Cape Peninsula
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The effect of different growth media and salinity on the vegetative growth *of Trachyandra ciliata* (Wild cabbage) in hydroponics

by

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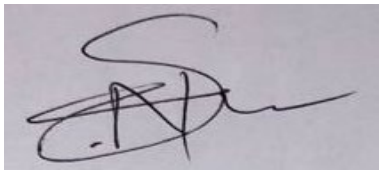
DECLARATION

I, Sihle Ngxabi, declare that the contents of this dissertation/thesis represent my own unaided work and that the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.



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ABSTRACT

Water scarcity and increasing salinization of agricultural lands will pose a huge threat to food production in the future. Increasing agricultural production, therefore, becomes a challenge to keep up with the increasing population and sustain nutrition and food security. The Western Cape Province of South Africa has been the most affected by water scarcity and it has been predicted that in a few years, this province will be unable to supply water for its agricultural needs. This phenomenon necessitates the development of more innovative techniques and the cultivation of halophytes to enhance sustainable crop production. *Trachyandra ciliata* is a halophytic plant with limited literature and it belongs to the Asphodelaceae family that is popular for the medicinal properties of its members. The existing literature suggests that it is edible but there is no scientific evidence of its biochemical properties and nutrient content. Hydroponically grown plants use 10 times less water than conventionally grown plants because in soil-grown plants water quickly leaches to the soil, while water is collected and circulated again in hydroponic cultivation.

The aim of this study was to determine the influence of salinity and soilless media on the vegetative growth, phytochemical and antioxidant capacity of hydroponically grown *T. ciliata* under a greenhouse environment, in order to determine the growth protocol for this plant. Four identically constructed Nutrient Film Technique (NFT) systems were used, with each system on a separate metal mesh steel table (2.5 m long). Nutrifeed™ served as a general hydroponics feed while different salt concentrations of Sodium chloride (NaCl) (100mM, 200mM, 400mM of NaCl) were used as treatments added into each sump, while nutrient solution without addition of NaCl was considered as control. Twenty treatments were evaluated with 10 replicates per treatment. All nutrient solutions containing NaCl were replaced and the system flushed every week to avoid the buildup of salts in the medium, pots, gutters, and reservoirs. The treatments were comprised of 3 different soilless media; Leca clay (LC), silica sand (SS), and a combination of peat: perlite: vermiculite (PPV) at a 1:1:1 ratio. A standard laboratory scale was used to measure the weight and the shoot length was manually using a standard measuring tape. The chlorophyll content of the plants was determined using a SPAD-502 Konica-Minolta meter. Antioxidant content and capacity of metabolites within the mixture of dried and ground roots and shoots were assessed by means of assays for total Flavonols, total polyphenols, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), ferric reducing antioxidant power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH).

Salinity significantly (P≤0.05) affected shoots wet weight, root wet weight, shoot dry weight, and both total wet and dry weight. The highest mean values for shoots wet weight, root wet weight, and total wet weight were observed at 100 mM salinity level, while high salinity levels recorded the lowest mean values. Growth media had no significant effect on biomass accumulation. Both salinity and soilless media showed a significant effect on shoot length and number of leaves with a combination of 100mM+PPV recording the highest mean values. Both salinity and soilless media significantly affected the inflorescence weight and the number of flowers with 100mM+PPV interaction recording the highest significant mean values. The chlorophyll content was significantly affected by salinity while soilless media did not affect chlorophyll accumulation. 0mM and 100mM recorded the highest SPAD values although there was no significant effect between the treatments. Salinity-induced oxidative stress and soilless media are significantly (P≤ 0.05) effective for the accumulation of polyphenols. The interaction of moderate (20mM) and PPV recorded the highest significant mean values for polyphenols, FRAP, ABTS and DPPH. The combination of 100mM+PPV recorded the highest significant mean value for the accumulation of flavonols. It was concluded that low salt levels (100mM) promote biomass accumulation while high salt levels (400mM) are toxic and decrease growth significantly. Soilless media has no significant effect on plant biomass except for the inflorescence, in which the combination of 100mM+PPV is recommended. Moderate salt levels (200mM) in conjunction with PPV are recommended for the accumulation of polyphenolic compounds(antioxidants) except for flavonols, where 100mM is recommended. It was concluded that moderate salt levels may not promote biomass accumulation but effective for antioxidant content.

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DEDICATION

This work is dedicated to:

- My late great-grandmother, Veleleni Gloria Ngxabi, a strong and gentle soul who taught me to always trust in God, believe in the power of prayer, believe in hard work and that so much could be done with little. May your soul Continue resting in peace.
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STRUCTURE OF THE THESIS

The thesis is drafted differently from the alternative of a traditional format for a thesis. The article-format thesis examples of published, co-published, and/or “ready-for-publication” articles were prepared during candidature and applies to the format prescribed by CPUT for 100% master's studies which complies with the following guidelines:

1. The overriding principle of the thesis is that it remains an original contribution to the discipline or field by the candidate.

2. Chapters containing the journal articles form a coherent and integrated body of work, which focused on a single project or set of related questions or propositions. All journal articles form part of the sustained thesis with a coherent theme.

3. The study does not include work published before the commencement of the candidature.

4. The number of articles included depending on the content and length of each article and take full account of the university’s requirements for the degree as well as the one article already published or “ready-for-publication” expected for a master’s degree in this discipline.

5. The thesis should be examined in the normal way and according to the normal requirements as set out by the “Guidelines for Examiners of Dissertations and Theses” (using form HDC 1.7).

The thesis consists of the following chapters, which are concisely discussed as:

Chapter One: This chapter provides the significance of the research, its aim, and the overall list of specific objectives, which guided the study.

Chapter Two: This chapter provides insight into the potential of *T. ciliata* **(**Wild cabbage) as an underutilized leafy vegetable crop. It also highlights its uses, propagation, distribution, the environmental effect on growth as well as potential cultivation methods including soilless media which could be recommended for commercial use. It further discusses how the introduction of this plant into the market can mitigate food insecurity and water scarcity.

Chapter Three: This chapter evaluated the effect of salinity stress and different growth media on the growth *of Trachyandra ciliata* L. (Aphodelaceae) in hydroponics. The research justification, materials and methods, results, and discussions are presented.

Chapter Four: This chapter evaluated the Effect of salinity and different soilless media on phytochemicals and antioxidant capacity of *Trachyandra ciliata* (L.f) Kunth (Asphodelaceae) grown in hydroponics. The research justification, materials and methods, results and discussions are presented.

Chapter Five: Results and discussions. This chapter deals with the general discussion of results, which connects the findings from previous chapters.

Chapter Six: Conclusion and Recommendations. This chapter deals with conclusions that were made from previous chapters and is followed by recommendations for further research.

Chapter Seven: References

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ACRONYMS AND ABBREVIATIONS

°C- Degrees Celsius

ABTS- 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid)

ANOVA- Analysis of variance

cm- Centimetres

DPPH- 2,2-diphenyl-1-picrylhydrazyl ethanol

EC- Electric Conductivity

ETOH- Ethyl Alcohol

FDA- Food and Drug Administration

FRAP- Ferric Reducing Antioxidant Power

G- Gutter

$K_2S_2O_8$- Potassium-Peroxodisulphate

LC- Leca Clay

LDPE- Low-Density Polyethylene

LSD- Least Significant Difference

NaCl- Sodium chloride

NFT- Nutrient Film Technique

pH- Potential of Hydrogen

PPV- Peat:Perlite:Vermiculite

PVC- Polyvinyl Chloride

ROS- Reactive Oxygen Species

SD- Standard deviation

SE- Standard Error

SS- Silica Sand

T- Treatment

WWF- World Wide Fund for Nature

CHAPTER ONE

PROBLEM STATEMENT, AIMS, HYPOTHESES, AND OBJECTIVES

PROBLEM STATEMENT, AIMS, HYPOTHESES AND OBJECTIVES

1.1 PROBLEM STATEMENT

Agricultural production in southern Africa is greatly limited by inadequate availability of water as the region is the third to be confronted by devastating water scarcity after North Africa and the Middle East (Turton *et al*., 2000; Rosa *et al*., 2020). South Africa is a very rich and diverse country with the capacity to produce food crops all year round depending on the season, favorable climate ranges, variation in vegetation types, and rich soil profiles (Turton *et al*., 2000). Amongst the agricultural aspects affected is the incapability of the country to maximize agricultural productivity in order to keep up with the increasing population growth and food security problems. This is partly due to water scarcity to supplement summer rains (Singh, *et al*., 2014). It has been reported that the water demand has been increasing since the 1950s, but the freshwater supply has been decreasing consistently ever since (Gleick, 2003a). It is predicted that South Africa will face physical water scarcity in the near future because of the rapid population growth and decreasing freshwater supply (WWF, 2017). In South Africa, the province that is extremely affected by water scarcity is the Western Cape and it is also predicted that by 2025, this province would be unable to cater for its agricultural needs (WWF, 2017). This then calls for the cultivation of plants that are tolerant to high levels of drought and salinity to be used for food (Ventura *et al*. 2011). Condon *et al.* (2004) found that of the world's allocated water resource, about 80% is currently dedicated to irrigated agriculture and this level of water usage by agriculture is not maintainable in the future. They further suggest that anticipated population growth of approximately 2 billion people within 2–3 decades to come will necessitate that more of the available water resource be redirected to municipal, domestic, industrial, and environmental needs.

Food security is regarded as a broad term, but the basic definition is that it is the accessibility of food to all people at all times for active healthy life (Anderson, 1990; Du Toit *et al*., 2011). Du Toit *et al*. (2011) also stated that food security is regarded as being closely related to poverty in a country. South Africa is one of the countries with high variations of income in the world and with high levels of poverty compared to other middle-income countries (Altman *et al*., 2009). In response to this problem, continuous efforts are being made by the government to obtain high productivity in order to increase profitability and to meet the constantly increasing demand for food.

Hydroponic propagation is turning to be a favorite method of cultivating plants at present in South Africa because it allows the production of plants and flowers all year round. In South Africa, there are many areas of dry land including deserts, semi-deserts as well as places with inadequate groundwater. In response to this, farmers most of the time have no other

options but to use halophytes, which use less amount of water (Van Zyl, 2012). Production of crops in controlled environments gives chances of improved growth, quality, purity, and consistency (Hayden, 2006).

This study was carried out to investigate the effects of different salinity levels and growth media on the vegetative growth of *Trachyandra ciliata* grown in hydroponics. This plant is less studied with little to no literature at all. However, it has been documented that this plant is edible and was used as food by Khoi-san people who lived on the western Cape coast of South Africa (De Vynck *et al*., 2016). This research is also conducted to test the precise salt tolerance of this plant since it grows alongside other halophytes in nature in an attempt to prove a speculative thought that this plant can be watered directly with seawater like other halophytes.

It has been found that plants that are grown in hydroponics use ten times less water than the conventionally grown plants because water leaches out to the soil in soil-based plant cultivation, while water is collected and recycled in hydroponics (Ortiz *et al*., 2009). Wahome *et al*. (2011) reported that hydroponically grown plants grow faster than conventionally grown plants because all required nutrients are readily available in the solution.

This study is aimed at confronting the issue of water scarcity by introducing a vegetable crop that is drought tolerant. It is also to confront the issue of water scarcity by introducing a vegetable crop that is salt tolerant and that can be watered directly by seawater or diluted seawater. It also aims to contribute to alleviating food insecurity by introducing a new crop that can be used as a green edible vegetable to ensure easier access to food for local people. In this study, it was hypothesized that the optimal hydroponic growth protocol of *T. ciliata* would be developed at the end of the experiment. This research is of high importance as the results of this study will be recommended to commercial growers as an optimal growth protocol for this plant.

1.2 AIM OF THE STUDY

This study aims to evaluate the effect of salt stress and different growing media on the growth of *T. ciliata* to establish a growth protocol for the species.

1.3 HYPOTHESIS

It is hypothesized that optimum salinity will positively affect the growth of *T. ciliata*. It is hypothesized that a high concentration will negatively affect the growth of *T. ciliata*. It is also hypothesized that salinity stress will positively affect the accumulation of antioxidants.

1.4 OBJECTIVES

1.4.1 Main objective

The purpose of this study was to investigate vegetative growth and nutrient contents of *T. ciliata* (L.F) Kunth (Wild cabbage) in response to different growth media and the salinity of an aqueous nutrient solution in order to establish an appropriate hydroponic growth protocol.

1.4.2 Specific objectives

- To determine the vegetative growth of the inflorescence size of *T. ciliata* in response to different growth media and salinity, in order to determine and establish an optimal hydroponic growth protocol for this plant.
- To determine aerial fresh and dry weight of *T. ciliata* in response to different growth media and salinity, in order to determine and establish an optimal hydroponic growth protocol for this plant.
- To determine root fresh and dry weight of *T. ciliata* in response to different growth media and salinity, in order to determine and establish an optimal hydroponic growth protocol for this plant.
- To quantify chlorophyll content of *T. ciliata* in response to different growth media and salinity, in order to determine and establish an optimal hydroponic growth protocol for this plant.
- To determine the number of leaves and shoot length of *T. ciliata* in response to different growth media and salinity, in order to determine and establish an optimal hydroponic growth protocol for this plant.
- To quantify the antioxidant capacity of *T. ciliata* in response to different growth media and salinity in order to determine treatments that produce high amounts of antioxidants in hydroponics.

CHAPTER TWO

TRACHYANDRA CILIATA (ASPHODELACEAE) – AN UNDERUTILIZED VEGETABLE CROP FROM SOUTH AFRICAN WESTERN CAPE COAST: A REVIEW

EDIBILITY OF TRACHYANDRA CILIATA (ASPHODELACEAE) – AN UNDERUTILIZED VEGETABLE CROP FROM SOUTH AFRICAN WESTERN CAPE COAST: A REVIEW

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2.1 Abstract

The aim of Sustainable Goal 2 (SDG2) is to end hunger, improve food security and nutrition and promote sustainable crop production by 2050. However, climate change, increasing soil salinization, and the inadequate availability of freshwater have negatively affected crop production around the world including South Africa, making it difficult to meet the required target. This necessitates the use of wild edible plants that are adapted to adverse conditions such as drought and salinity in order to mitigate this problem. The genus *trachyandra* (Asphodelaceae) consists of three edible species (*T. ciliata, T. divaricata and T. falcata*) which are native to the dry saline environments of the western Cape coastal dunes. The genus is less studied with no record of cultivated species, although the existing literature states that *T. ciliata*, (wild cabbage) was originally used as a food source by the indigenous Khoi-san people who lived on the South African Cape coast. This review explores the importance of Asphodelaceae family, *T. ciliata* as a vegetable crop, and its potential as a pharmaceutical candidate. Furthermore, this review examines potential technological advances such as hydroponics that could be used for sustainable crop production of *T. ciliata*. The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) was utilized in the selection of articles in this review. The existing literature provided useful information on the potential of *T. ciliata* as a vegetable crop and the importance of using halophytes to achieve food security. This appraisal is expected to serve as a template for researchers, food enthusiasts, potential farmers, and policy makers who may be keen on exploring further nutritional composition and medicinal potential of this plant.

Keywords: Asphodelaceae, food security, halophytes, hydroponics, water scarcity

2.2 Introduction

Agricultural production in southern Africa is greatly limited by inadequate availability of water as the region is the third to be confronted by devastating water scarcity after North Africa and the Middle East (Turton *et al*., 2000). Amongst the agricultural aspects affected is the incapacity of the state to maximize agricultural production in order to keep up with the increasing population growth to sustain food security. This is partly due to water scarcity to supplement summer rains (Singh, *et al*., 2014). Demands for water have tripled since the 1950s while freshwater supplies have been declining consistently ever since (Gleick, 2003a). It is predicted that South Africa will face physical water scarcity by 2025 because of the rapid population growth and decreasing freshwater supply (WWF, 2017). In South Africa, the province that is extremely affected by water scarcity is the Western Cape and it is also predicted that in the near future, this province would be unable to cater for its agricultural needs (WWF, 2017). This then calls for the cultivation of halophytic plants that are tolerant to high levels of salinity and drought to be used for food (Ventura *et al*., 2011). Condon *et al.* (2004) reported that out of the world's water resource, about 80% is presently dedicated to irrigated agriculture and this level of usage by agriculture is not maintainable in the future. They further suggested that anticipated population growth of about two billion people within 2–3 decades to come will necessitate that more of the available water resource be used for environmental, domestic, industrial, and municipal needs. It is predicted that by 2050, crop production must increase by at least 50 to 100% in order to cater for the constantly growing global population, but the current increase is around 1 to 1.5% and therefore it is a major challenge to guarantee sustainable agriculture (WWF, 2017).

