essential part of aerobic life and metabolism [19]. Antioxidants derived from plant extracts are more preferred compared to synthetic ones because of health and safety reasons [11,20]. It has been reported that the consumption of fruit and vegetables containing phenolic compounds with antioxidant activity can reduce the risk of chronic infections and diseases [19,21,22]. These health factors have given rise to the extensive screening of plants for antioxidants and other pharmaceutical properties.

Continuous drought and high salinity levels, therefore, call for a need to evaluate and develop new protocols for future food production [23]. Several authors, including [24] and [23] suggest that the cultivation of indigenous, salt, and drought-tolerant halophytic plants for food could be one of the approaches to sustainable food and nutrition security. Tolerance and sensitivity to salinity may differ according to the type of salinity, different plant species, type of growing medium, and the plant's stage of development [25]. It has been found that plants grown in hydroponics use ten times less water than conventionally grown plants [26]. This is because, in soil-based plant cultivation, water leaches out, whereas water is collected and recycled in hydroponics [26]. Wahome et al. [27] reported that hydroponic plants grow faster than conventionally grown plants because all required nutrients are readily available in the solution.

Trachyandra is a genus of more than 50 species under the Asphodelaceae (Aloe) family. Species of *Trachyandra* are found throughout Southern Africa but the majority of them are restricted and endemic to the winter rainfall area of the South Western Cape and very few extend further northwards, with only one extending as far as Ethiopia [28,29]. Over the years, the Asphodelaceae family has been extensively studied based on medicinal properties and are widely used in the pharmaceutical and beverage industries. However, *Trachyandra* is a less studied genus with little documented literature available on medicinal use and cultivation.

T. ciliata commonly known as wild cabbage or Veldkool (Afrikaans), is a halophytic species in Asphodelaceae [30]. The plant is underexploited with little to no literature at all, and there are no *Trachyandra* species that are currently cultivated [28,31]. However, it has been documented that the inflorescence of *T. ciliata* was used by the indigenous Khoi-san people as a vegetable before colonization that led to the erosion of knowledge about native plants [29,31]. As drought and salt-tolerant species, it is a good candidate needed to ensure increased agricultural production and relieving pressure on the demand for freshwater for irrigation in an attempt to address the issue of water scarcity and food insecurity. However, knowledge on the responses of this crop to salinity is imperative to improve

its performance and to plan new areas for expanding its production for marketability considering the existing knowledge gap in botanical literature. This study was, therefore, undertaken to evaluate the effect of various salinity concentrations and different growth media on physiological parameters and bioactivity of *T. ciliata* grown in hydroponics under greenhouse conditions, to develop an efficient growth protocol for this plant.

2. Materials and Methods

This experiment was carried out in the research nursery of the Cape Peninsula University of Technology at Bellville campus, Cape Town, South Africa, located at 33°55′48.8″ S, 18°38′32.7″ E. The temperatures in the experimental greenhouse in which the study was conducted were kept between 21 and 26 °C during the day and 12 and 18 °C at night with the use of environmental control. The relative humidity average was kept at 60%.

2.1. Experimental Design

In this experiment, 4 identically constructed nutrient film (NFT) systems were used, with each system on separate wire mesh square tables (2.5 m) that provided a flat surface (Figure 1). The treatments were labeled as T1–T4. Each system had its low-density polyethene (LDPE) 50 L reservoir in which the nutrient solution was prepared. There were 3 Polyvinyl Chloride (PVC) square gutters (2 m), put in place with cable ties on each table, in which 3 different substrate combinations were tested. The gutters were sealed with PVC adhesive to prevent leaks. The gutters were labeled G1, G2, and G3. In the construction of each system, a 1 × 2000 L/h submersible pump with 2.5 m head capacity, 20 mm LDPE irrigation piping, 4 × 20 mm elbow irrigation fittings and 4 × 20 mm flow regulators were used.

Figure 1. The layout of the experiment showing replicates before the addition of NaCl. n = 10 replicates (Picture: Ngxabi).

Gutters were fitted with 1 outlet that returned the solution to the reservoir (Figure 1). Every gutter on each system had 10 pots (12.5 cm height × 12.5 cm length × 12.5 cm width) with a different substrate to test for the best medium. Every gutter was then covered with a black plastic bag to provide a dark conducive environment for the roots and to also

discourage the growth of algae by depriving it of direct sunlight, which is necessary for photosynthesis. One side of the table was slightly elevated to allow the flow of the nutrient solution, creating a spontaneous circulating system. A 1 × 2000 L/h submersible pump with 2.5 m head capacity was used to circulate the nutrient solution for 24 h from the beginning to the end of the experiment. Electrical conductivity (EC) in the nutrient solution was monitored daily with a calibrated hand-held digital EC meter (Hanna instruments®TM HI 98312). The pH of the solution was monitored with a calibrated hand-held digital pH meter (Eurotech®TM pH 2 pen). Potassium hydroxide was used to elevate pH, while phosphoric acid was used to decrease the pH of the nutrient solution [32].

2.1.1. Plant Material

The plant material of *T. ciliata* was obtained from a local nursery. The plant material was propagated by the division technique because it has rhizomes. A total of 120 plants were then transferred into the hydroponic system. It was ensured that the plants were as genetically identical as possible. Divided plantlets were placed in each pot, resulting in 30 plants for each treatment with 10 repetitions and 120 plants for the whole experiment and arranged in a randomized block design.

2.1.2. Nutrient Solutions

Nutrifeed™ fertilizer supplied by Starke Ayres, Cape Town, has been certified as containing all the essential nutrients necessary for healthy and vigorous plant growth and is now widely used to make hydroponic aqueous solutions. It has the following specifications: 65 g/kg N, 27 g/kg P, 130 g/kg K, 70 mg/kg Ca, 20 mg/kg Cu, 1500 mg/kg Fe, 10 mg/kg Mo, 22 mg/kg Mg, 240 mg/kg Mn, 75 mg/kg S, 240 mg/kg B and mg/kg Zn. The fertilizer group 1 Reg No: K2025 (Act 36/1947) was applied in this experiment to supply all the nutrients to the nutrient solution equally in all the sumps. The system ran with tap water for the first 4 weeks to reduce transplant shock. Nutrifeed aqueous solution was prepared manually using normal tap water as stipulated by the manufacturer.

2.1.3. Medium Treatments

The pots were placed in each gutter, and the medium combinations were manipulated as follows:

Silica sand (100% sand),
PPV (1:1:1—Peat: Perlite: vermiculite),
Clay (100% Leca clay).

Shade cloth was placed at the base of each pot to prevent leakage of the medium through drainage holes of the pot.