Salinity presents limitations in plants and leads to a decline in general yield, leaf production, delayed flowering, and abortion of flower buds (Sharma & Ramawat, 2013; Abdallah *et al*., 2016). It is therefore a huge challenge for scientists to determine sustainability with regards to water resource preservation and food availability for underprivileged populations in the future. The adoption of the phenomenon to use salt and drought tolerant crops to ensure increased agricultural production while preserving water resources should be extensively researched in preparation for the future (Ventura & Sagi, 2013). Coastal lands represent large hectares that are currently underexploited for conventional agriculture but have the potential of being used for halophytic food crops (Silveira et al., 2009). Halophytes are highly productive under saline conditions and that could be the solution to the issue of freshwater depletion and their use could ensure the productivity of saline soils (Yamaguchi & Blumwald, 2005; Silveira *et al*., 200).

Furthermore, the increasing population has led to the industrialization of lands that were used for agricultural purposes (Rosa *et al*., 2020). An increase in soil salinization together

with the growing scarcity of freshwater stimulates the need to develop more innovative techniques to enhance sustainable crop production (Ventura & Sagi, 2013). Therefore, technological advances and improvements are key factors in determining sustainability and agricultural production in the future (Ventura & Sagi, 2013).

Hydroponic propagation is turning to be a favourite method of cultivating plants at present in South Africa because it allows the production of plants and flowers throughout the year. In South Africa there many areas of dry land including deserts, semi-deserts as well as places that have inadequate groundwater. In response to this, farmers in most cases have no other options but to use high-density crops that use less amount of water (van Zyl, 2012). Production of crops in controlled environments gives chances of improved growth, quality, purity, and consistency (Hayden, 2006). It is imperative that techniques that have the potential to increase agricultural production and ensure food security be studied extensively so that the risk of unavailability of food in the future is mitigated. Ventura *et al*., (2011) suggested that the cultivation of indigenous salt and drought tolerant halophytic plants for food could be one of the approaches to sustain food security.

Figure 2.1: *Trachyandra ciliata* plant growing on the sand dunes of the Agulhas Plain. (Picture: Laubscher)

2.3 Materials and methods

The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) was utilized in the selection of articles in this review, Following the method defined by Moher *et al.,* 2009. Various scientific reports, online databases, and theses/dissertations were explored using the library of the Cape Peninsula University of Technology. Search engines such as Scopus, Web of Science (WOS), and AgriFor were primarily used to conduct a

thorough search, while other quick checks were conducted in google scholar, science direct, and web of science. For this review, the search was limited to Southern Africa with special attention given to South Africa and the literature search period was from 1984 to 2020.

2.4 Food Security

Strassburg *et al*., (2014), Pimentel and Burgess, (2013), and Schultz and de Wrachien (2002) reported that the present world food production derived from 1.5 billion hectares of land, and that is about 12% of the global land area. It has been reported that in the last 50years, cultivated land has been reduced by 13% and that global agricultural productivity growth is projected to decrease by 1.5% every year until 2030, the further decline by 0.9% to 2050, compared to 2.3% growth per year since 1961 (FAO, 2008 & Hanjra; Qureshi, 2010; Rosa *et al*, 2020). Continuous declines in agricultural progress will one way or the other affect world food production (Narayanamoorthy, 2007). Future food supply will depend on the proper management of agricultural resources and investments in machinery as well as strict policies to try and achieve reasonable increases in food production (Herrero *et al*., 2010).

Food security is regarded as a broad term, but the basic definition is that it is the accessibility to food at all times by all people for active healthy life (Anderson, 1990; Du Toit *et al*., 2011). Du Toit *et al*. (2011) further stated that food security is also regarded as being closely related to poverty in a country. South Africa is amongst the nations that have high proportions of income variation in the world and it has high levels of poverty compared to other middle-income countries (Altman *et al*., 2009). In response to this problem, continuous efforts are being made by the government to obtain high productivity in order to increase profitability and to meet the constantly increasing demand for food.

At the national level, food security means a situation in which the country has the ability to produce, import, sustain, and retain food that is needed to support its people with minimum per capita dietary standards (du Toit *et al*., 2011). At a community level, it means a situation in which the people in a particular community are able to access food that is safe and nutritionally satisfactory through a viable system that ensures sustainability within the community. At a household level, food security refers to a situation in which people can access food in their homes and are not threatened by hunger or undernourishment (du Toit *et al*., 2011).

Food insecurity and the aspects of it are most experienced at household and individual levels (Misselhorn, 2005). Aliber and Hart (2009) reported that South Africa is regarded as a food secure country at a national level because of its capacity to produce enough staple food and having the ability to import from other countries in order to cover the demand for food. This statement was supported by Du Toit *et al*. (2011) but argued that the same cannot be

reported at a household level and poor rural areas in the country. It was then reported that 20% of South African homes have insufficient access to food (Statistics South Africa, 2009).

According to Hanjra and Qureshi (2010) “the key drivers which have recently impacted and will impact on food production and supply include: (a) water (and to some extent land) crisis; (b) climate change crisis; (c) energy prices and (d) credit crisis”. They further report that competition among different segments, regions, countries, and human activities associated with water is already occurring. This is supported by Yoffe *et al*. (2004) and Khan *et al*. (2009a) who reported that about 40% of the global population live in areas where they directly compete for water resources and that China and Africa are already experiencing water shortages.

The food security problems propagate the need for the introduction of drought and salt-tolerant plants to substitute and add capacity to sustain agricultural production to counter the projected shortage of food in the future (Chinnusamy *et al*., 2005; Yamaguchi & Blumwald, 2005; Chaurasia *et al*., 2020). Asphodelaceae is a family of succulent plants that have been extensively studied throughout the years because of the high medicinal properties they contain, and they are a big part of the world's pharmaceutical industry. Also, *Trachyandra* species could be having numerous applications such as a novel vegetable and a source of secondary metabolites with numerous uses in the pharmaceutical industry.

2.5 *Trachyandra* genus and species *T. ciliata*

The genus *Trachyandra,* also known as wild cabbage is a genus that consists of more than 50 species, belonging to the Asphodelaceae family (Smith & Van Wyk, 1998). The name *Trachyandra* is derived from Greek words, trachy, which means rough, and andro, which means male, and refers to the hairy filaments. *Trachyandra* species are perennial plants that are woody or herbaceous with granular pubescence in some species. They are deciduous plants because they lose their leaves during cold seasons (Obermeyer, 1962). The root systems are fibrous or spindle-shaped and sometimes swollen near the tips to form thin tubers. The stems are upright and woody or herbaceous mostly naked but sometimes covered with old leaf bases, or mostly developed as a vertical or rarely horizontal rhizome (Smith & Van Wyk, 1998).

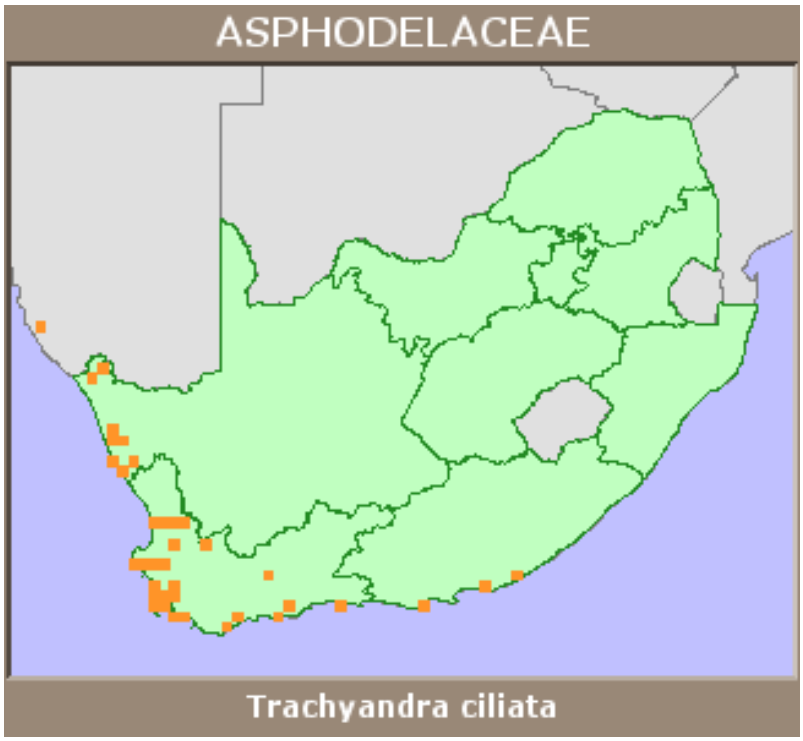

Figure 2.2: Distribution of *T. ciliata* in South Africa.
(Picture: http://redlist.sanbi.org/species.php?species=2203-18)

This is largely a South African genus with the majority of species found in the south Western Cape (Figure 2.2). *Trachyandra* species are found all over South Africa and southwest Africa and few species extending as far as southern Zimbabwe, Angola, Kenya, and Ethiopia (Smith & Van Wyk, 1998). Species of *Trachyandra* are occurring 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 (Smith & Van Wyk, 1998). As this plant grows on the Cape coast dunes it is also a good addition to coastal gardens; it can be thought that it is highly tolerant to salinity because most plants that grow in this environment are halophytes.

Figure 2.3: *T. ciliata* growing on coastal sand dunes in the habitat.
(Picture: Laubscher)

Trachyandra as a genus is less studied with little to no literature available and there are no *Trachyandra* species that are currently cultivated (Manning, 2007; De Vynck *et al*., 2016). However, it has been documented that *T. ciliata*, *T. divaricata,* and *T. falcata* are edible and were used as food by Khoi-san people that lived on the South African cape coast (De Vynck *et al*., 2016). Colonization and removal of indigenous people from cultural lands led to erosion and detachment from the knowledge of the land and its useful plants (Janssen, 2010; Cavanagh, 2013; Kuhnlein *et al*., 2013; Van Rooyen, 2016). Current information indicates that the inflorescence can be steamed and eaten as a vegetable or added into a stew (De Vynck *et al*., 2016) (Figure 2.2). However, the nutrient contents of these plants are yet to be scientifically proven, hence further studies are needed to support the current information. Throughout the years the Asphodelaceae family has been intensively studied based on medicinal properties and is widely used in the pharmaceutical and beverage industry (Van Wyk, 2011). This could be the case for these undocumented *Trachyandra* species, and they might have numerous applications such as novel vegetables and a source of secondary metabolites with uses in the pharmaceutical industry. Ventura *et al*., (2011) suggest that the cultivation of indigenous salt and drought tolerant halophytic plants for food could be one of the approaches to sustain food security. *T. Ciliata* has no literature relatingting to its nutritional properties and potential medicinal properties. Therefore, studying its closely related species in the Asphodelaceae family may also lead to scientific justification of its edibility and potential as a pharmaceutical candidate.

Figure 2.4: Edible inflorescence of *T. ciliata* showing the asparagus type florescence. (Picture: http://www.fynboshub.co.za/catablog-items/trachyandra-ciliata/)

2.6 Asphodelaceae Family

Asphodelaceae is a family of angiosperms in the order asparagales. The family is made up of 12 genera and approximately 1060 species native to Africa, Arabian Peninsula, Central Asia, the Mediterranean basin, west, and central Europe, Madagascar, New Zealand, and Australia. Klopper *et al.* (2013) and Angiosperm Phylogeny Group (2016) reported that the name Asphodelaceae was first scientifically published in Jussieu's *Genera Plantarum* in 1789 and this date is regarded as the starting date for the nomenclature of this family. Within the family, there are two sub-families namely Asphodeloideae and Alooideae (Chase, 2000).

Members of the Asphodelaceae family are very broad with a few characteristics common to all members. They range from small to medium-sized plants that are often succulent, herbs, or large trees with leaf arrangements of terminal rosettes on fibrous and woody stems rather than succulent. According to Smith and van Wyk (1998) "Leaves dorsiventral, lanceolate-acuminate, linear or subulate, terete, often succulent and thickly conical, spirally arranged or distichous as in some species of Alooideae, amplexicaul, margins toothed, serrate or entire, sharply pointed, parallel veins often obscure".

2.6.1. The importance of Asphodelaceae species in the food industry

Although plants in the Asphodelaceae family are popular with high medicinal properties, recent studies, investigating the potential of South African indigenous species as food crops have shown that some species could be valuable products for the food and beverage industries (Van Wyk, 2011). Chen *et al*. (2012) and Cock (2015) described the use of the inner, non-bitter gel of *Aloe* plants as a food supplement and as new development. There is

little documented evidence of this species being used as a traditional food except for the use as preservatives by Cape farmers in the production of jam (Chen *et al*., 2012). Cock (2015) reported the presence of flavonoids in Aloe plants and what this finding adds to the popular use of the genus. As reported earlier *Trachyandra* can be eaten as a vegetable and was used by Khoi-san people traditionally (De Vynck *et al*., 2016). The species grows naturally in highly saline soils of the south-western Cape coast which makes this one of the candidates as a salt-tolerant food crop to be introduced into the food market, especially while drought and salinity of agricultural lands are constantly increasing (De Vynck *et al*., 2016). Hence studies to evaluate salt tolerance and nutrition, research studies on this plant should be conducted to introduce *T. ciliata* as a salt-tolerant food crop.

2.6.2 Nutritional value Asphodelaceae plants

Researchers have determined the health benefits of food and beverage products that are comprising of the leaf parenchyma of Aloe species (Kleinschmidt, 2004; Cock, 2015). The Food and Drug Administration has certified and granted permission for the use of *A. faro* as a direct food additive for human consumption (FDA, 2002). Cock (2015) reported the presence of some essential vitamins (vitamin C, vitamin B1, vitamin B2, vitamin B6, vitamin B12, and vitamin E) for the efficiency of the human body to be contained in unspecified Aloe leaf gels. He further reported that leaf gel of aloe plants contains essential minerals including zinc, iron, manganese, phosphorous, molybdenum, sodium, potassium, magnesium, calcium, and copper. As it is documented that *Trachyandra* is an edible vegetable, more focus and more studies should be carried out to evaluate the nutrient content of plants in this genus. Equally so, the issue of the water crisis that is threatening agricultural production needs to be addressed and explored in order to develop techniques that will increase production without exerting more pressure on the demand for freshwater.

2.7 The water crisis in south-western cape Mediterranean climate regions

Arguments about world water scarcity and food security have intensified lately and the exact future demand for water and food supplies is unclear (Hanjra & Qureshi., 2010). Globally, 1.1 billion hectares out of 1.5 billion hectares of cultivated land is depending on rain water without any irrigation systems which makes up to about 80% of the world's cultivated land that produces 60% of global staple foods (Schultz & De Wrachien, 2002; FAO, 2008). Thenkabail *et al*. (2010) reported that irrigated agricultural land covers 19% of the cultivated land which contributes about 40% to the global agricultural productivity. Astonishingly, irrigated agricultural land is also responsible for 70% of water withdrawals from the world’s rivers despite contributing so little to global food production (Molden *et al*., 2007).

Water scarcity and food insecurity are inter-linked because water is very important for future global food security and water scarcity reduces agricultural productivity and thus contribute to the increasing trend of food security problems (Hanjra & Qureshi, 2010). The demands of irrigation water for other industries such as environmental developments and urbanization exerts huge pressure on the agricultural industry and global food security (Molden *et al*., 2007; Hanjra & Qureshi, 2010). It is predicted that South Africa will face physical water scarcity by 2025 because of the rapid population growth and decreasing freshwater supplies (WWF, 2017). In South Africa, the province that is extremely affected by water scarcity is the Western Cape and it is also predicted that in the near future, this province would be unable to cater for its agricultural needs (WWF, 2017). Salinity is also a growing threat to agricultural production as many agricultural lands have turned highly saline and cannot be used to produce plants any further. This then calls for the cultivation of plants that are tolerant to high levels of drought and salinity to be used for food (Ventura *et al*. (2011). Condon *et al.,* (2004) found that of the world's allocated water resource, about 80% is currently dedicated to irrigated agriculture and this level of usage by agriculture is not viable in the future. They further suggest that predictable population growth of about 2 billion people within 2–3 decades to come will necessitate that more of the available water resources be used for municipal, industrial, domestic, and environmental needs.

Falkenmark and Molden (2008) suggested that enhanced water management and new investments in irrigation infrastructure should be put in place in trying to minimize the influence of water shortage and partially meet the demand for food production. Currently, hydroponic cultivation is a popular technique to ensure maximum agricultural production while reducing the demand for fresh water in irrigation (Savvas, 2002). It is therefore paramount to extensively study and explore the potential of hydroponic techniques to grow salt-tolerant vegetable crops.

2.8 The importance of growing halophytic plans hydroponically

Dr. W.F Gericke who conducted several experiments on planting crops using water was the person who gave the term "hydroponics" in 1936 (Abdullah, 2001). Hydroponics is a substitute for traditional plant production and is defined as the growing of plants in a soilless medium or an aquatic-based environment (Anderson *et al*., 1989; Montanari *et al*., 2008; Venter, 2010). In this case, an artificial medium is used to provide structural support to the plants and not to provide essential nutrients to the plant (Venter, 2010). Furthermore, all the essential nutrients needed by the plant are blended with water and are made available to the plant (Wahome *et al*., 2011). It has been reported that hydroponics allows for the attainment of high yields without compromising the quality of crops (Savvas, 2002).

Hydroponic production of plants is becoming popular in the modern world with ecological imbalances such as extreme temperatures, drought, chemical toxicity, and oxidative stress threatening conventional agricultural practices (Putra & Yuliando, 2015). Hydroponics can be applied in places where there is limited availability of space, where there is a shortage of water, where the soil is chemically imbalanced, and where there are high levels of pathogen infestation (Correa *et al.*, 2012). In South Africa, challenges like population growth, water scarcity, and increasing demand for food have raised a necessity for sustainable and efficient cultivation methods (Molden *et al*., 2007; Hanjra & Qureshi., 2010; WWF, 2017). Ortiz *et al*. 2009) reported that hydroponically grown plants use 10 times less water than soil-grown plants because in soil-grown plants water quickly leaches to the soil while in hydroponics, water is collected and circulated again. It is further reported that hydroponic cultivation limits the loss of water and nutrients (Siddiqi *et al*., 1998; Van Os, 1999). It has been found that hydroponically grown plants grow faster and healthier because they get nutrients in exact amounts according to the grower (Ortiz *et al*., 2009; Wahome *et al*. 2011).

Considering water scarcity that is currently hitting South Africa especially the western Cape, Hydroponic cultivation of vegetable crops can be the solution because it has been proven that it is the most sustainable, efficient, and effective cultivation method in the agriculture industry of today (Van Os, 1999; Ortiz *et al*., 2009; Putra and Yuliando, 2015). Furthermore, hydroponic cultivation of halophytes is a field that needs to be explored in an attempt to address the water crisis and food insecurity simultaneously. As the cultivation of edible halophytic crops (e.g. *T. cilata*) has been regarded as the possible solution for the sustainability of food production in the future, it is very key to start developing hydroponic growth protocols for these plants, in order to better understand their nutrient uptake and salt tolerance.

For an efficient nutrient uptake, hydroponic cultivation is regarded as an ideal method to manipulate or optimize both the supply of nutrients and the root environments (Adams, 1993). Continuous replacement of nutrients in the hydroponics system is important because it allows for efficient nutrient uptake and prevents toxins of waste nutrients (Ho & Adams, 1995). Well-managed watering intervals can help achieve a desirable balance between foliage growth and reproductive growth and that is usually realized through the use of an irrigation computer that can be set to water according to the stage in which the plant is (Anon, 1986; Cooper, 1978). These researchers further explain that the water supply and nutrient supply have to be matched with carbon assimilation or growth rates in order to achieve balanced growth. Likewise, it is important to understand the general hydroponic nutrient supply in order to plan protocols for the cultivation of halophytes.

The introduction of hydroponic systems has allowed for the determination of optimal requirements for the basic nutrients needed for plant growth (Adams, 1993). The required

concentration of nutrients in the aqueous solution is determined by the rate of uptake by the plant, which differs from plant to plant (Adams & Gimmett, 1984). In most cases, potassium supply is not necessarily for high yields but uniform flowering and ripening, and the addition of NaCl in the solution reduces the K concentration. So that means an addition of NaCl should be carried out with care as it reduces the acidity of the solution, thus reducing the uptake of K (Ho & Adams, 1995). *T. ciliata* and other halophytes naturally grow in saline conditions, in which the uptake of K is limited. There is a necessity to reduce nitrate concentration in the solution as lower nitrate content of vegetable crops is recommended for health and it is to reduce the negative greenhouse effect on the environment K (Ho & Adams, 1995). Furthermore, this has called for partial replacement of KNO_3 and NO_3 by KCl, $CaCl_2$, and NaCl in the nutrient solution. The acidity and alkalinity (EC) of the nutrient solution in the cultivation of halophytes needs to be handled with care, as this may be the deciding factor on the successful cultivation of these plants (Cooper, 1978; Anon, 1986; Farber *et al.*, 2020).

2.9 Salinity and EC in relation to nutrient uptake

In recent years, electric conductivity (EC) has been increased by adding NaCl to the solution but later studies proved that NaCl induced EC decreases the uptake of Calcium (Ca), which can result in devastating outcomes (Adams, 1991; Ho & Adams, 1995). Adjusting the electrical conductivity of the solution is a more precise method of controlling plant development. Measuring EC in the root zone is a practical method to adjust nutrient supply because the conductivity of the solution is directly proportional to the amount of salt dissolved in the solution (Anon, 1986). This means if the solution has high conductivity, there is a high concentration of salts. The salinity of the solution has an intense effect on osmotic potential because osmotic stress decreases the uptake of nutrients and water (Ho & Adams, 1995). It has also been reported that salinity or high EC reduces the nutritional status and yield (Adams, 1991). However, the effect of salt differs from species to species. Halophytes are a group of plants that have evolved to withstand highly saline conditions and the effect of salinity is minimal compared to normal plants. This means thorough research needs to be conducted per species in order to determine the precise tolerance to salinity.

Not all plants are sensitive to high salt levels, some plants have evolved in saline regions and prefer high salt levels in their root zone such as halophytes including *T. ciliata*. Growth media that are used in hydroponics usually have a neutral EC and pH (Fonteno, 1996). It is therefore important not to ignore the role growth media plays in ensuring the successful hydroponic cultivation of halophytes and plants in general. The growth medium plays an important role in successful cultivation as it plays the role of being an anchor and allows for gas exchange and holds water and nutrients (Fonteno, 1996; Hussain *et al*., 2014).

2.10 The importance of growing substrates in hydroponics

Soil is a natural growth media for all plants because all plants naturally occur in different areas in which different soil types are found. However, the use of soil in the greenhouse and commercial cultivation has created many problems and come with different disadvantages such as undesirable microbial activities, soil-borne diseases, and nematodes, and thus varying salinity, acidity levels, poor nutrient levels poor drainage, and undesirable soil characters (Nelson, 1991; Ananda & Ahundeniya, 2012). In the most advanced technology of plants growing in greenhouses, numerous inorganic and organic substrates such as Rockwool, vermiculite, perlite, peat, leca clay, silica sand, and others have replaced the soil (Lin *et al*., 1996; Jankauskienė *et al*., 2015). This is aided by the fact that these substrates are disease and pest-free materials that can hold the plant and sufficient moisture required plants to grow and most can be reused for many years (Asaduzzaman *et al.,* 2015). The origin of these media is different, some of them are of natural origin and some are produced artificial and they are also different in their chemical, physical, and biological properties. Consequently, a medium selection is one of the most significant factors influencing plant growth and development in the greenhouse and affecting crop quality (Ananda & Ahundeniya, 2012).

Growing substrate plays four significant roles in the plant. It supplies nutrients to the plant, allows for the provision of water to and from the plant roots, allows for gas exchange, and provides support as an anchor for the plant (Fonteno, 1996; Xego, 2017). Hussain *et al*. (2014) state that the use of soilless growing medium allows for more effective and efficient use of water and fertilizer, and thus minimize the need for use of chemicals for pests and diseases. *T. Ciliata* naturally grows on the sand dunes (Smith & Van Wyk, 1998), but studies have shown that plants can perform better in other soils in greenhouse cultivation compared to their natural soils (Abad *et al*., 2002; Hanna, 2010; Venter, 2010; Hussain *et al*., 2014).

2.11 Conclusion

Water scarcity and increasing salinization of agricultural lands in Mediterranean areas around the southern coastlines of Africa are posing a huge threat to food production in the future. It has been predicted that South Africa could face physical water scarcity by 2025 due to the rapid population growth and decreasing freshwater supplies. Highly saline and coastal lands occurring in abundance around the coast of South Africa are seen as unusable production areas for conventional crop cultivation. The potential of this land can be explored to experiment with possible salt-tolerant crops as a substitute to the conventional vegetable crops which demand high water supplies in cultivation. This also necessitates the development of more efficient technological innovations to counter the predicted water crisis. An example such as hydroponics has proven to be a popular cultivation method to ensure

efficiency in water usage. Water savings using up to 10 times less water could be ensure compared to conventional cultivation methods as irrigation water in hydroponic systems is circulated and reused. Halophytic plants such as *T. ciliata* have the potential to become an important vegetable crop in the predicted dry seasons because of their resiliency to withstand highly saline and dry conditions. This phenomenon, therefore, calls for more research studies to be conducted on edible salt-tolerant halophytic plants. In the recent past, Asphodelaceae has been extensively studied mainly for the medicinal properties of many of its members. *T. ciliata*, also belonging to the same family has no records in the literature, but little information state that it is edible and was used by the past Khoi-san people who lived on the western Cape coast in the past. The information and the findings of this review are recommended as points of reference for researchers, food enthusiasts, potential farmers, and policy makers who may be keen on exploring further nutritional composition and medicinal potential of this plant. The information gathered makes this plant a candidate as a commercial vegetable crop because of its ability to withstand highly saline and dry coastal conditions. This warrants the need to conduct studies on the hydroponic propagation of *T. ciliata* and determine its hydroponic growth protocol in an attempt to partially address the issue of water scarcity and food insecurity. It is recommended that further studies be conducted on the breeding of this plant to produce varieties with high yields and nutrition.

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CHAPTER THREE

THE EFFECT OF SALT STRESS AND DIFFERENT GROWTH MEDIA ON THE GROWTH *OF TRACHYANDRA CILIATA* IN HYDROPONICS

SUBMITTED TO: HORTICULTURAE

THE EFFECT OF SALT STRESS AND DIFFERENT GROWTH MEDIA ON THE GROWTH *OF TRACHYANDRA CILIATA* IN HYDROPONICS

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3.1 Abstract

Agricultural production in the southern African region is greatly threatened by increasing salinization in cultivation lands and persistent water scarcity issues. This then calls for the implementation of strategies to use halophytic plants to substitute the affected crops due to their ability to tolerate drought and salinity. *Trachyandra ciliata*, a species that is endemic to the south western Cape coastal areas has been found to be edible but there is no literature on its cultivation. The current study was undertaken to determine the effect of salinity stress and different soil-less media on the vegetative growth of *Trachyandra ciliata* (L.f.) Kunth under hydroponics conditions; in order to develop a growth protocol for the species. Four identically constructed nutrient film (NFT) systems were used, with each system on a separate metal mesh steel table (2.5 m long). NUTRIFEED™ served as a general hydroponics feed while different salt concentrations of Sodium chloride (NaCl) (100mM, 200mM, 400mM of NaCl) were used as treatments added into each sump while nutrient solution without addition of NaCl was considered as control. The experiment was carried over 22 weeks. Low salinity levels significantly increased plant height, the number of leaves, plant wet weight, and the production in the inflorescence at $P \leq 0.05$. In contrast, the control was the most productive treatment on plant dry weight except for the inflorescence. Production in the inflorescence is the most important aspect of this experiment and the yield from PPV soil-less mixture was significantly productive compared to other media. Therefore, *T. ciliata* proved to strive and be more productive under low salinity levels in combination with PPV soil-less media.

Keywords: inflorescence; nutrient solution; sodium chloride; soilless media

Abbreviations: Perlite (P), Peat (P), Vermiculite (V), Silica Sand (SS), Treatment (T), Sodium Chloride (NaCl), PPV (Peat: Perlite: Vermiculite).

3.2 Introduction

Agricultural production in Southern Africa is greatly limited by drought. The region is the third most water-scarce-prone region after the Middle East and North Africa (Turton *et al.,* 2000). 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 (Singh, 2014). Water demands have tripled since the 1950s while freshwater supplies have been declining consistently (Gleick, 2003a). The Western Cape Province of South Africa has been the most affected by water scarcity. Furthermore, it has been predicted that in a few years, this province will be unable to supply water for its agricultural needs (WWF, 2017). An increase in soil salinization together with the growing scarcity of freshwater stimulates the need to develop more innovative techniques to enhance sustainable crop production (Ventura & Sagi, 2013). Accumulation of salt in agricultural lands results in salt toxicity, which then reduces the efficiency of stomatal conductance, photosynthesis rate, transpiration, and respiration (Rengasamy *et al*., 2003; Koryo, 2006). The buildup of salts in the root zone reduced the water holding capacity of the plant, leading to reduced yields. It has been found that plants that are grown in hydroponics use ten times less water than conventionally grown plants (Wahome *et al.*, 2011). This is because, in soil-based plant cultivation, water leaches out whereas water is collected and recycled in hydroponics (Ortiz *et al*., 2009). Wahome *et al.* (2011) reported that hydroponic plants grow faster than conventionally grown plants because all required nutrients are readily available in the solution.

Continuous drought and high salinity levels call for a need to evaluate and develop new protocols for future food production (Ventura *et al*., 2011). Several authors including Ventura *et al*. (2011) and Klados and Tzortzakis (2014) 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 fluctuate according to different plant species, the plant's stage of development, the type of salinity, and the type of growing medium (Botia *et al.*, 1998).

The genus *Trachyandra,* also known as wild cabbage is a genus that consists of more than 50 species, belonging to the Asphodelaceae family (Smith & Van Wyk 1998). Throughout the years the Asphodelaceae family has been intensively studied based on medicinal properties and is widely used in the pharmaceutical industry. This is a less studied genus with little documented literature available (Manning, 2007; Boatwright & Manning, 2010). However, there is literature indicating that *T. ciliata*, *T. divaricata,* and *T. falcata* are edible and were used as food by Khoi-san people that lived on the South African Cape coast where colonization led to the detachment from indigenous knowledge of these plants (De Vynck *et al*., 2016). The inflorescence is steamed, boiled, or added into a stew as a vegetable (De Vynck *et al*., 2016). The species in this genus are found all over 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 (Smith & Van Wyk, 1998). *Trachyandra ciliata* grows naturally in highly saline soils of the South Western Cape coast (Smith & Van Wyk 1998) which makes it a candidate as a salt-tolerant food crop to be introduced into the market. This therefore would be very useful in ensuring increased agricultural production and relieving pressure on the demand for freshwater for irrigation in an attempt to address the issue of food insecurity and water scarcity. However, understanding the responses of this crop to salinity is imperative to improve its performance and to plan new areas for expanding its marketability. However, there is no information in the literature on the salt tolerance of *Trachyandra* species. This study was therefore undertaken to evaluate the effect of various salinity concentrations and different growth media on physiological parameters of *T. ciliata* grown in hydroponics under greenhouse conditions.

3.3 Materials and method

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 ºC during the day and 12 and 18 ºC at night with the use of environmental control. The Relative Humidity average was kept at 60%.

3.3.1 Experimental design

In this experiment, four 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 3.1). The treatments were labeled as T1–T4. Each system had its low-density polyethylene (LDPE) 50-liter reservoir in which the nutrient solution was prepared. There were three Polyvinyl Chloride (PVC) square gutters (2 m), put in place with cable ties on each table, in which three 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 x 2000 L/h submersible pump with 2.5 m head capacity, 20 mm LDPE irrigation piping, 4 x 20 mm elbow irrigation fittings, and 4 x 20 mm flow regulators were used. Gutters were fitted with 1 outlet that returns the solution to the reservoir.

Every gutter on each system had 10 pots (12.5 cm height x 12.5 cm x 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 x 2000 L/h submersible pump with 2.5 m head capacity

was used to circulate the nutrient solution for 24 hours from the beginning to the end of the experiment.

EC in the nutrient solution was monitored daily with calibrated a hand-held digital EC meter (Hanna instruments®™ HI 98312). The pH of the solution was monitored with a calibrated hand-held digital pH meter (Eurotech®™ pH 2 pen). Potassium hydroxide was used to elevate pH, while phosphoric acid was used to decrease the pH of the nutrient solution.

Figure 3.1: The layout of the experiment showing replicates before the addition of NaCl (Picture: Ngxabi).

3.3.2 Plant Material

The plant material of *T. ciliata* was obtained from a local nursery. The plant material was further 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.

3.3.3 Nutrient solutions

Nutrifeed fertilizer has been certified as containing all the essential nutrients necessary for healthy and vigorous plant growth and is now wildly used to make hydroponic aqueous solutions. This fertilizer was used 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.

3.3.4 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.

3.3.5 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^{TM}. Three salt concentrations (100mM, 200mM, 400 mM of NaCl) were tested in this experiment and added into each sump while 0mM of NaCl was considered as a control (Figure 3.2). The saline solutions were prepared using tap water. All nutrient solutions containing NaCl were replaced every week to avoid the buildup of salts in the soil, pots, gutters, and reservoirs. The pH was maintained at 6.0.