2.1.4. Salinity Treatments

Different salt concentrations were manipulated using Sodium chloride (NaCl) in the Nutrifeed nutrient solutions. NaCl was added at week 6 after the system had been running for a month with tap water and one more week with the addition of Nutrifeed™. Three salt concentrations (100 mM, 200 mM, 400 mM of NaCl) were tested in this experiment and added into each sump, while 0 mM of NaCl was considered as a control (Figure 2). The saline solutions were prepared using tap water. All nutrient solutions containing NaCl were replaced weekly to avoid the accumulation of salts in the medium, pots, gutters, and reservoirs. The pH was maintained at 6.0. A 400 mM of NaCl was prepared by dissolving 23.38 g of NaCl in 1 L of water (1 M of NaCl contains 58.44 g). This was serially diluted to achieve lower concentrations of desired salinity.

Figure 2. Plant response to salinity at week 9 of the experiment showing signs of wilting on the first table due to higher salt concentration. (Picture: Ngxabi). n = 10 replicates.

2.2. Determination of Growth Parameters

2.2.1. Plant Weight

A standard laboratory scale was used to determine the weight of the plants before planting to ensure uniformity of the samples. After the experiment, inflorescence shoot and root systems were separated, and their weight was recorded. The loose plant material was separately dried at 50 °C using a LABTECH™ model LDO 150F (Daihan Labtech India. Pty. Ltd. 3269 Ranjit Nagar, New Dehli, 110008) oven until moisture was completely removed from the tissues; dry weights were then measured and recorded [33].

2.2.2. Shoot Length

The length of the shoots was used to determine the height of the plants. Shoot length was measured (cm) manually using a standard measuring tape every second week and recorded on a data spreadsheet.

2.2.3. Number of Leaves

The leaves were counted every second week and recorded on a data spreadsheet.

2.2.4. Chlorophyll Content of Leaves

The chlorophyll content of the plants was determined using a SPAD-502 Konica-Minolta meter. The average readings of 2 fully developed leaves from each plant were determined and recorded on a data spreadsheet. The readings were recorded during the midday with average daylight levels of 10 Klux (light intensity) [34].

2.2.5. Formulation of Crude Extract

Crude extracts were obtained by stirring the finely ground plant material (whole plant harvested at week 22) in ethyl alcohol (EtOH) and centrifuged at 4000 rpm for 5 min. The supernatant was filtered through a Whatman No. 1 filter paper, which was placed in a Buchner funnel connected to an electric vacuum pump. This was conducted to remove

unmacerated tissue and other debris. The resulting crude extracts used for all analyses were utilized to perform phytochemical and antioxidant assays [10].

2.3. Determination of Phytochemical and Antioxidant Contents

Phytochemical content and antioxidant capacity of metabolites in the plant extract were assayed for total flavonols, total polyphenols, ferric reducing antioxidant power (FRAP), ABTS, and DPPH.

2.3.1. Total Polyphenol Assay

The total polyphenol essay of the extracts was performed using the Folin–Ciocalteu method as reported by [35,36]. About 25 µL of the sample was mixed with 125 µL Folin–Ciocalteu reagent (Merck, Johannesburg, South Africa) that was diluted 10 times with distilled water. Then 7.5% sodium carbonate (Sigma, Alberton, South Africa) solution was prepared and added in a 96-well microplate with extracts. The plate was incubated for 2 h at room temperature and the absorbance was then measured at 765 nm in a Multiskan spectrum plate reader (Thermo Electron Corporation, Waltham, MA, USA). The standard curve was prepared using 0, 20, 50, 100, 250, and 500 mg/L gallic acid (Sigma, South Africa) in 10% EtOH, and the results were expressed as mg gallic acid equals per g dry weight (mg GAE/g DW).

2.3.2. Estimation of Flavonol Content

The flavonol content of the extracts was determined using quercetin 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol (Sigma-Aldrich, Johannesburg, South Africa) as standard. About 12.5 µL of the crude sample extracts were mixed with 12.5 µL 0.1% HCl (Merck, South Africa) in 95% ethanol, 225 µL 2% HCl for each sample. The extracts were then incubated for 30 min at room temperature. The absorbance was read at 360 nm at a temperature of 25 °C [37]. The results were expressed as mg quercetin equivalent per g dry weight (mg QE/g DW).

2.3.3. Determination of Ferric Reducing Antioxidant Power (FRAP)

The method of [38] was used to perform the FRAP assay. FRAP reagent was prepared by mixing 30 mL Acetate buffer (0.3 M, pH 3.6) (Merck, South Africa) with 3 mL 2,4,6-tripyridyl-s-triazine (10 mM in 0.1M Hydrochloric acid) (Sigma, South Africa), 3 mL Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) (Sigma, South Africa), 6 mL of distilled water and incubated for 30 min. at 37 °C [20]. Then, 10 µL of the crude sample extract was mixed with 300 µL of the FRAP reagent in a 96-well plate. The absorbance was then measured at 593 nm in a Multiskan spectrum plate reader. An L-Ascorbic acid (Sigma-Aldrich, South Africa) was used as a standard to calculate the FRAP sample values, with the concentration curve varying from 0 to 100 µM. The results were expressed as µM ascorbic acid equivalents (AAE) per g dry weight (µM AAE/g DW).

2.3.4. Determination of ABTS Antioxidant Capacity

The ABTS antioxidant capacity was assayed through a method described by [39] with slight modification. The stock solutions included a 7 mM ABTS and 140 mM Potassium-peroxodisulphate ($K_2S_2O_8$) (Merck, South Africa) solution. The solution in the experiment was then prepared by adding 88 µL $K_2S_2O_8$ to 5 mL ABTS solution. These 2 solutions were mixed and left to react for 24 h in the dark at room temperature. Trolox (6-Hydrox-2,5,7,8-tetramethylchroman-2- 20 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 ABTS in the dark at room temperature for 5 min before the absorbance was read at 734 nm at 25 °C in a microplate reader. The results were expressed as µM/Trolox equivalent per g dry weight (µM TE/g DW).

2.3.5. Antioxidant Capacity of DPPH Radicals

The DPPH radical was generated from a solution of 0.135 mM DPPH prepared in a dark bottle [40]. About 300 µL of DPPH solution was reacted with graded concentrations (0 and 500 µM) of Trolox standard (6-Hydrox-2,5,7,8-tetramethylchroman-2- 20 carboxylic acid) solution and 25 µL of crude extract. The mixtures were incubated for 30 min, after which absorbance was taken at 517nM [33]. The results were expressed as µM/Trolox equivalent per g dry weight (µM TE/g DW).

2.4. Statistical Analysis

A two-way analysis of variance (ANOVA) was used to determine the interaction between salinity and growth media on some growth parameters and phytochemicals and antioxidant capacity of *T. ciliata*. The significant differences between treatment means at $p \leq 0.05$ were compared using Fisher's least significant difference (LSD). All the calculations were conducted on a computer software program STATISTICA version 10.

3. Results

3.1. Effects of Salt Stress and Soilless Media on Vegetative Growth

3.1.1. The Effects of Salinity and Growth Media on Leaf Number

The interaction of salinity and media also did not have any significant effect on the number of leaves produced. However, 100 mM concentration combined with PPV medium recorded the highest mean value of 42.80 followed by sand in the control, while 400 mM showed salt toxicity and recorded the lowest means across all growth media mixtures, including the control when combined with Leca clay. The results further showed that different salinity levels significantly affected ($p \leq 0.05$) the number of leaves (Table 1). In contrast, the results showed that soil-less media did not have a significant influence on the number of leaves produced by the plants (Table 1).