100 mM of NaCl

0.1 M= 58.44g Formula: n=n/v

0.1= n/1

N= 0.1

0.1=m/58.44

m= 5.844 g of NaCl per Littre

Figure 3.2: 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)

3.3.6 Statistical analysis

A two-way analysis of variance (ANOVA) was used to determine the interaction between salinity and growth media on growth parameters. The significant differences between treatment means at $p \leq 0.05$ were compared using Fisher's least significant difference (LSD). All the calculation was done on a computer software program STATISTICA version 10.

3.3.7 Determination of plant growth

3.3.7.1 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 for 7 days 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.

3.3.7.2 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.

3.3.7.3 Number of leaves

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

3.3.7.4 Chlorophyll content of leaves

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

3.4 RESULTS

3.4.1 The effects of salinity and growth media on leaf number

The results obtained from the experiment show that different salinity levels significantly affected (P≤ 0.05) the number of leaves (Table 3.3 and Figure 3.3). In contrast, the results show that soil-less media did not have a significant influence on the number of leaves produced by the plants. The interaction of salinity and media also did not have any significant effect on the number of leaves produced (Table 3.3). 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 (Figure 3.3).

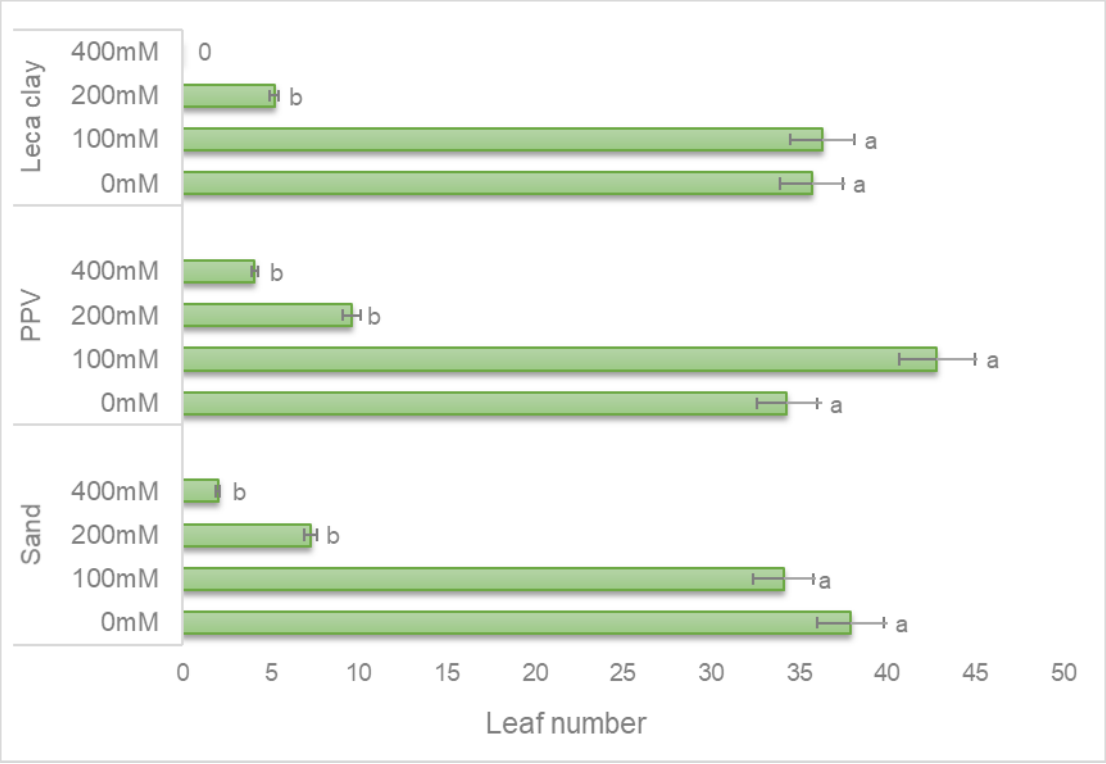

Figure 3.3: Influence of salinity and growth media on leaf number in hydroponically grown *T. ciliata.* The mean values with different letters are significantly different at P ≤0.05.

3.4.2 The effects of salinity and growth media on plant height

The results gathered from the current trial indicate that soil-less media significantly (P≤ 0.05) influenced shoot length (Table 3.3). The results also show that different salinity levels had a significant (P≤ 0.05) influence on shoot length. However, there was no interaction between soil-less media and salinity on plant height (Table 3.4). Also, 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. Also, a 200 mM salinity had an equivalent effect on plant height in sand and PPV media while 200mM salinity in Leca clay yielded equivalent height as 400 mM salinity in the sand and PPV (Table 3.3 and Figure 3.4).

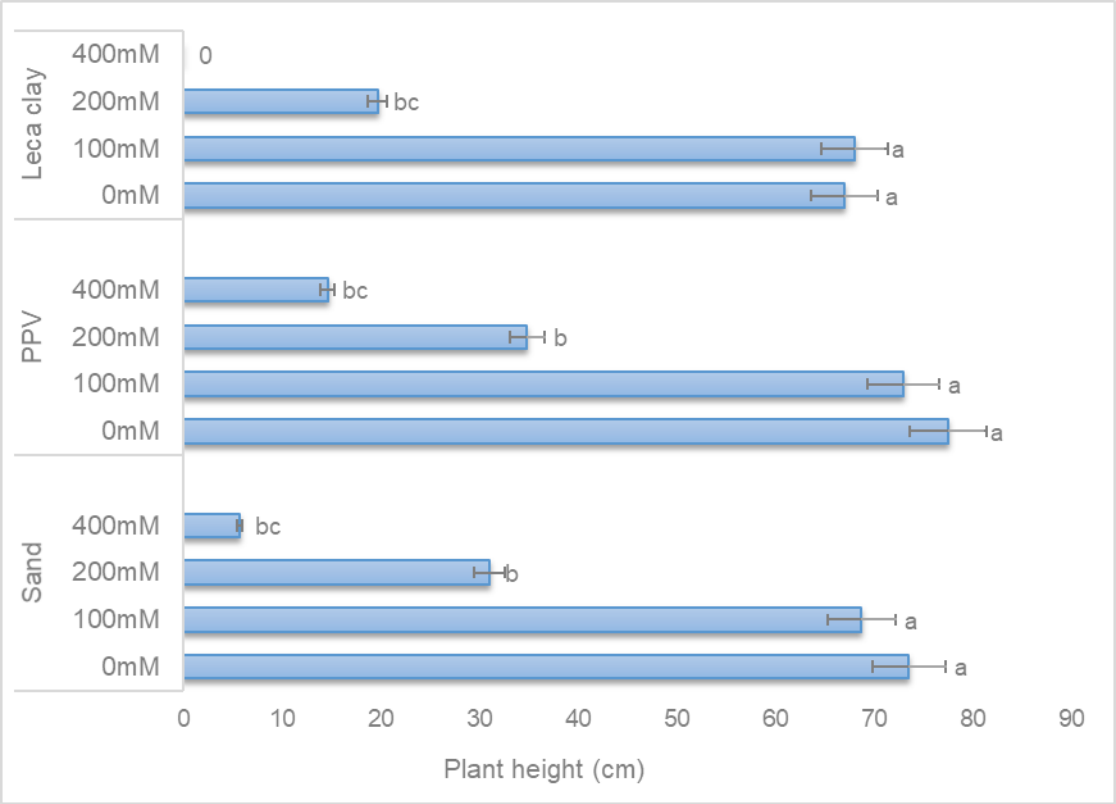

Figure 3.4: Influence of salinity and growth medium on height in hydroponically grown *T. ciliata*. The mean values with different letters are significantly different at P ≤0.05.

3.4.3 The effects of salinity and growth media on shoot wet weight

The results obtained from the present experiment also shows that different salinity levels significantly affected shoot wet weight at P≤ 0.05. In contrast, the results show that soil-less media did not have a significant influence on wet shoot weight. Also, there was no interaction between soil-less media and salinity on wet shoot weight. However, 100 mM+LC recorded the highest mean shoot weight (144.02g) while the least equivalent values were respectively recorded in 200 mM salinity in Leca clay, and 400 mM salinity in sand and PPV. Similarly, PPV and sand yield equal shoot weight at 0 mM and 100 mM.

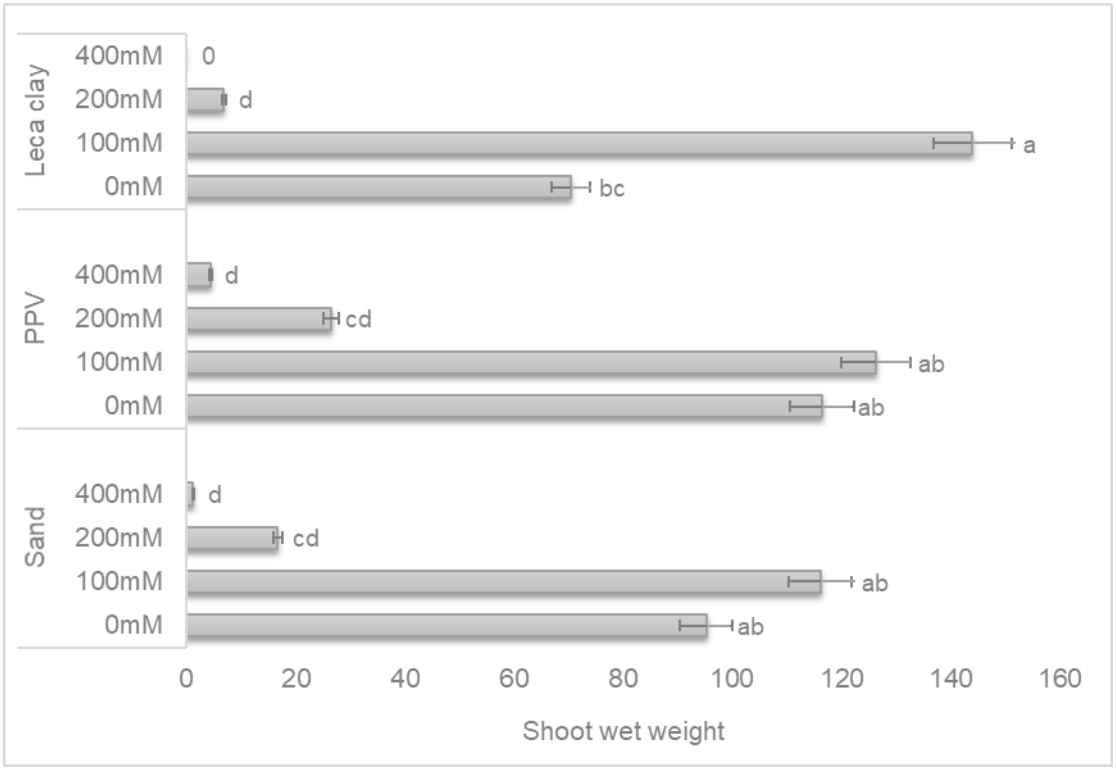

Figure 3.5: Influence of salinity and growth medium on shoot wet weight in hydroponically grown *T. ciliata.* The mean values with different letters are significantly different at P ≤0.05.

3.4.4 The effects of salinity and growth media on shoot dry weight

The data gathered from the present trial indicate that different salinity levels significantly affected (P≤ 0.05) shoot dry weight (Table 3.1). Like wet weight, the results indicate that soil-less media did not have a significant influence on dry shoot weight. Also, there was no interaction between soil-less media and different salinity levels on the dry weight. In addition, 0mM and 100 mM salinity had equivalent weight in the dry shoot, while the remaining salt concentrations had the least dry shoot weight.

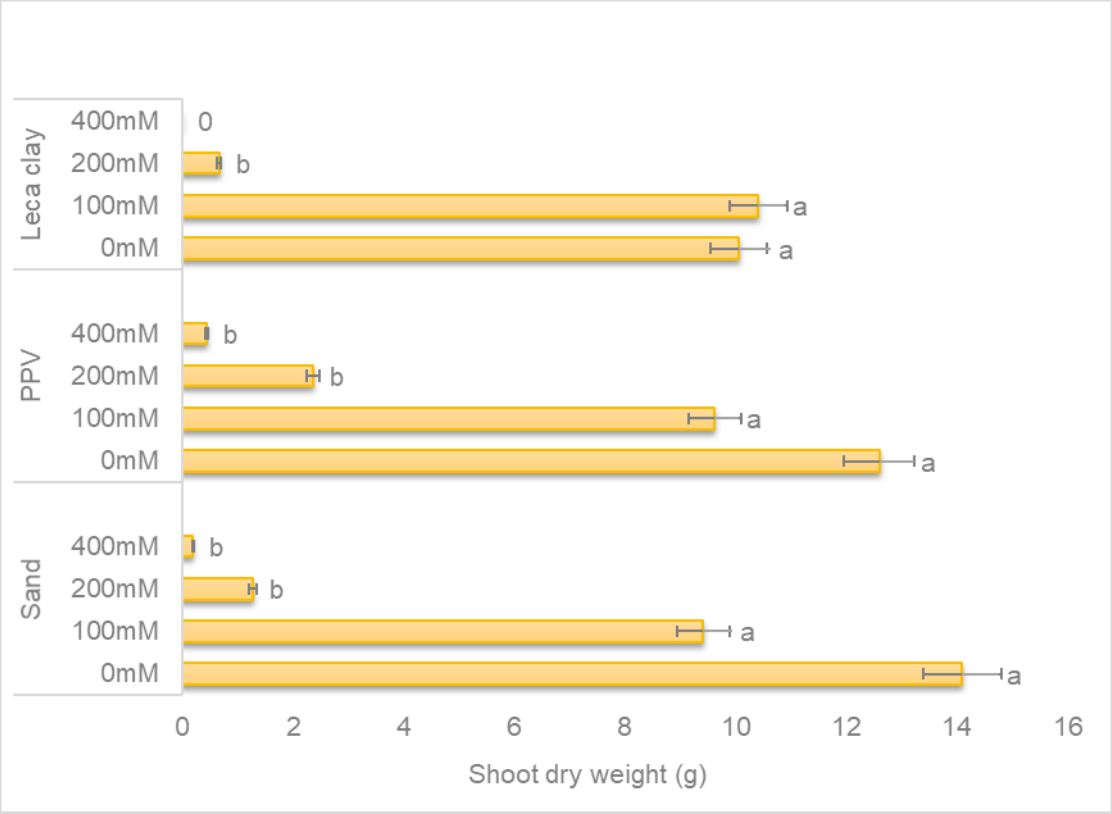

Figure 3.6: Influence of salinity and growth medium on shoot dry weight in hydroponically grown *T. ciliata*. The mean values with different letters are significantly different at P ≤0.05.

3.4.5 The effects of salinity and growth media on root wet weight

As shown in Table 3.1, different salinity levels significantly (P≤ 0.05) influenced root wet weight. In addition, growth media did not significantly affect wet root weight. The results indicate that there was an interaction between growth media and salinity on wet root weight (P≤ 0.05). Treatment 100 mM+LC recorded the highest mean value (39.09) followed by 0mM+SS and 100 mM+PPV with 35.78 and 29.88 respectively while 400 mM recorded the lowest mean values in all soil-less mixtures (Figure 3.7).

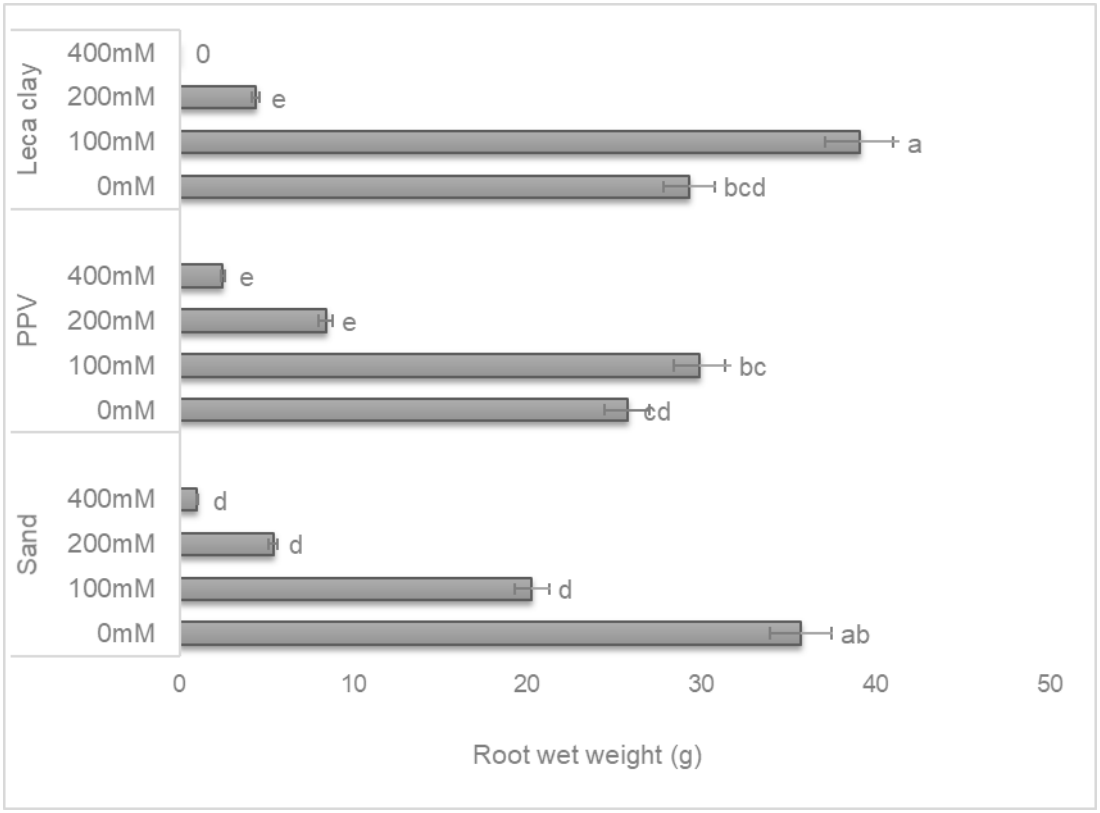

Figure 3.7: Influence of salinity and growth medium on root wet weight in hydroponically grown *T. ciliata*. The mean values with different letters are significantly different at P ≤0.05.

3.4.6 The effects of salinity and growth media on root dry weight

The results obtained from the current experiment indicate that salinity significantly influenced (P≤ 0.05) root dry weight. On the contrary, soil-less media proved not to have any significant effect on root dry weight. The results show that soil-less media and its interaction with different salinity levels had a significant influence (P≤ 0.05) on root dry weight (Table 3.1). Surprisingly, the combinations of 0 mM+SS recorded the highest mean value (5.52) followed by 100mM+LC, which was the exact opposite, compared to root wet weight. In addition, 400 mM recorded the lowest mean values across all soil-less treatments (Figure 3.8).

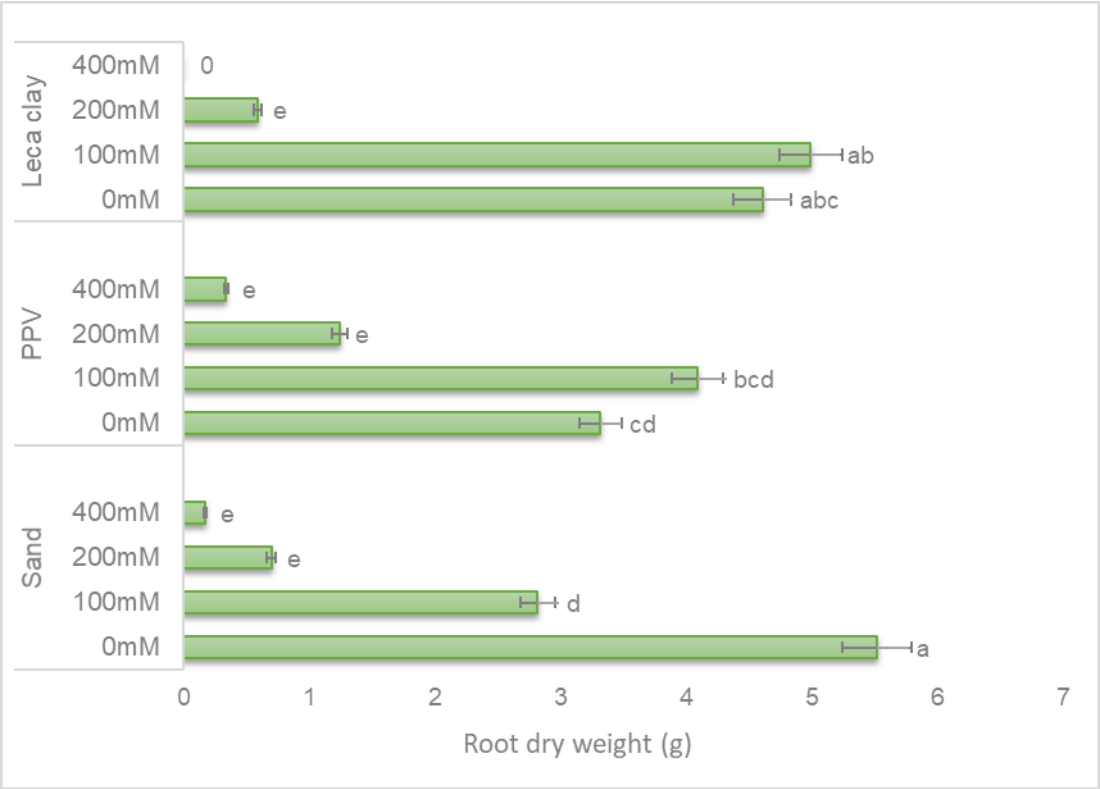

Figure 3.8: Influence of salinity and growth medium on root dry weight in hydroponically grown *T. ciliata.* The mean values with different letters are significantly different at P ≤0.05.

3.4.7 The effects of salinity and growth media on the total wet weight

The results gathered from the present experiment indicate that salinity significantly affected (P≤ 0.05) *T. ciliata* total wet weight (Table 3.3). Contrary to that, soil-less media did not show any significant effect on total wet weight. In addition, soil-less media and its interaction with different salinity levels did not have any significant effect on total wet weight. The combination of 100mM+LC recorded the highest mean value (183.11) followed by 100 mM+PPV with a mean value of 156.31. Salinity 400 mM recorded the lowest mean values across all soil-less mixtures (figure 3.9).

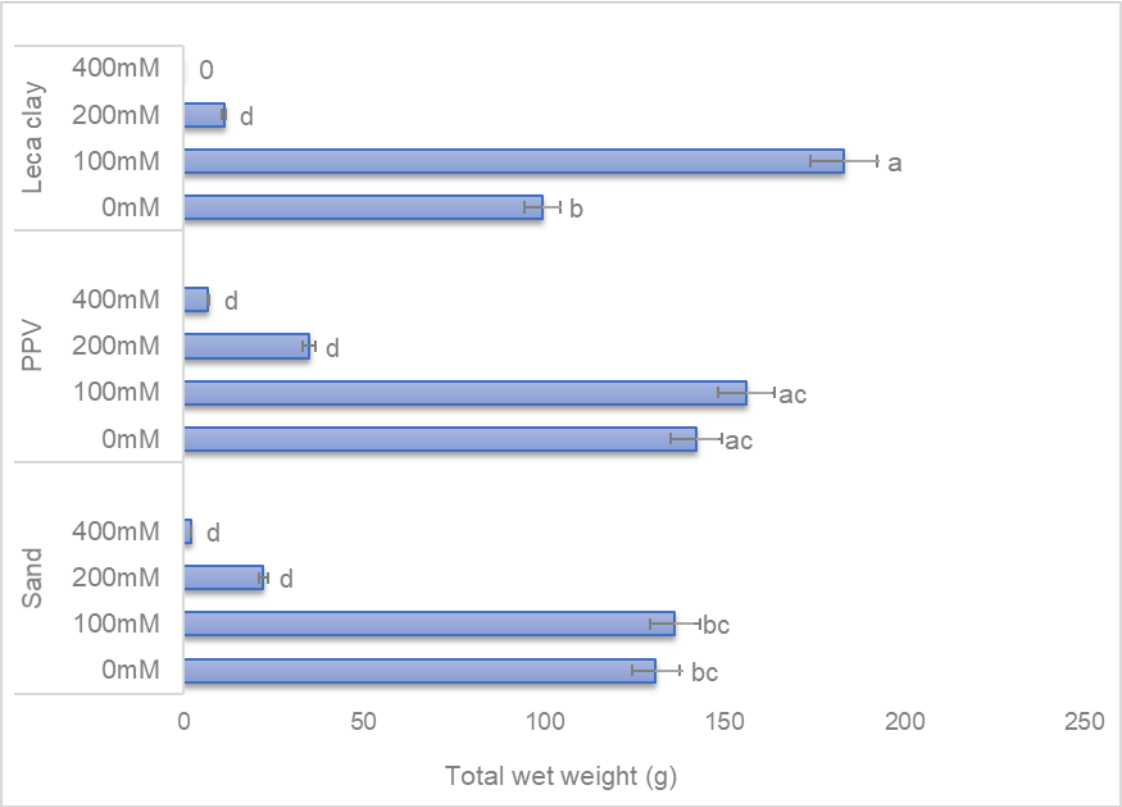

Figure 3.9: Influence of salinity and growth medium on total wet weight in hydroponically grown *T. ciliata.* The mean values with different letters are significantly different at P ≤0.05.

3.4.8 The effects of salinity and growth media on the total dry weight

Results of the present trial show that salinity significantly affected (P≤ 0.05) total dry weight in *T. ciliata* (Table 3.3). Contrary to that, soil-less media did not show any significant effect on total wet weight. In addition, soil-less media and its interaction with different salinity levels did not have any significant effect on total wet weight. In contrary to the total wet weight results, the interaction of 0 mM+SS recorded the highest mean value (19.62) followed by 0 mM+PPV with a mean of 15.92. Similar to the weight results, 400 mM recorded the lowest mean values across all soil-less mixtures (Figure 3.10).

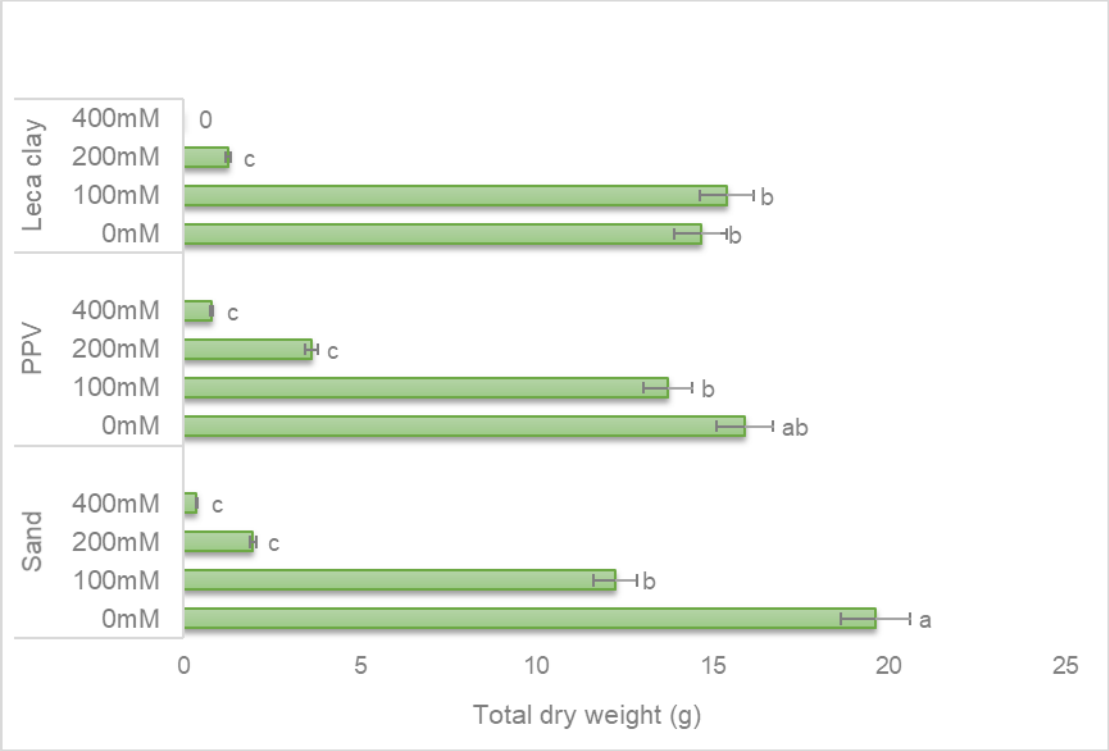

Figure 3.10: Influence of salinity and growth medium on total dry weight in hydroponically grown *T. ciliata.* The mean values with different letters are significantly different at P ≤0.05.

3.4.9 The effects of salinity and growth media on chlorophyll content

The results obtained from the current experiment indicate that salinity had a significant influence (P≤ 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 (Table 3.2). The interaction of 0mM+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. Salinity 400 mM recorded the lowest mean values across all soil-less treatments (Figure 3.12).

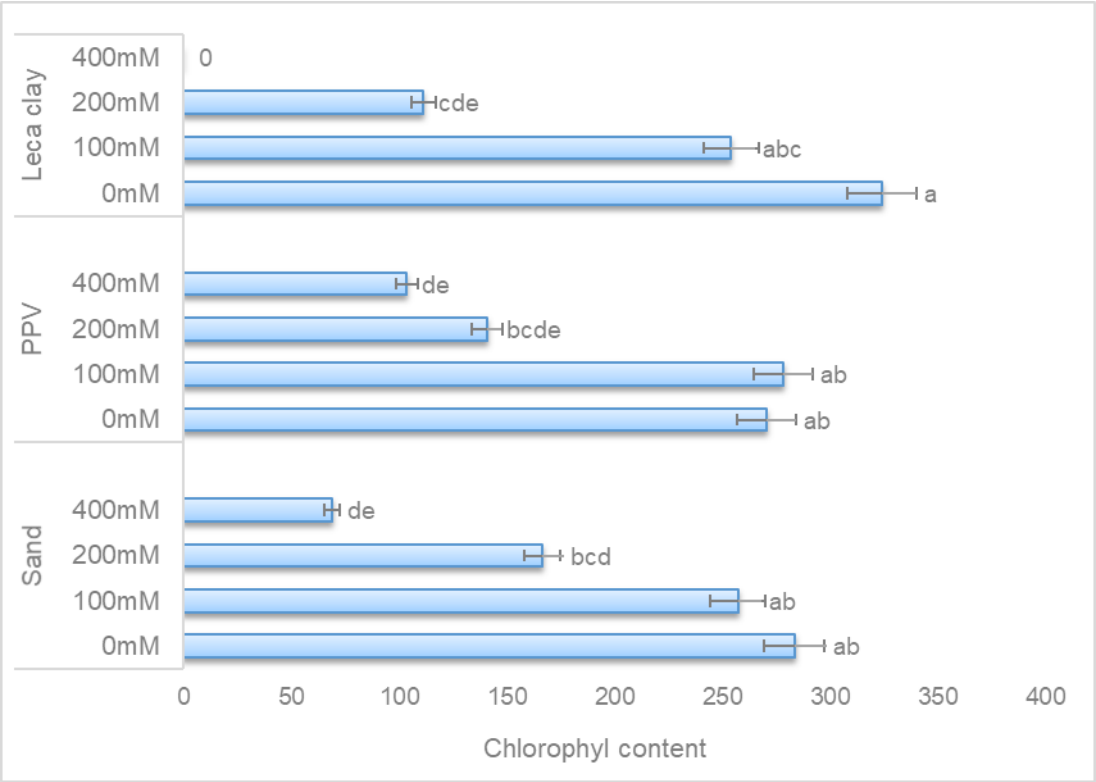

Figure 3.11: Influence of salinity and growth medium on chlorophyll content in hydroponically grown *T. ciliata.* The mean values with different letters are significantly different at P ≤0.05.

3.4.10 The effects of salinity and growth media on the number of flowers

The results of the current trial show that only the control and 100mM saline treatment produced flowers (Figure 3.13). Soil-less media did not have any significant effect on the number of flowers produced. On the contrary, Salinity had a significant effect (P≤ 0.05) on the number of flowers produced by the plants. Furthermore, the interaction of soil-less media and salinity had a significant effect on the number of flowers produced. The interaction of 100mM+PPV recorded a significantly higher number of flowers in comparison with any other treatment (Table 3.2). The interactions of 100mM+SS and 100mM+LC recorded the least number of flowers with means of less than one for both.