Table 1. Effect of four varied salinity levels and different soil media on total dry weight, total fresh weight, and number of leaves of hydroponically grown *T. ciliata*.

Soil-Less Medium	NaCl Conc. (mM)	Total Dry Weight (g)	Total Fresh Weight (g)	Leaf No.	Shoot Length (cm)
Silica Sand	0	19.62 ± 2.56 a	131.02 ± 20.2 bc	37.90 ± 3.78 a	73.50 ± 1.61 e
	100	12.24 ± 1.79 b	136.40 ± 21.44 bc	34.10 ± 5.7 a	68.70 ± 8.72 e
	200	1.97 ± 0.76 c	22.15 ± 7.54 d	7.30 ± 2.1 b	31.00 ± 8.66 ab
	400	0.3 ± 0.19 c	2.15 ± 1.13 d	2.00 ± 1.04 b	5.70 ± 3.02 cd
PPV	0	15.92 ± 2.08 ab	142.16 ± 18.47 ac	34.30 ± 6.71 a	77.50 ± 8.81 e
	100	13.72 ± 0.98 b	156.31 ± 14.16 ac	42.80 ± 3.89 a	72.90 ± 3.34 e
	200	3.62 ± 1.11 c	34.91 ± 11.22 d	9.60 ± 2.66 b	34.80 ± 9.67 a
	400	0.78 ± 0.3 c	6.88 ± 2.81 d	4.10 ± 1.64 b	14.60 ± 5.04 bcd
LECA Clay	0	14.66 ± 1.58 b	99.68 ± 13.31 b	35.70 ± 3.01 a	67.00 ± 1.91 e
	100	15.40 ± 2.53 b	183.11 ± 29.95 a	36.30 ± 6.59 a	68.00 ± 8 e
	200	1.26 ± 0.44 c	11.17 ± 3.76 d	5.20 ± 1.81 b	19.70 ± 6.62 abc
	400	0.00 ± 0 c	0.00 ± 0 d	0.00 ± 0 b	0.00 ± 0 d
Two-way ANOVA F-Statistics					
Soil-less Medium		0.30 ns	0.84 ns	0.82 ns	3.17 *
NaCl Conc.		92.02 *	77.24 *	70.74 *	77.62 *
Soil-less Medium * NaCl Conc.		1.55 ns	0.19 ns	0.46 ns	0.20 ns

Mean values ± SD are shown in columns. The mean values followed by different letters down the columns are significantly different at $p \leq 0.05$ (*) and ns = not significant as calculated by Fisher's least significant difference. n = 10 replicates.

3.1.2. The Effects of Salinity and Growth Media on Plant Height

The results gathered from the current trial indicate that soil-less media significantly ($p \leq 0.05$) influenced shoot length. The results also showed that different salinity levels had a significant ($p \leq 0.05$) influence on shoot length. However, there was no interaction between soil-less media and salinity on plant height. In addition, the highest height was

recorded in treatments with 0 mM and 100 mM salinity in all soilless media as there was no significant difference in the results obtained. In addition, a 200 mM salinity had an equivalent effect on plant height in sand and PPV media, while 200 mM salinity in Leca clay yielded equivalent height as 400 mM salinity in the sand and PPV (Table 1).

3.1.3. The Effects of Salinity and Growth Media on the Total Fresh Weight

Soil-less media and its interaction with different salinity levels did not have any significant effect on total fresh weight. The combination of 100 mM + LC recorded the highest mean value (183.11 g) followed by 100 mM + PPV with a mean value of 156.31 g. The results gathered from the present experiment indicate that salinity significantly affected ($p \leq 0.05$) *T. ciliata* total fresh weight. Contrary to that, soil-less media did not show any significant effect on total fresh weight. Salinity 400 mM recorded the lowest mean values across all soil-less mixtures (Table 1).

3.1.4. The Effects of Salinity and Growth Media on the Total Dry Weight

In contrary to the total fresh weight results, the interaction of 0 mM + SS recorded the highest mean value (19.62 g) followed by 0 mM + PPV with a mean of 15.92 g. Results of the present study showed that salinity significantly affected ($p \leq 0.05$) the total dry weight in *T. ciliata*. Contrary to that, soil-less media did not show any significant effect on total fresh weight. In addition, soil-less media and its interaction with different salinity levels did not have any significant effect on total fresh weight. Comparable to the fresh weight results, 400 mM recorded the lowest mean values across all soil-less mixtures (Table 1).

3.1.5. The Effects of Salinity and Growth Media on Chlorophyll Content

The interaction of 0 mM + clay recorded the highest SPAD-502 value (324) followed by 0 mM + SS and 100 mM + PPV with SPAD-502 values of 283.40 and 278.30, respectively. The results also indicate that salinity had a significant influence ($p \leq 0.05$) on *T. ciliata* chlorophyll content. On the contrary, soil-less media did not significantly affect the chlorophyll content. Moreover, the interaction of soil-less media and salinity did not have any significant effect on the chlorophyll content. Salinity 400 mM recorded the lowest mean values across all soil-less treatments (Table 2).

Table 2. Effect of four varied salinity levels and different soil media on the number of flowers, inflorescence fresh weight, inflorescence dry weight, and chlorophyll content of hydroponically grown *T. ciliata*.

Soil-Less Medium	NaCl Conc. (mM)	Number of Flowers	Inflorescence Fresh Weight (g)	Inflorescence Dry Weight (g)	Chlorophyll
Silica sand	0	1.20 ± 0.42 b	33.2 ± 13.17 ab	2.08 ± 0.99 bc	283.40 ± 9.89 ab
	100	0.30 ± 0.15 c	7.07 ± 4.02 c	0.74 ± 0.41 cd	257.30 ± 29.7 ab
	200	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 d	166.60 ± 46.42 bcd
	400	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 d	69.00 ± 35.9 de
PPV	0	0.90 ± 0.28 b	40.14 ± 13.19 ab	3.47 ± 1.15 b	270.60 ± 33.5 ab
	100	1.80 ± 0.25 a	46.73 ± 7.9 a	5.14 ± 0.79 a	278.30 ± 7.91 ab
	200	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 d	141.00 ± 39.52 bcde
	400	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 d	103.70 ± 36.64 de
LECA Clay	0	0.90 ± 0.23 b	27.77 ± 6.86 b	2.67 ± 0.67 b	324.00 ± 16.14 a
	100	0.30 ± 0.21 c	7.20 ± 6.27 c	0.60 ± 0.52 cd	254.20 ± 28.91 abc
	200	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 d	111.30 ± 38.18 cde
	400	0.00 ± 0 c	0.00 ± 0 c	0.00 ± 0 d	0.00 ± 0 f
Two-way ANOVA F-Statistics					
Soil-less Medium		4.34 ns	4.78 ns	8.15 *	0.84 ns
NaCl Conc.		22.87 *	19.2 *	19.38 *	38.99 *
Soil-less Medium * NaCl Conc.		5.72 *	2.79 ns	4.78 *	1.35 ns

Mean values ± SD are shown in columns. The mean values followed by different letters down the columns are significantly different at $p \leq 0.05$ (*) and ns = not significant as calculated by Fisher's least significant difference. n = 10 replicates.