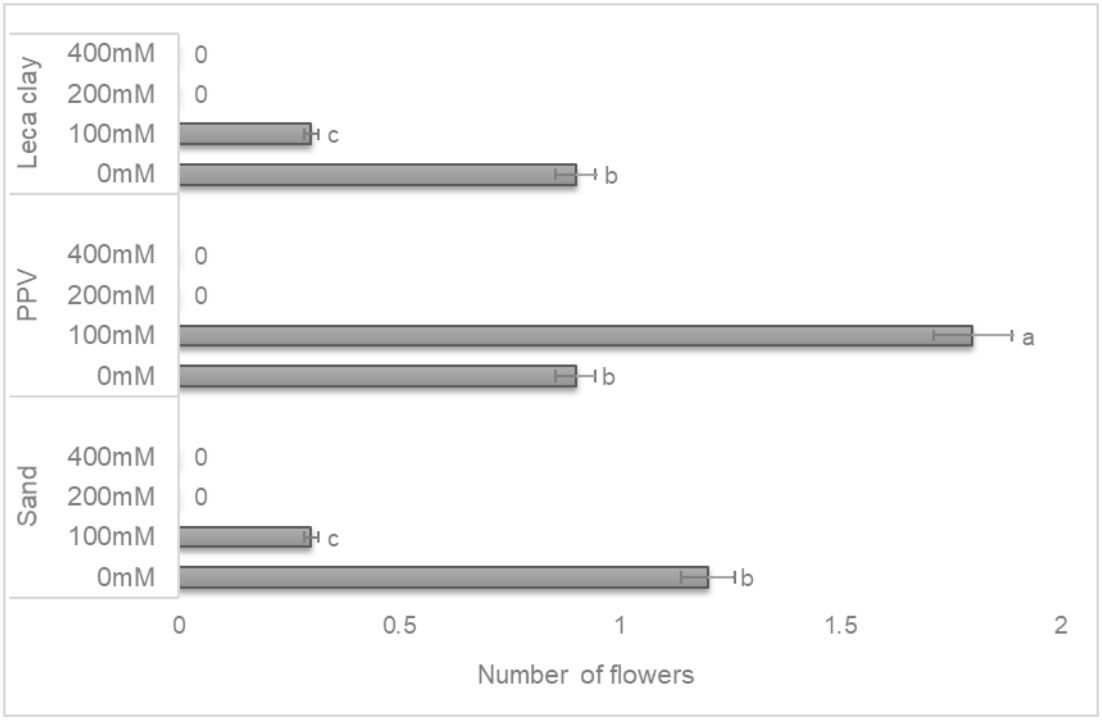

Figure 3.12: Influence of salinity and growth medium on the number of flowers in hydroponically grown *T. ciliata.* The mean values with different letters are significantly different at P ≤0.05.

3.4.11 The effects of salinity and growth media on inflorescence wet weight

The results of the current experiment indicate that salinity had a significant influence (P≤ 0.05) on inflorescence wet weight (Table 3.2). In contrast, soil-less media did not have a significant effect on inflorescence wet weight. Moreover, the interaction of soil-less media and salinity did not have any significant effect on inflorescence wet weight. The highest mean value was observed in the interaction of 100mM+PPV followed by 0mM+PPV with 46.73 and 40.14, respectively. The interaction of 100mM+LC and 100mM+SS recorded the lowest mean values of 7.20 and 7.07 respectively (figure 3.14).

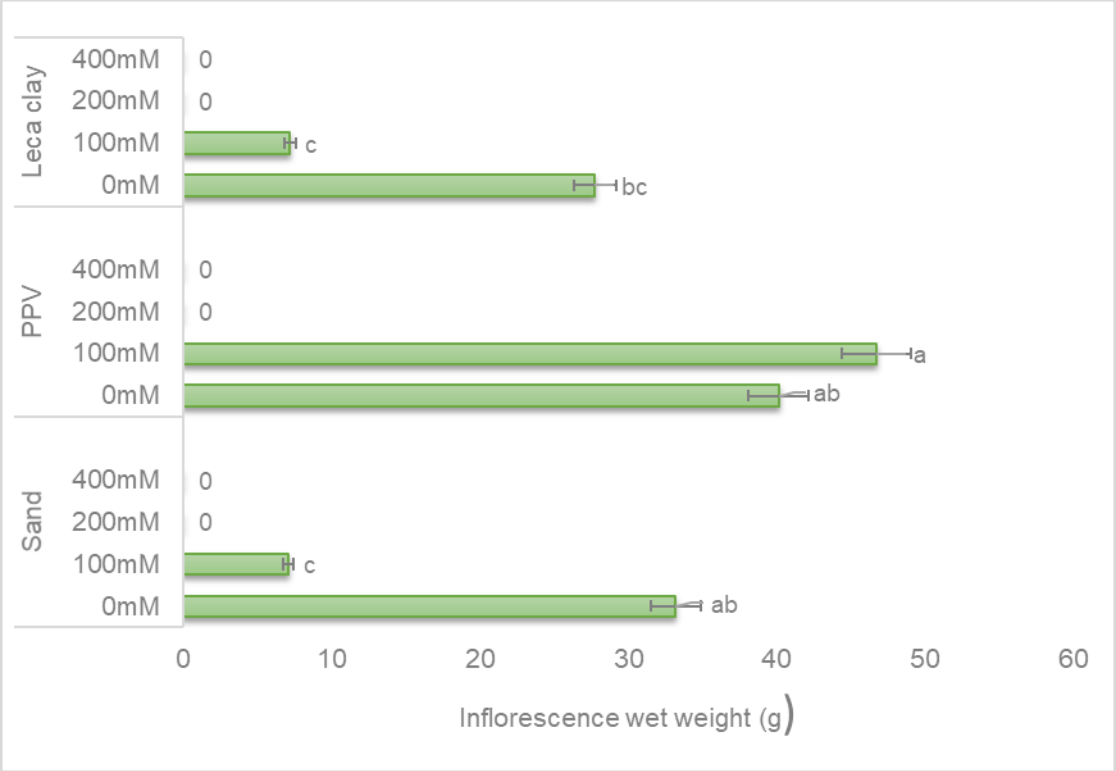

Figure 3.13: Influence of salinity and growth medium on inflorescence wet weight in hydroponically grown *T. ciliata.* The mean values with different letters are significantly different at P ≤0.05.

3.4.12 The effects of salinity and growth media on inflorescence dry weight

The results of the current trial interestingly indicated that soil-less media significantly influenced inflorescence dry weight at (P≤ 0.05). Salinity also significantly affected the inflorescence dry weight (P≤ 0.05), which conforms with the results obtained for wet weight. Another interesting aspect was that the interaction of soil-less media and salinity had a significant effect (P≤ 0.05) on the inflorescence dry weight (Table 3.2). Again, the interaction of 100mM+PPV recorded a significantly high value (5.14) followed by 0mM+PPV and 0mM+LC whose means were 3.47 and 2.67 respectively. The interaction of 100mM+SS and 100mM+LC recorded the lowest mean values of 0.74 and 0.60 respectively (Figure 1.15).

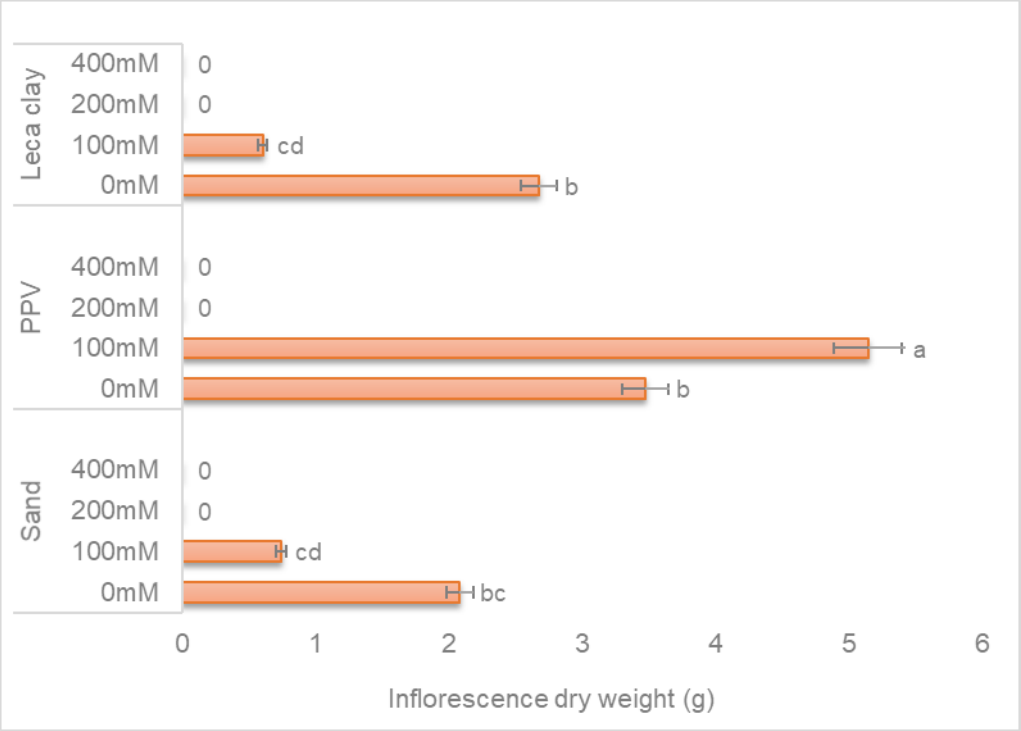

Figure 3.14: Influence of salinity and growth medium on inflorescence dry weight in hydroponically grown *T. ciliata*. The mean values with different letters are significantly different at P ≤0.05.

Table 3.1 The effects of salinity and growth media on the shoots and root weight of hydroponically grown *T. ciliata.*

Soil-less Medium Conc.	NaCl	Shoot weight (wet)	Shoot Weight (Dry)	Root weight (wet)	Root Dry weight
Silica Sand	0.00	95.24±14.38ab	14.10±1.61a	35.78±6.2ab	5.52±1.04a
	100mM	116.14±19.4ab	9.42±1.51a	20.26±2.6d	2.82±0.38d
	200mM	16.75±5.72cd	1.27±0.49b	5.40±1.86e	0.70±0.29e
	400mM	1.12±0.59d	0.19±0.1b	1.03±0.54e	0.17±0.09e
PPV	0.00	116.42±15.64ab	12.60±1.65a	25.74±3.19cd	3.32±0.46cd
	100mM	126.43±13.03ab	9.63±0.9a	29.88±2.4bc	4.09±0.33bcd
	200mM	26.50±8.54cd	2.37±0.71b	8.41±2.77e	1.24±0.4e
	400mM	4.39±2.17d	0.44±0.2b	2.49±0.84e	0.34±0.12e
LECA Clay	0.00	70.40±10.86bc	10.06±1.19ba	29.28±2.97bcd	4.61±0.52abc
	100mM	144.02±23.68a	10.41±1.71a	39.09±6.47a	4.99±0.85ab
	200mM	6.80±2.36d	0.66±0.23b	4.37±1.49e	0.59±0.21e
	400mM	0.00±0	0.00±0	0.00±0	0.00±0
Two-way ANOVA F-Statistics					
Soil-less Medium		1.3ns	1.11ns	0.64ns	0,43ns
NaCl Conc.		74.7*	95.31*	67.33*	59.89*
Soil-less Medium*NaCl Conc.		1.41ns	1.17ns	3.61*	3.45*

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference.

Table 3.2 Mean squares from the analysis of variance for the effect of four varied salinity levels and different soil media on the number of flowers, inflorescence wet weight, inflorescence dry weight and chlorophyll of hydroponically grown *T. ciliata*.

Soil-less Medium Conc.	NaCl	Number of Flowers	Inflorescence wet weight	Inflorescence Dry weight	Chlorophyll
Silica sand	0.00	1.20±0.42b	33.2±13.17ab	2.08±0.99bc	283.40±9.89ab
	100mM	0.30±0.15c	7.07±4.02c	0.74±0.41cd	257.30±29.74ab
	200mM	0.00±0	0.00±0	0.00±0	166.60±46.42bcd
	400mM	0.00±0	0.00±0	0.00±0	69.00±35.9de
PPV	0.00	0.90±0.28b	40.14±13.19ab	3.47±1.15b	270.60±33.5ab
	100mM	1.80±0.25a	46.73±7.9a	5.14±0.79a	278.30±7.91ab
	200mM	0.00±0	0.00±0	0.00±0	141.00±39.52bcde
	400mM	0.00±0	0.00±0	0.00±0	103.70±36.64de
LECA Clay	0.00	0.90±0.23b	27.77±6.86b	2.67±0.67b	324.00±16.14a
	100mM	0.30±0.21c	7.20±6.27c	0.60±0.52cd	254.20±28.91abc
	200mM	0.00±0	0.00±0	0.00±0	111.30±38.18cde
	400mM	0.00±0	0.00±0	0.00±0	0.00±0
Two-way ANOVA F-Statistics					
Soil-less Medium		4.34ns	4.78ns	8.15*	0,84ns
NaCl Conc.		22.87*	19.2*	19.38*	38.99*
Soil-less Medium*NaCl Conc.		5.72*	2.79ns	4.78*	1.35ns

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference.

Table 3.3 Mean squares from the analysis of variance for the effect of four varied salinity levels and different soil media on total dry weight, total wet weight, number of leaves and shoot length of hydroponically grown *T. ciliata*.

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

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference.

3.5 DISCUSSION

As agricultural productivity is threatened by increasing salinity levels in the soil, there is a need to discover and grow vegetable crops that are salt-tolerant to guarantee the world, 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, (2006) and Rengasamy *et al*. (2003) who found that low salinity increases productivity and higher levels reduce growth in halophytic plants.

Moreover, the results obtained from the experiment also proved that low levels of salinity (100mM) do enhance the production of *T. ciliata* and that high levels of salinity reduce production. In this experiment, salinity significantly affected shoots wet weight, shoot dry weight, root wet weight, shoots wet weight, and both total wet and dry weight. The highest mean values for shoots wet weight, root wet weight, and total wet weight were observed at 100mM salinity level, while high salinity levels recorded the lowest mean values. These results are in correspondence with the findings of Klados and Tzortzakis (2014) who concluded that low salinity levels enhance growth and high levels reduce production considerably (Pardossi *et al.*, 1999; Andriolo *et al*., 2005; Chondraki *et al*., 2012). Adams (1991) and Sayyad-Amin *et al*. (2016) reported that salinity or high EC reduces crop yield. The control produced the highest mean values for shoot dry weight, root dry weight, and total dry weight although the margins were close to those of 100 mM salinity treatment. Plants respond differently to salinity as affected by different salt levels, including growth reduction resulting from nutritional differences (Grattan & Grieve, 1999; Song *et al.*, 2006). In addition, khan *et. al*. (2000) reported that the water potential and osmotic potential of a halophytic plant (*Suaeda fruticosa*) became more negative as salinity was increased, hence the decrease in weight. Growth media had no significant effect on biomass. The highest means were observed in Leca clay for shoots and root wet weights while sand recorded the highest means for shoots and root dry weights. Other studies also show that plant biomass increases with increasing salinity in *Solanum melongena* L. (Shalhevet *et al.*, 1993), *Phaseolus vulgaris* L. (Gama *et al*., 2007), and *Solanum Lycopersicum* L. (Hayward & Long, 1941). The findings of this trial correspond to those of Koryo (2006) and Khan *et. al*., (2000) who worked on a halophytic plant and found that low salinity increases productivity and higher levels reduce plant growth. This phenomenon of salt toxicity is related to the efficiency of stomatal conductance, photosynthesis rate, transpiration, and respiration as affected by high salinity levels (Rengasamy *et al*., 2003; Koryo, 2006).

Likewise, the number of leaves increased at low salt levels and declined because of salt toxicity. The most productive soil-less medium was a mixture of perlite: peat: vermiculite at

100mM 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 (Hanna, 2010; Venter, 2010; Rapaka, 2013). Both salinity and soil-less media showed a significant effect on shoot height and interestingly, 0 mM and 100 mM concentrations showed no significant effect amongst each other in all media mixtures. PPV showed to be the most effective soil-less medium and recorded the highest mean values when in conjunction with the 0mM and low salinity levels (100 mM). These results correspond with the findings of Klados and Tzortzakis (2014), Sayyad-Amin *et al*. (2016), and Zapryanova and Atanassova (2009) who concluded that low salinity levels increase leaf length and high salt levels reduced the leaf length. Gama *et al*. (2007), Hayward and Long (1941), and Shalhevet *et al*. (1993) further reported that salinity has different effects on growth, and effects may differ according to species.

The inflorescence is the most important aspect because it is the only part that is edible and can be used as a vegetable, hence the significance of its results. Low salinity (±100mM) 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 (Koryo, 2006). The 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*., (2014), Zapryanova and Atanassova (2014), Blitz and Gallagher (1991), and Stanton *et al.,* (1997) 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 flowering or delay flowering. In addition, Zapryanova and Atanassova (2009) also reported that increasing salinity results in delayed and reduction of flowering.

Furthermore, salinity had a significant effect on the chlorophyll content under different salinity levels 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 (Jamil, 2007; Heidari, 2012; Sayyad-Amin *et al*., 2016). Heidari (2012) further suggested that increased salinity may reduce chlorophyll content in normal plants and may increase it in salt-tolerant plants. In addition, Dhanapackiam and Ilyas (2010) reported a decreased chlorophyll pigment with increasing salinity. Salinity reduced stomatal conductance, water potential, osmotic potential, and nitrogen concentration on the leaves, thereby reducing chlorophyll content (Koryo, 2006).