3.1.6. The Effects of Salinity and Growth Media on the Number of Flowers

The interaction of soil-less media and salinity had a significant effect on the number of flowers produced. The interaction of 100 mM + PPV recorded a significantly higher number of flowers in comparison with any other treatment. The interactions of 100 mM + SS and 100 mM + LC recorded the least number of flowers with means of less than one for both. Furthermore, the results showed that only the control and 100 mM saline treatment produced flowers (Table 2). Soil-less media did not have any significant effect on the number of flowers produced. On the contrary, salinity had a significant effect ($p \leq 0.05$) on the number of flowers produced by the plants.

3.1.7. The Effects of Salinity and Growth Media on Inflorescence Fresh Weight

The interaction of soil-less media and salinity did not have any significant effect on inflorescence fresh weight. The highest mean value was observed in the interaction of 100 mM + PPV followed by 0 mM + PPV with 46.73 g and 40.14 g, respectively. The interaction of 100 mM + LC and 100 mM + SS recorded the lowest mean values of 7.20 g and 7.07 g, respectively. Moreover, the results of the current experiment indicated that salinity had a significant influence ($p \leq 0.05$) on inflorescence fresh weight. In contrast, soil-less media did not have a significant effect on inflorescence fresh weight (Table 2).

3.1.8. The Effects of Salinity and Growth Media on Inflorescence Dry Weight

The interaction of 100 mM + PPV recorded a significantly high value (5.14 g) followed by 0 mM + PPV and 0 mM + LC whose means were 3.47 g and 2.67 g, respectively. The interaction of 100 mM + SS and 100 mM + LC recorded the lowest mean values of 0.74 g and 0.60 g, respectively. However, findings from this study indicated that soil-less media significantly influenced inflorescence dry weight at ($p \leq 0.05$). Salinity also significantly affected the inflorescence dry weight ($p \leq 0.05$), which conforms with the results obtained for fresh weight. Another interesting aspect was that the interaction of soil-less media and salinity had a significant effect ($p \leq 0.05$) on the inflorescence dry weight (Table 2).

3.2. Effects of Salt Stress on Phenolic Content and Antioxidant Capacity

3.2.1. Effect of Salinity and Soilless Media on the Accumulation of Polyphenols

The results obtained from this experiment showed that salinity and soilless media had a significant effect ($p \leq 0.05$) on the total polyphenol content (Figure 3). The interaction of 200 mM NaCl and PPV medium significantly produced the highest concentration of polyphenols (11.07 mg GAE/g DW) compared to other treatments, while 400 mM NaCl yielded the lowest content of polyphenol in all soilless media, especially in LECA clay as revealed by Tukey least significant difference ranking. Likewise, 0 mM and 100 mM NaCl yielded an equivalent polyphenol in the sand medium; 0 mM and 200 mM NaCl produced no significant difference in polyphenol content in LECA clay; thus also, 100 mM of NaCl in PPV and Leca clay yielded an equal amount of polyphenol as shown by Tukey least significant difference ranking. The lowest content of polyphenol was recorded in the interaction of 400 mM and PPV, whereas no growth was recorded in Leca clay with 400 mM NaCl treatment, hence no polyphenol.

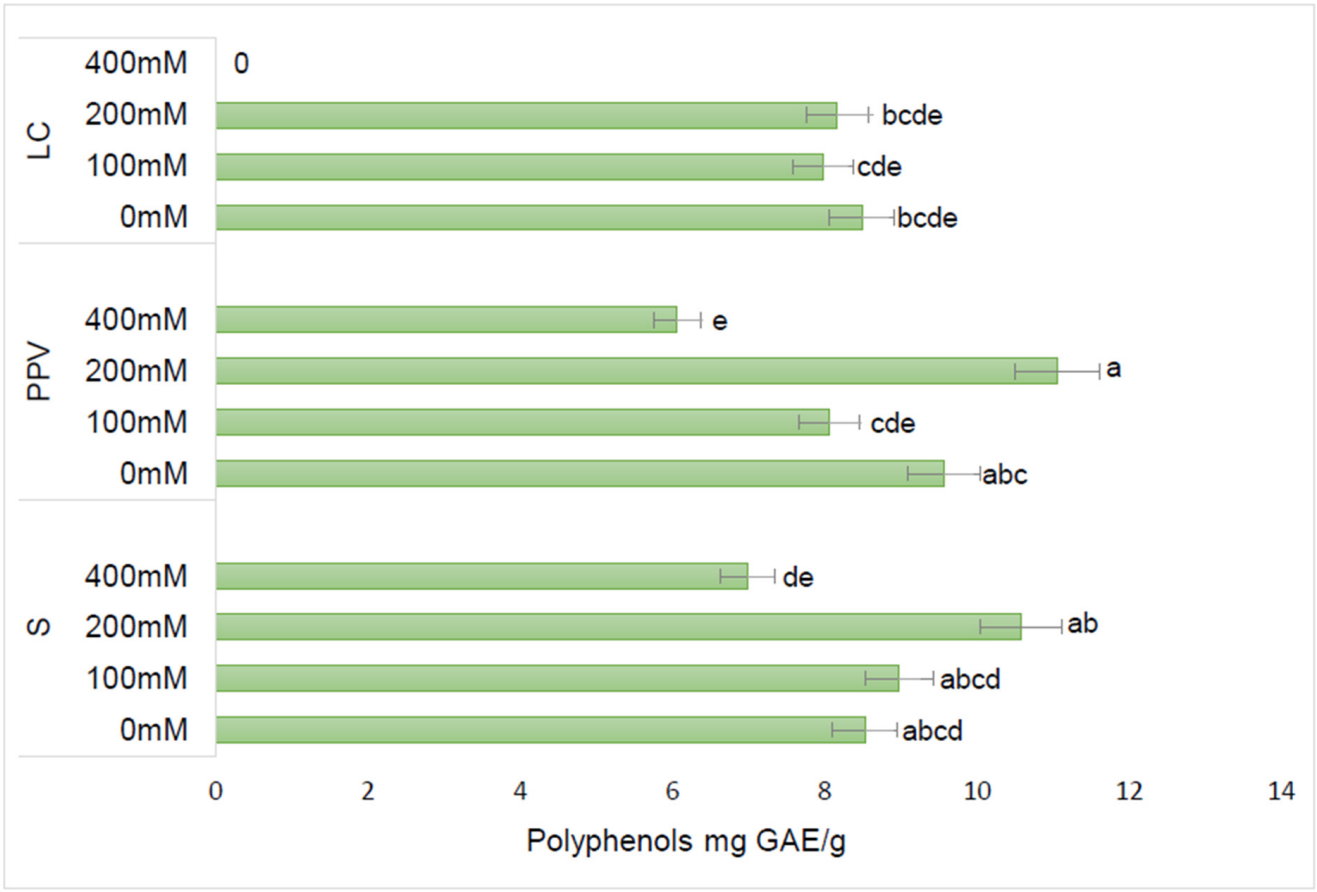

Figure 3. Influence of salinity (NaCl) and growth medium on the polyphenol content of *T. ciliata.* The mean values with different letters are significantly different at $p \leq 0.05$. $n = 10$ replicates.

3.2.2. Effect of Different Salinity Levels and Soilless Media on Flavonols

There was no significant difference in the interaction between 100 mM NaCl and 200 mM salt with sand and PPV, respectively. Similarly, an equivalent flavonol yield was found in the interaction of the control samples of zero salt concentration with sand, PPV, and Leca clay. Both salinity and soilless media were found to have a significant influence on the flavonol content of *T. ciliata*. Their interaction also had a significant effect on flavonol concentration at $p \leq 0.05$. The highest mean flavonol value (8.01 mg QE/g of the pulverized sample) was recorded in the interaction of 100 mM salt and PPV compared to all other treatments, while the lowest mean value (2.38 mg QE/g DW) was observed in 200 mM in Leca clay (Figure 4).

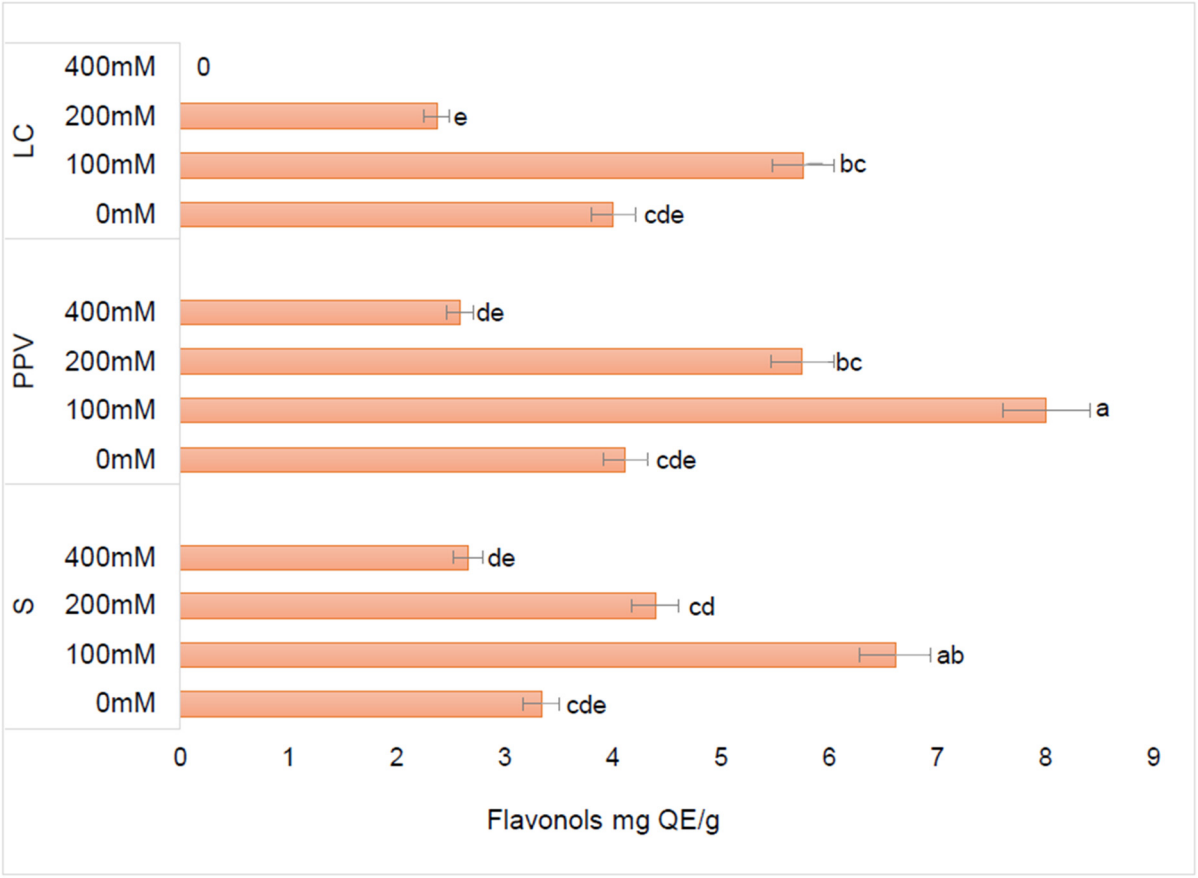

Figure 4. Influence of salinity (NaCl) and soilless media on flavonol content. The mean values with different letters are significantly different at $p \leq 0.05$. $n = 10$ replicates.

3.2.3. The Effect of Salinity and Soilless Media on FRAP

The lowest mean value was observed in the interaction of 400 mM + SS (31.71 µM AAE/g DW), and as stated in earlier results, no growth was recorded in 400 mM with Leca clay plants died because of salt toxicity. Similarly, interactions of sand and Leca clay with 100 mM of salt had equivalent FRAP activity with 400 mM in PPV. However, sand and Leca clay had equivalent FRAP activity at zero salt concentration. Results also showed that both salinity and soilless media had a significant influence ($p \leq 0.05$) on the FRAP values of *T. ciliata*. The 200 mM NaCl recorded the highest mean FRAP values in Leca clay and PPV, whereas the same salt concentration was of lesser effect with sand (Figure 5).