3.6 CONCLUSION

To overcome the growing challenges of food insecurity and to maximize agricultural production, alternative uses of less quality or salty water and salt-tolerant crops will become very important in the future. Furthermore, it will be necessary to mitigate the negative effects of salinity by applying strategies such as diluting seawater with suitable nutrient solutions to relieve pressure on the need for freshwater for irrigation. 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. Since this plant belongs to the highly medicinal *Aloe* family (Asphodelaceae), it is therefore recommended that further studies be conducted on the effect of salt stress on the nutritional compositions, anti-oxidant capacity, and anti-microbial activities to support its cultivation. Furthermore, examining the effect of salt stress on the water influx in the root zone is also recommended to evaluate the drought tolerance of this plant. It is also recommended that smaller amounts of NaCl ranging from 0 to 200 mM be used in further experiments because of the level of salt toxicity in higher concentrations.

3.7 Acknowledgements

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CHAPTER FOUR

EFFECT OF SALINITY AND DIFFERENT SOILLESS MEDIA ON PHYTOCHEMICALS AND ANTIOXIDANT CAPACITY OF *TRACHYANDRA CILIATA* (L.F) KUNTH (ASPHODELACEAE) GROWN IN HYDROPONICS

SUBMITTED TO: HORTICULTURAE

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4.1 ABSTRACT

Salinity stress induces a decline in photosynthetic efficiency resulting in the diversion of carbohydrate synthesis to the production of antioxidants and phytochemicals that are useful for plants and human health. *Trachyandra ciliata* commonly known as Wild cabbage or Veldkool (Afrikaans) is a halophytic plant that belongs to the Asphodelaceae (Aloe) family, which is reported to be edible although there is no literature available on its phytochemicals. This then prompted a need for phytochemical screening of this plant and examination of its antioxidant capacity *in-vitro* using 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) assays, 2,2-diphenyl-1-picrylhydrazyl ethanol (DPPH), and ferric reducing antioxidant power (FRAP) assays. This study, therefore, investigated the effects of salinity and different soilless media on antioxidant properties and phytochemical content of *T. ciliata* grown in hydroponics to explore its potential as a natural source of secondary metabolites since there are risks in the use of synthetic phytochemicals. The results show that salinity and soilless media had a significant effect ($P \leq 0.05$) on the antioxidant activity and phytochemical content of *T. ciliata*. It was also observed that 200mM of NaCl treatment produced significantly higher mean values for polyphenols, FRAP, ABTS, and DPPH while 100mM recorded the highest mean value for flavonols. PPV soilless medium was proved to be the best for the production of polyphenols, FRAP, ABTS, and flavonols while the highest mean value was recorded in silica sand for DPPH. It was concluded that a combination of 200mM+PPV can be recommended for maximum production of antioxidants for *T. ciliata*.

Keywords: carbohydrates, polyphenols, salinity, soilless media

Abbreviations: Ferric Reducing Antioxidant Power (FRAP) Perlite (P), Peat (P), Vermiculite (V), Silica Sand (SS), Treatment (T), Sodium Chloride (NaCl), PPV (Peat: Perlite: Vermiculite), Reactive oxygen species (ROS).

4.2 INTRODUCTION

Accumulation of salt in agricultural lands results in salt toxicity, which then reduces the efficiency of stomatal conductance, photosynthesis, transpiration, and respiration (Stefanov *et al*., 2016). A significant buildup of salts in the root zone results in reduced water holding capacity, leading to reduced yield (Silveira *et al*., 2009). Plants respond differently to salinity as affected by different salt levels, including growth reduction resulting from nutritional differences (Penella *et al*., 2016). It is reported that environmental factors such as drought, salinity, low or high pH, and high temperature may result in increased production of reactive oxygen species (ROS), which often result in oxidative stress when available in high concentrations (Bravo, 1998; Daniels *et al*., 2011). 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 (Balasundram *et al.*, 2006; Ksouri *et al*., 2007). 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 form of fruits and vegetables (Yang *et al*., 2014; Mazouz *et al*., 2020).

Reactive oxygen species (ROS) are derivatives produced during aerobic breakdown and are active in numerous essential signaling pathways within plants (Schieber & Chandel, 2014; Mittler, 2017; Forrester *et al*., 2018). Reactive oxygen species are valuable to plants because they support cellular proliferation, biological functions, and sustainability (Forrester *et al*., 2018). Therefore, maintaining a basic level of ROS in all cells is important for life (Mittler, 2017). Although ROS are beneficial to life, extreme concentration in the cell can lead to damage of lipids, proteins, DNA, and nucleic acids (Mittler, 2002; Mazouz *et al*., 2020). Oxidative stress is believed to be accountable for the initiation or advancement of some of the diseases in plants and humans because it reflects an imbalance in reactive oxygen species, which leads to damage in cells and lipids (Diplock *et al*., 1998; Schieber & Chandel, 2014). This imbalance gives the impression to free radicals (molecules with unpaired electrons) which can react with oxygen to deprive other cells of electrons, resulting in undesirable compounds that can damage tissues and cause many diseases (Phaniendra *et al.*, 2015; Forrester *et al*., 2018).

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 shikimate, phenylpropanoid, and pentose phosphate pathways in plants (Kähkönen *et al*., 1999; Randhir *et al.*, 2004). 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 (Bravo, 1998; Jimoh *et al*., 2019a). 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 (Adegbaju *et al*., 2020; Adewusi *et al*., 2011; Daniels *et al*., 2011). Oxidation is regarded as the process in which electrons are transferred from one atom to the other and that is an important part of metabolism and aerobic life (Zheng & Wang, 2001). Antioxidants derived from plant extracts are more preferred compared to synthetic ones because of health and safety reasons (Ksouri *et al*., 2007; Jimoh *et al*., 2020). It has been reported that consumption of fruit and vegetables that are comprising of phenolic compounds that have antioxidant activity reduces the risk of chronic infections and diseases (Finley, 2004; Idris *et al*., 2019; Jimoh *et al*., 2019b; Salami & Afolayan, 2020). These health factors have given rise to the extensive screening of plants for antioxidants and other pharmaceutical properties.

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 (Manning and Goldblatt, 2007; Smith & Van Wyk, 1998; Van Jaarsveld, 2020). *T. ciliata* commonly known as Wild cabbage or Veldkool (Afrikaans) is a halophytic species in Asphodelaceae which has gained a reputation in research throughout the years based on the medicinal properties of most of its members that are widely used in pharmaceutical and beverage industries (Van Wyk, 2011). The plant is less studied with little to no literature at all and there are no *Trachyandra* species that are currently cultivated (Manning & Goldblatt, 2007; De Vynck *et al*., 2016). However, it has been documented that the inflorescence of *T. ciliata* was used by the Khoi-san people as a vegetable before colonization that led to the erosion of knowledge about indigenous plants (Kuhnlein *et al*., 2013; De Vynck *et al*., 2016; Van Jaarsveld, 2020). Other vegetable crops such as cabbage and spinach have been proven to contain high levels of antioxidants. This could be the case for this undocumented *T. ciliata* species.

Plants that grow under salt stress tend to produce secondary metabolites such as phenolic compounds that are beneficial for human health (Yang *et al*., 2014; Mazouz *et al.*, 2020;). This, therefore, inspires the need for screening of this plant for phytochemical and antioxidants content as affected by salinity. Growing substrate plays four significant roles to the plant; it allows for the provision of water to the plant roots, gas exchange to and from the roots, supplies nutrients to the plant, and provides support as an anchor for the plant (Fonteno, 1996; Jordan *et al*., 2018; Faber *et al*., 2020). This study aims to determine the effect of salinity and different soilless media on phytochemical contents and antioxidant capacity of *T. ciliata* grown in hydroponics and investigate variability in phytochemicals and antioxidant content of the plant at varying salt concentrations.

4.3 MATERIALS AND METHODS

This experiment was carried out in the research nursery at the Bellville campus of the Cape Peninsula University of Technology (CPUT), South Africa (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 ºC during the day and 12 and 18 ºC at night with the use of environmental control. The Relative Humidity average was kept at 60%. After harvesting, phytochemical and antioxidant assays were carried out in the Oxidative Stress Research Centre, Faculty of Health and Wellness Sciences, CPUT.

4.3.1 Hydroponic system design

In this experiment, four 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 3.1). The treatments were labeled as T1–T4. Each system had its low-density polyethylene (LDPE) 50-liter reservoir in which the nutrient solutions were prepared. There were three Polyvinyl Chloride (PVC) square gutters (2 m), put in place with cable ties on each table, in which three 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 x 2000 L/h submersible pump with 2.5 m head capacity, 20 mm LDPE irrigation piping, 4 x 20 mm elbow irrigation fittings, and 4 x 20 mm flow regulators were used. Gutters were fitted with 1 outlet that returns the solution to the reservoir.

Every gutter on each system had 10 pots (12.5 cm height x 12.5 cm x 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 and creating a spontaneous circulating system. A 1 x 2000 L/h submersible pump with 2.5 m head capacity was used to circulate the nutrient solution for 24 hours from the beginning to the end of the experiment.

EC in the nutrient solution was monitored daily with a calibrated hand-held digital EC meter (Hanna instruments®™ HI 98312). The pH of the solution was monitored with a calibrated hand-held digital pH meter (Eurotech®™ pH 2 pen). Potassium hydroxide was used to elevate pH, while phosphoric acid was used to decrease the pH of the nutrient solution (Butcher et al., 2017).

4.3.2 Plant Material

The plant material of *T. ciliata* was obtained from a local nursery and was further 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 arranged in a randomized block design.

4.3.3 Nutrient solutions

Nutrifeed fertilizer which has been certified as containing all the essential nutrients necessary for healthy and vigorous plant growth was used to make hydroponic aqueous solutions. An equal amount of Nutrifeed was added to the nutrient solution 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.

4.3.4 Soilless media treatments

The pots (12.5 cm height x 12.5 cm x 12.5 cm width) were placed in each gutter and the medium combinations were manipulated as follows:

- Sand (100% silica 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.

4.3.5 Salinity Treatments

Different salt concentrations were manipulated using sodium chloride (NaCl) in the Nutrifeed nutrient solutions. Sodium chloride 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. The saline solutions were prepared using tap water. All nutrient solutions containing NaCl were replaced every week to avoid the accumulation of salts in the medium, pots, gutters, and reservoirs. The pH was maintained at 6.0.

4.3.6 Formulation of crude extract

Crude extracts were obtained by stirring the finely ground plant material (whole plant) in ethyl alcohol (ETOH) and centrifuged at 4000 rpm for 5 min. The supernatant was filtered through a Whatman No. 1 filter paper placed in a Buchner funnel connected to an electric vacuum pump to remove unmacerated tissue and other debris. The resulting crude extracts were used for all analyses were utilized to perform phytochemical and antioxidant assays (Daniels *et a*l., 2011).

4.3.7 Determination of antioxidant capacity

Phytochemical content and antioxidant capacity of metabolites within the mixture of roots and shoots were assayed for total flavonols, total polyphenols, ferric reducing antioxidant power (FRAP), ABTS, and DPPH.

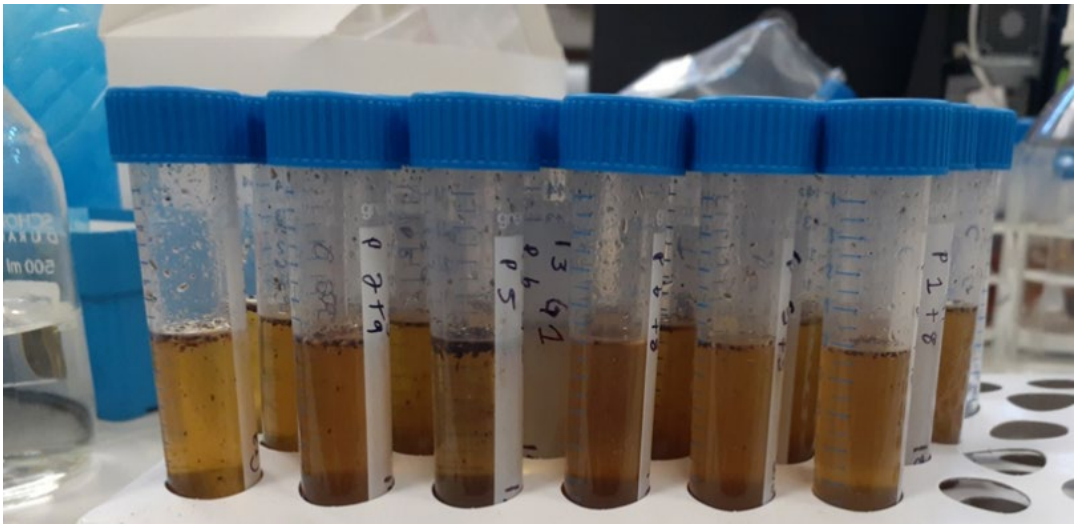

Figure 4.1: Collection of samples extracted with 60% Ethanol.
(Picture: Ngxabi)

4.3.7.1 Polyphenol assays

The total polyphenol essay of the extracts was performed using the Folin Ciocalteu method as reported by Ainsworth & Gillespie, (2007) and Singleton et al., (1999). About 25 µL of the sample was mixed with 125 µLFolin-Ciocalteu reagents (Merck, South Africa) that was diluted 10 times with distilled water. Then 7.5% sodium carbonate (Sigma, South Africa) solution was prepared and added to 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, 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 presented in a form of mg gallic acid equals per g dry weight (mg GAE/g DW).

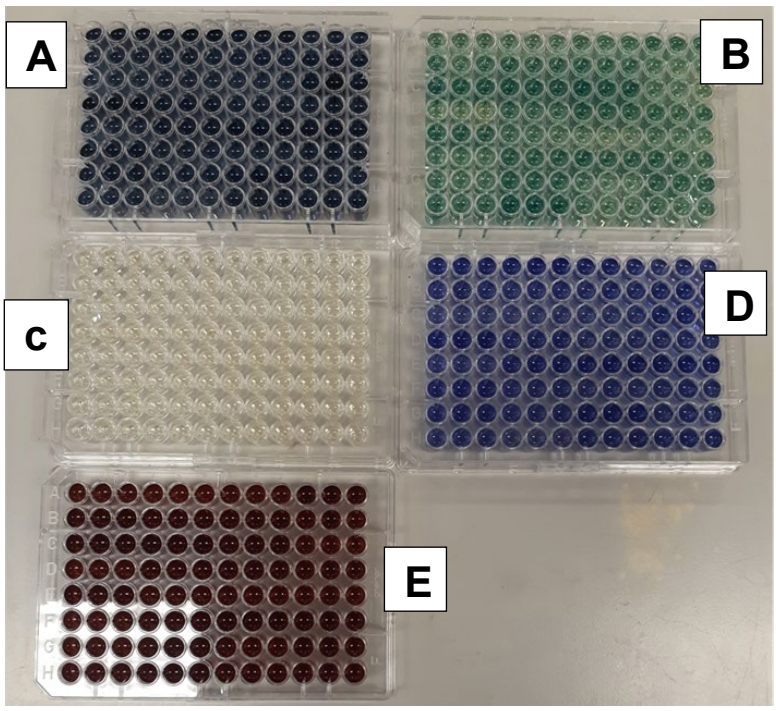

Figure 4.2: Polyphenol assays. A; Polyphenols assay, the darker the color the more polyphenols the sample contains. E; ABTS assay, the lighter the color the more antioxidants the sample contains. C; Flavonols assay, no color as this assay is measured in the UV range which the human eye cannot see. D; FRAP assay, the darker the color the more antioxidants the sample contains. E; DPPH assay, the lighter the color the more antioxidants the sample contains. (Picture: Ngxabi)

4.3.7.2 Estimation of flavonol content

The flavonol content of the extracts was evaluated using quercetin 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol (Sigma-Aldrich, South Africa) as standard. 12.5 µL of the crude sample extracts was 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 minutes at room temperature. The absorbance was read at 360 nm, at a temperature of 25 °C (Mazza et al., 1999). The results were presented in a form of mg quercetin equivalent per g dry weight (mg QE/g DW).

4.3.7.3 Determination of Ferric Reducing Antioxidant Power (FRAP)

The method of Benzie and Strain (1999) was used to perform the FRAP assay. FRAP reagent was prepared by mixing 30 mL Acetate buffer (0.3M, pH 3.6) (Merck, South Africa) with 3 mL 2,4,6- tripyridyl-s-triazine (10mM in 0.1M Hydrochloric acid) (Sigma, South Africa), 3 mL Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H2O$) (Sigma, South Africa), 6 mL of distilled water and incubated for 30 minutes. at 37 °C. Then 10 µL of the crude sample extract was mixed with 300 µL of the FRAP reagent in a 69-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 a

concentration curve varying from 0 to 100µM. The results were presented in a form of µM ascorbic acid equivalents (AAE) per g dry weight (µM AAE/g DW).