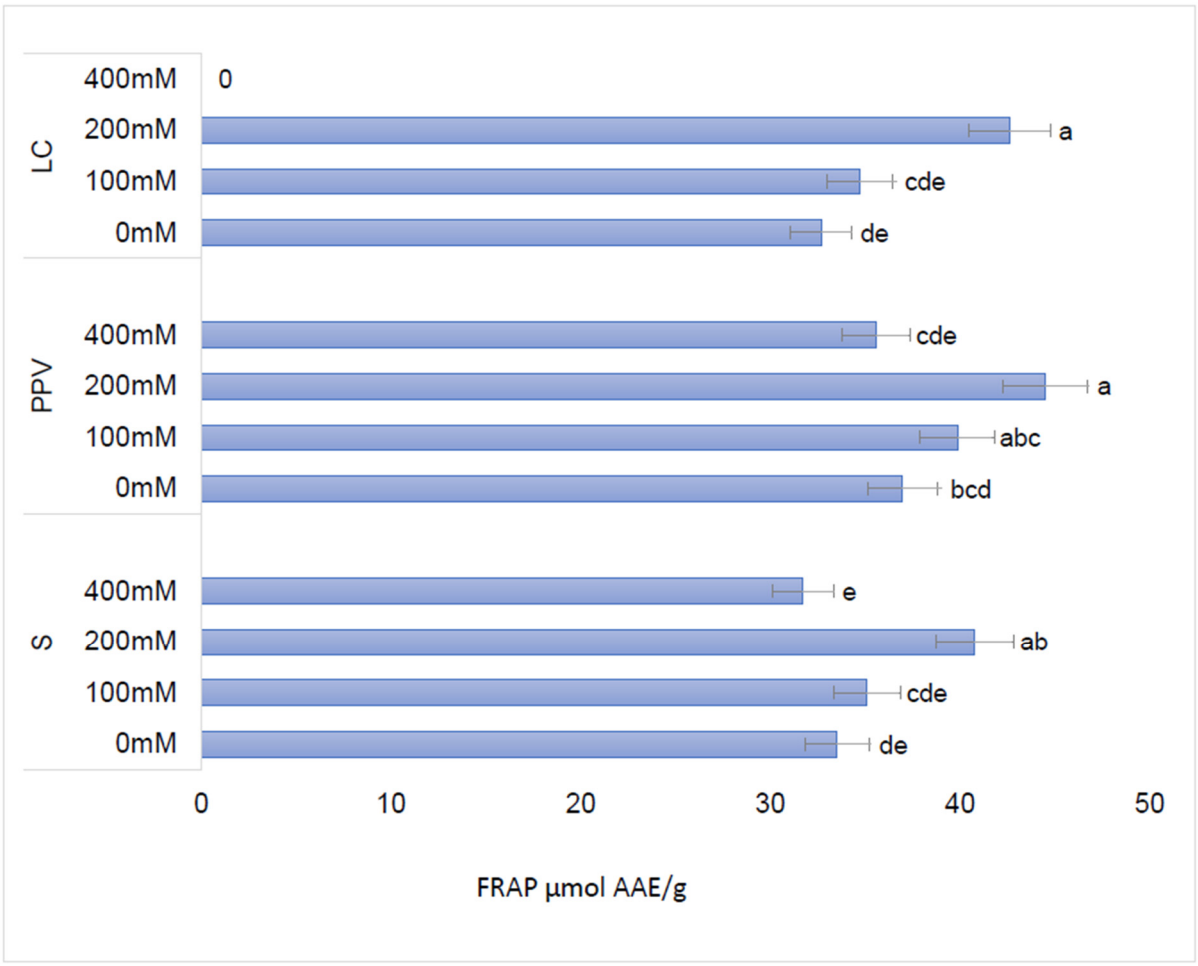

Figure 5. Influence of salinity (NaCl) and soilless media of FRAP. The mean values with different letters are significantly different at $p \leq 0.05$. $n = 10$ replicates.

3.2.4. Influence of Different Salinity Levels and Soilless Media on ABTS

Results of this experiment also revealed that both salinity and soilless media had a significant effect on the ABTS values. Figure 6 shows that the 200 mM salt concentration had the highest values in PPV (40.79 µM TE/g DW) and lowest in the sand with 400 mM. Results further showed significant variability in ABTS capacity in all concentrations tested between soilless media.

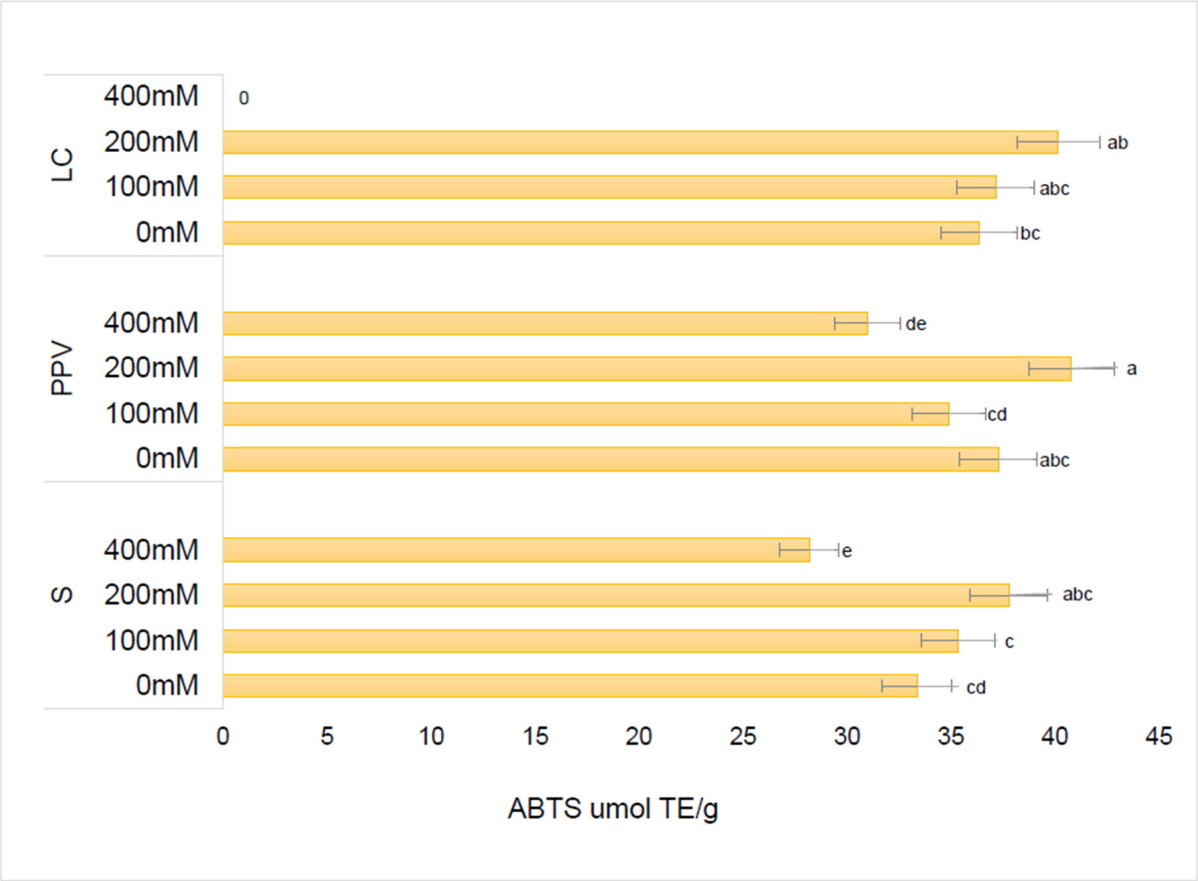

Figure 6. The influence of salinity (NaCl) and soilless media on ABTS. The mean values with different letters are significantly different at $p \leq 0.05$. $n = 10$ replicates.

3.2.5. Effect of Different Salinity Concentrations and Soilless Media on DPPH

The highest mean was obtained in the interaction of 200 mM + SS (19.02 µmol TE/g), while the lowest was recorded in the interaction of 400 mM + PPV (9.39 µmol TE/g). The results obtained showed that different salinity levels had a significant influence on DPPH values. Results also showed that soilless media had significantly influenced the DPPH values. The 200 mM concentration recorded the highest mean values in sand medium, although, at this salinity, Leca clay and PPV had equivalent effects on DPPH radical, which is almost similar to sand while 400 mM recorded significantly lowest values in PPV (Figure 7). There was no significant difference between 0 mM and 100 mM salinity levels in the LECA clay medium. However, 0 mM and 200 mM salinity levels in PPV had an equivalent effect on DPPH with 100 mM salt in sand medium (Figure 7).

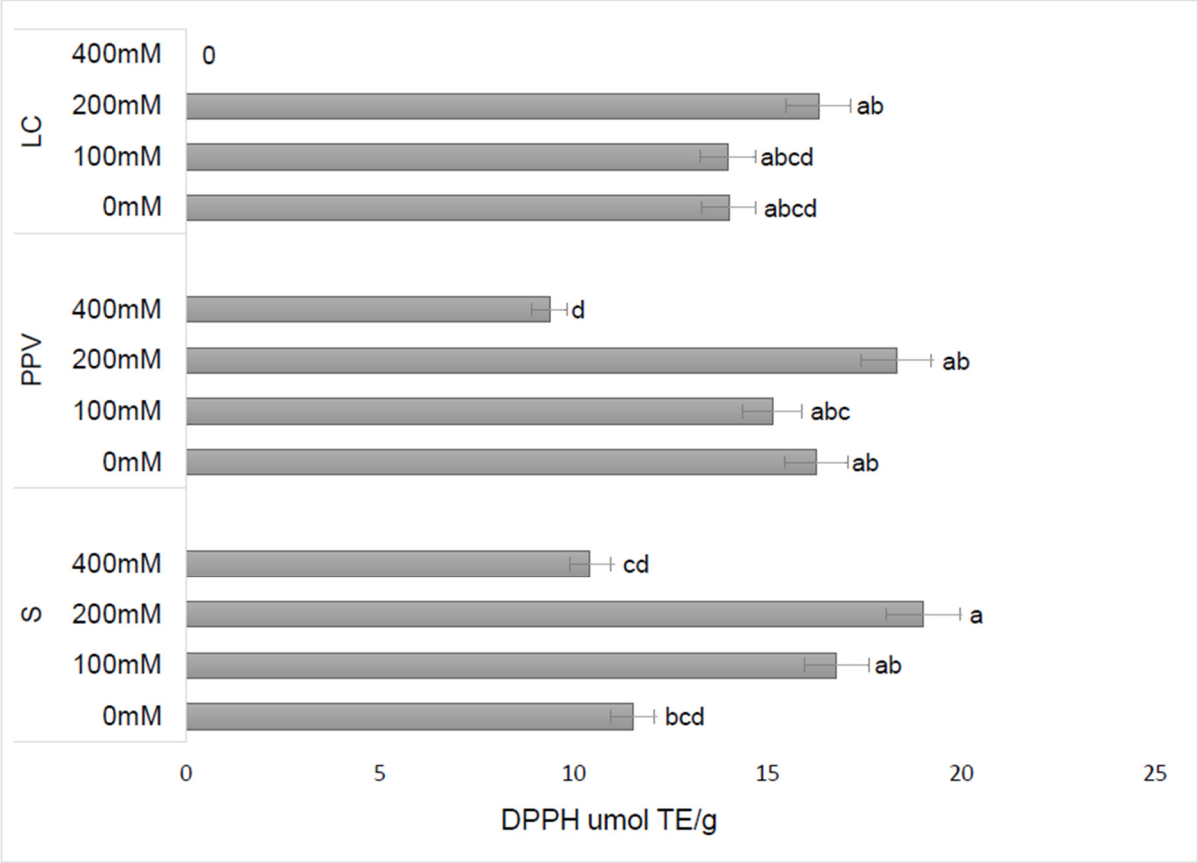

Figure 7. Influence of salinity (NaCl) and soilless media on DPPH. The mean values with different letters are significantly different at $p \leq 0.05$. $n = 10$ replicates.

4. Discussion

As agricultural productivity is threatened by increasing salinity levels in the soil, there is a need to grow vegetable crops that are salt-tolerant to supplement the existing crop plants to guarantee the world's food and nutrition security. From this study, it can be reported that *T. ciliata* is indeed a true halophyte because of its ability to survive and complete its life cycle under highly saline conditions. This agrees with previous reports by Koryo [41] and Rengasamy et al. [42] on the growth response of halophytes to high saline conditions. In addition, the growth medium plays an important role in successful cultivation as it serves as an anchor that allows for gas exchange and holds water and nutrients for the plants [43]. Medium selection is one of the most significant factors influencing plant growth and development in the greenhouse and affecting crop quality. It is, therefore, important to consider types of growth substrates in greenhouse cultivation because different plants require different growth substrates and, naturally, are adapted to certain types of soils [32].

The results obtained from this study also suggested that low levels of salinity (100 mM) enhance the vegetative production of *T. ciliata*, while high levels of salinity reduced vegetative growth. In this experiment, salinity significantly affected both the fresh and dry weight of the shoot. The highest mean values for total fresh weight were observed at 100 mM salinity level, while the lowest mean values were recorded at high salinity. These results agree with the findings of Klados and Tzortzakis [24], who concluded that low salinity levels enhance growth and high levels reduce production considerably. Adams [44] and Sayyad-Amin et al. [45] reported that salinity or high EC reduces crop yield. The control with 0 mM salinity produced the highest mean values for total dry weight, although almost equivalent to the mean weight recorded in 100 mM salinity treatment. In addition, Khan et al. [46] reported that water and osmotic potentials of a halophytic plant (*Suaeda fruticosa*) became

more negative as salinity was increased, hence the decrease in weight whereas an increase in NaCl heightened the reproductive capacity in *Suaeda salsa* [47]. Growth media had no significant effect on the biomass of *T. ciliata*. The highest biomass means were observed in Leca clay for fresh weight, while for dry biomass, the highest mean values were recorded in plants grown in sand. Other studies also show that plant biomass increases with increasing salinity in *Solanum melongena* L. [48] and *Phaseolus vulgaris* L. [49] whereas biomass in *Lycopersicon esculentum* (tomato) decreases with increased salinity [50]. The findings of this research also agree with those of Koryo [41] and khan et al. [46], who worked on halophytic plants and found that low salinity increases productivity, and at higher salinity, plant growth reduces. Invariably, this phenomenon of salt toxicity is related to the efficiency of stomatal conductance, photosynthetic rate, transpiration, and respiration as affected by high salinity levels [41,42].

Likewise, the number of leaves increased at low salt levels and declined due to salt toxicity. The most productive soilless medium was a mixture of perlite, peat, and vermiculite at 100 mM salt level. This is because this mixture has a high water holding capacity and is porous at the same time, which increases aeration in the root zone [51,52]. Both salinity and soilless media showed a significant effect on shoot height, and interestingly, 0mM and 100 mM concentrations showed no significant effect amongst each other in all media mixtures. PPV proved to be the most effective soil-less medium and recorded the highest mean values when in conjunction with the 0 mM and low salinity levels (100 mM). These results correspond with the findings of Klados and Tzortzakis [24], Sayyad-Amin et al. [45], and Zapryanova and Atanassova [53], who concluded that low salinity levels increased leaf length while high salt levels reduced it. However, [49] and [48] further reported that salinity had different effects on growth, and this may differ from species to species.

The inflorescence of *T. ciliata* is the most important part being the only part that is edible and can be used as a vegetable [31], hence the significance of its results. Low salinity ($\pm$100 mM) has significantly proven to enhance flower development and weight, while high salinity prevents flower development. Treatments with high salt levels (200 mM and 400 mM) were not able to develop any flowers. This phenomenon might be related to the absence of hormones that are responsible for flower development due to salt toxicity. It is reported that a reduction in plant productivity with increasing salinity is related to reduced osmotic potential and reduced stomatal conductance [41]. PPV soil mixture significantly proved to be the most effective growth medium when compared to other soilless media. The findings of this study correspond to that of Ventura et al. [54], Zapryanova and Atanassova [53], and Stanton et al. [55], who found that the development of flowering buds and inflorescence weight increases in low NaCl levels and decrease as salinity increases. These authors further reported that salt toxicity causes flower abortion and inhibits or delays flowering.

Moreover, different levels of salinity had a significant effect on the chlorophyll content, although there was no difference between the control and low salt level (100 mM) across all soilless media. Chlorophyll content then decreased as salinity levels increased. The growth media did not affect the chlorophyll content. Salt toxicity in the root zone leads to a reduction in photosynthesis and consequently may have resulted in reduced chlorophyll content in the leaves [45]. It was further suggested that increased salinity might reduce chlorophyll content in normal plants and may increase it in salt-tolerant plants. Salinity reduced stomatal conductance, water potential, osmotic potential, and nitrogen concentration on the leaves, thereby reducing chlorophyll content [41].

Furthermore, results from this study also suggested that soilless media had a significant effect on the accumulation of phytochemicals and antioxidants. The highest mean values in all parameters were recorded in PPV soilless medium, and this medium was the most consistent. The trend observed was that sand recorded average mean values, while Leca Clay recorded slightly lower values. These findings are related to the fact that the PPV medium has everything needed by plants as it is made up of peat, perlite, and vermiculite. The medium has a high water holding capacity, high capillary action, and is porous at the

same time because of perlite [52]. Literature reports that the most beneficial component of PPV is peat because it promotes the growth of beneficial bacteria that promote growth and has anti-fungal properties that protect plants from lethal fungi [56]. The dominance of this medium can also be associated with the fact that it is a mixture of different media that have different benefits to plants.

Generally, moderate salt concentration (200 mM) showed a significantly positive effect on polyphenols. There was not much of a difference between 0 mM and low salt concentration (100 mM), while high salt concentration produced significantly lower means. These findings corroborate an earlier report by [57] that moderate salt concentration promoted the production of phenolic compounds within a halophytic plant, *Cynara scolymus* L. Ben-Abdallah et al. [58] and Ksouri et al. [11] found similar results and reported that high salinity levels restricted the accumulation of phenolic compounds in a halophytic plant, *Cakile maritima* Scop. It has been reported that salinity stress reduces plant biomass and photosynthesis [50]. As a result, plants divert the production of carbohydrates and produce secondary metabolites [59]. It was also reported that the decline in the production of polyphenols at high salt concentration is caused by restricted uptake of phosphorus and potassium, which are principal elements for the production of secondary metabolites such as polyphenols [60]. Ben Abdallah et al. [61] and Wong et al. [62] further stated that disturbances in enzyme activity at high salt concentrations lead to reduced photosynthesis, causing a reduction in growth, polyphenol content, and antioxidant capacity. The excessive presence of ROS leads to oxidative stress, which results in DNA damage and cell death, hence the reduction in polyphenolic compounds [60].

In contrast, there was a different observation regarding flavonol content, which was opposite to the trend that was observed in other parameters. The results, just as in all other parameters, proved that salinity, as well as soilless media, have a significant influence on the accumulation of flavonols. However, in this case, low salt concentration (100 mM) recorded significantly higher mean values in all soil mixtures. This is opposite to what was observed in other parameters where moderate salinity provided high mean values in phytochemical yield. High salt levels (200 mM +) proved not to be effective for the accumulation of flavonols. These findings agree with Ksouri et al. [11], who reported that 100 mM was the most effective concentration on the antioxidant capacity of a halophyte (*Cakile maritima*). The lowest mean value was surprisingly recorded in the treatment of 200 mM + clay. This is also in agreement with earlier reports that low to moderate salt concentrations positively contributes to the accumulation of flavonols [57].

Ferric reducing antioxidant power (FRAP) and ABTS were significantly affected ($p \leq 0.05$) by salinity and soilless media. The highest FRAP and ABTS values were observed in moderate salt concentration (200 mM) across all soilless media. The lowest FRAP value was surprisingly observed in the combination of 0 mM and Leca clay followed by high salt concentration in all other media, while it was observed in the interaction of 400 mM and Leca clay for ABTS. Low salt concentration (100 mM) recorded slightly higher FRAP and ABTS values but significantly lower than those of moderate salinity. These results may be associated with the fact that low to moderate salt concentration does enhance the presence of polyphenolic compounds and antioxidants, while salt toxicity reduces the antioxidant capacity of halophytes [11]. Salt toxicity on the root zone of plants negatively affects water and nutrient osmotic potential because water is taken out of cells around root hairs, resulting in dehydration and reduced photosynthesis rates [63]. Furthermore, high salinity can be toxic and lead to reduced photosynthesis, resulting in decreased synthesis of secondary metabolites [11].

Similarly, the DPPH radical-scavenging activity was significantly affected by salinity and soilless media. The highest radical scavenging activity was observed in the moderate salt concentration (200 mM) across all soilless media treatments, while the lowest was observed in the highest concentration (400 mM). The trend was the same as in polyphenols regarding 0 mM and 100 mM concentrations. These findings agree with [57], who reported that moderate salinity concentration had the highest radical-scavenging activity. This may

be related to the rupturing of cells and inhibition of nutrient and water uptake at high salt levels [62,64]. The results of this experiment agree with the correlation between FRAP and DPPH that was reported by Sharma and Ramawat [65]. It is reported that not all plants respond to salinity the same way; others show a significant increase in production of radical-scavenging activity, while others may show a significant decrease in biological activity [65].

5. Conclusions

This current study reveals that low salinity levels may be used successfully in hydroponics in conjunction with a mixture of perlite as well as peat and vermiculite (1:1:1) for the cultivation of *T. ciliata* on a commercial scale. Besides, moderate salinity-induced oxidative stress is useful to produce antioxidants. It can be concluded that low salinity is required for optimal vegetative yield in wild cabbage, and the plant is a potential source of natural antioxidants. Further studies are required to understand the distribution of sodium ions and changes in nutrient content as affected by salinity. Studies to evaluate the enzymatic activities and the mechanisms involved in the accumulation of antioxidants are also recommended.

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