4.3.7.4 Determination of ABTS antioxidant capacity

The ABTS antioxidant capacity was assayed utilizing a method described by Idris et al., (2017) with slight modification. The stock solutions included a 7mM ABTS and 140mM Potassium-peroxodisulphate ($K_2S_2O_8$) (Merck, South Africa) solution. The solution the experiment was then prepared by adding 88 µL $K_2S_2O_8$ to 5 mL ABTS solution. These two 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 plate reader. The results were presented as µM/Trolox equivalent per g dry weight (µM TE/g DW).

4.3.7.5 Antioxidant capacity of DPPH radicals

The DPPH radical was generated from a solution of 0.135 mM DPPH prepared in a dark bottle (Olatunji & Afolayan, 2019; Unuofin et al., 2017). 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. Mixtures were incubated for 30min after which absorbance was taken at 517nM. The results were expressed in a form of µM/Trolox equivalent per g dry weight (µM TE/g DW).

4.3.8 Statistical analysis

A two-way analysis of variance (ANOVA) was used to determine the interaction between salinity and growth media on growth parameters. The significant differences between treatment means at $P \leq 0.05$ were compared using Fisher's least significant difference (LSD). All the calculation was done on a computer software program STATISTICA version 10.

4.4 RESULTS

4.4.1 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 4.3). The interaction of 200mM NaCl and PPV medium significantly produced the highest concentration of polyphenols (11.07 mg GAE/g DW) compared to other treatments while 400mM NaCl yielded the lowest content of polyphenol as revealed by Tukey least significant difference ranking. Likewise, 0mM and 100mM NaCl yielded an equivalent flavonol in the sand medium; 0mM

and 200mM NaCl produced no significantly different flavonol in Leca clay; so also, 100mM 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 400mM and PPV whereas no growth was recorded in Leca clay with 400mM NaCl treatment, hence there is no polyphenol record (Table 4.1).

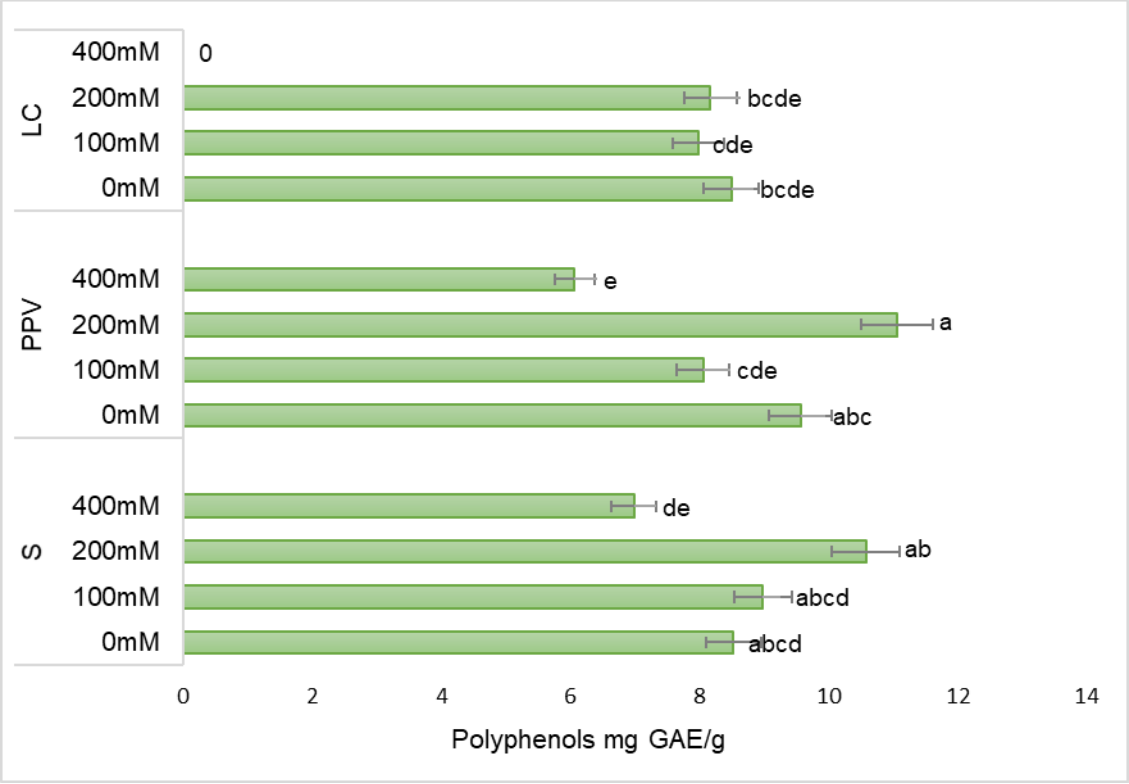

Figure 4.3: Influence of salinity and growth medium on the polyphenol content of *T. ciliata.* The mean values with different letters are significantly different at P ≤0.05.

4.4.2 Effect of different salinity levels and soilless media on flavonols

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≤ 0.05. The highest mean flavonol value (8.01 mg QE/g of the pulverized sample) was recorded in the interaction of 100mM salt and PPV compared to all other treatments while the lowest mean value (2.38 mg QE/g DW) was observed in 200mM in Leca clay (figure 4.7). There was no significant difference in the interaction between 100mM NaCl and 200mM salt with sand and PPV respectively (Table 4.1). Similarly, an equivalent flavonol yield was found in the interaction of the control samples of zero salt concentration with sand, PPV, and Leca clay.

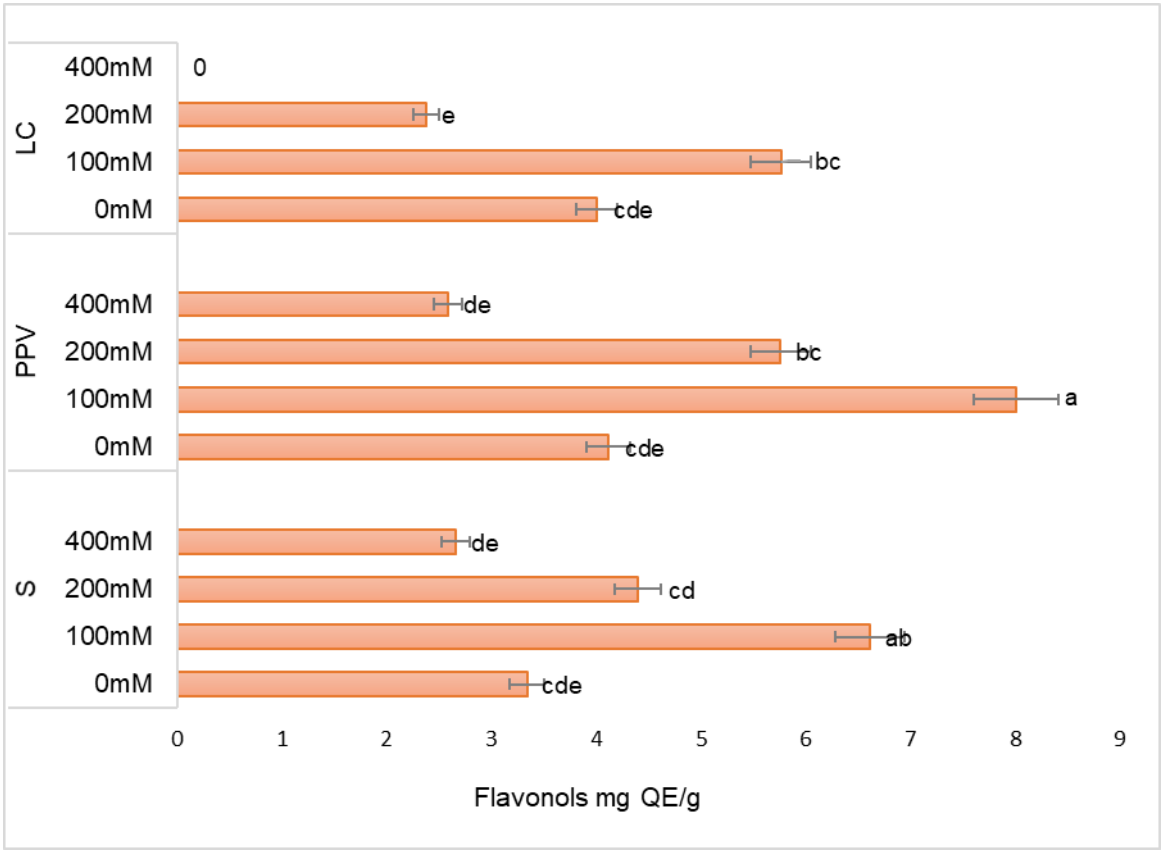

Figure 4.4: Influence of salinity and soilless media on flavonol content. The mean values with different letters are significantly different at P ≤0.05.

4.4.3 The effect of salinity and soilless media on FRAP

As presented in Figure 4.4, results show that both salinity and soilless media had a significant influence (P≤ 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. 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 400mM 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. (Figure 4.5).

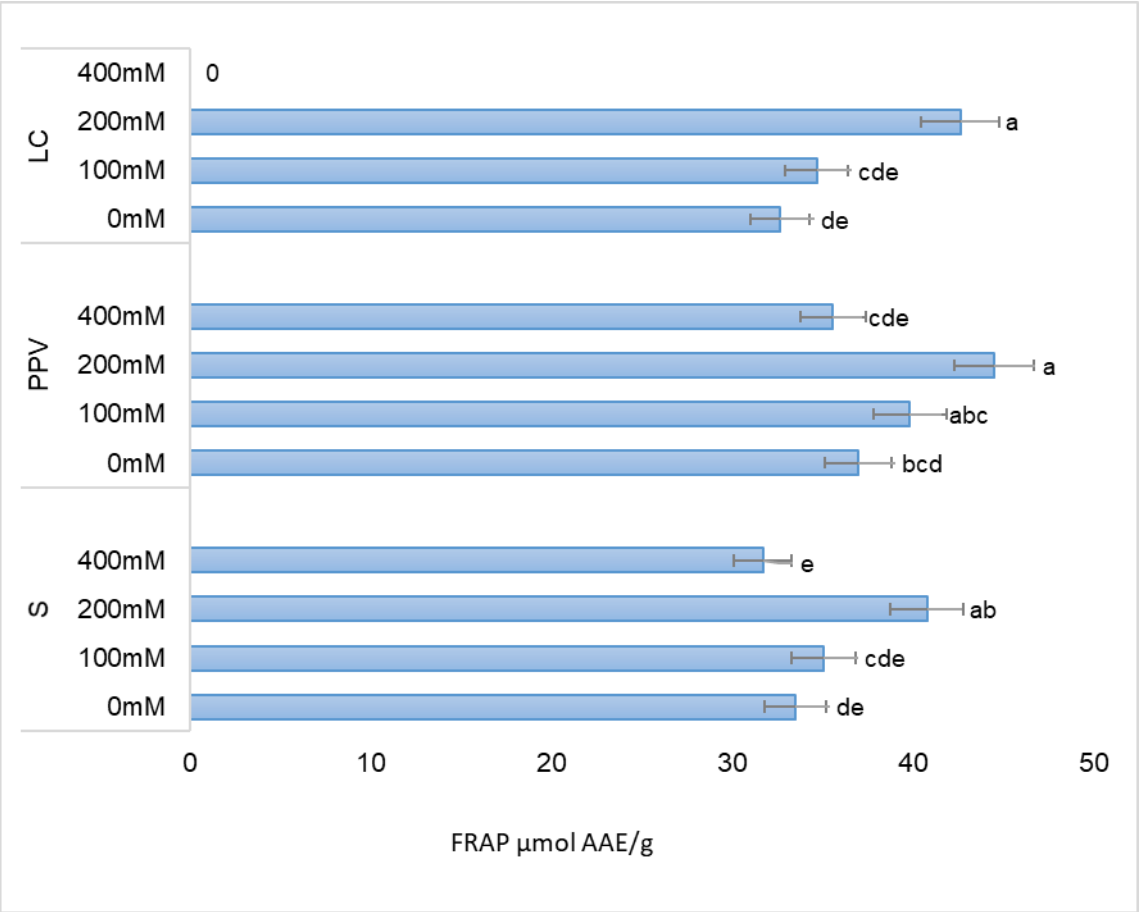

Figure 4.5: Influence of salinity and soilless media of FRAP. The mean values with different letters are significantly different at P ≤0.05.

4.4.4 Influence of different salinity levels and soilless media on ABTS

Results of this experiment revealed that both salinity and soilless media had a significant effect on the ABTS values (Table 4.5). Figure 4.5 shows that the 200mM salt concentration had the highest values in PPV (40.79 µM TE/g DW) and lowest in sand with 400mM. There was no significant difference between 0mM, 100mM, and 200mM in PPV respectively with Leca clay, sand media and in LC so also at 0mM and 100mM concentrations with sand and PPV, respectively. Besides, other samples showed significant variability in ABTS capacity.

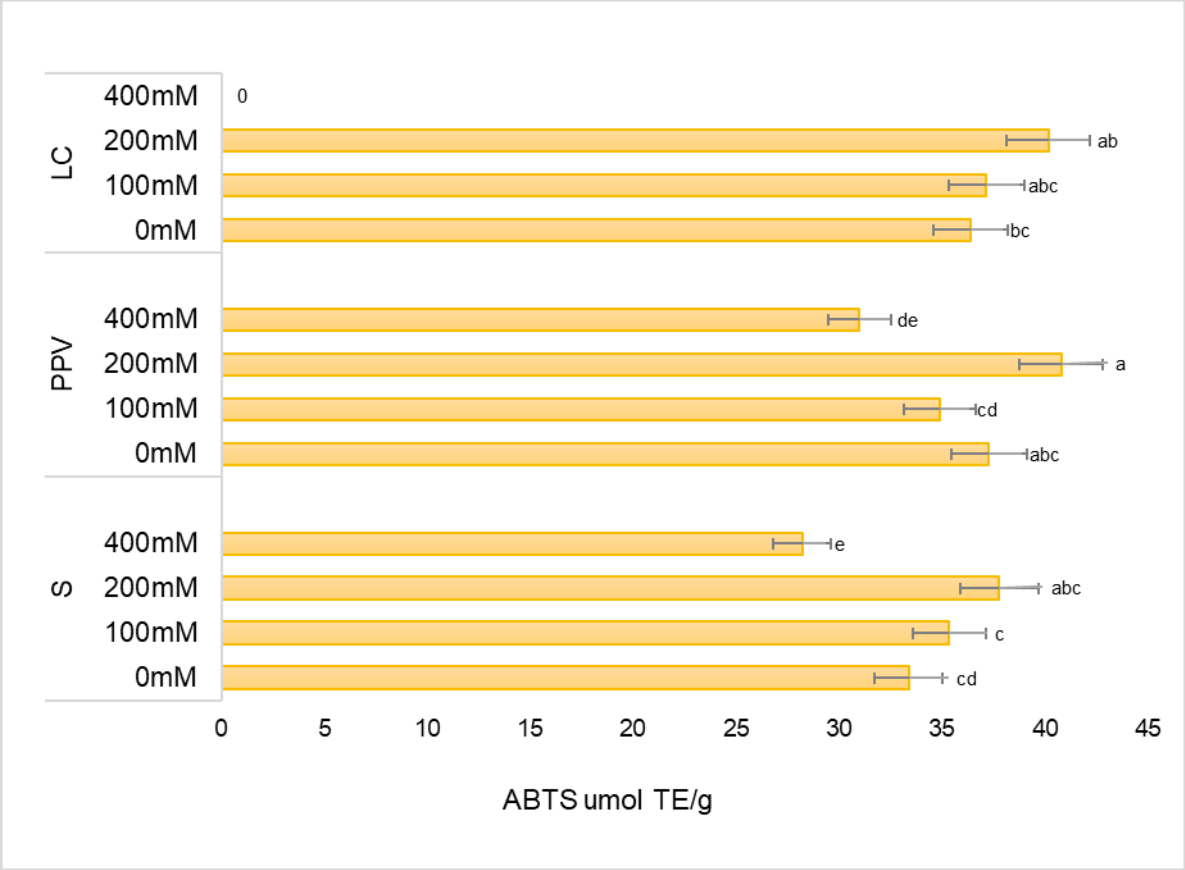

Figure 4.6: the influence of salinity and soilless media on ABTS. The mean values with different letters are significantly different at P ≤0.05.

4.4.5 Effect of different salinity concentrations and soilless media on DPPH

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 200mM 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 400mM recorded significantly lowest values in PPV (Figure 4.6). Similarly, the highest mean was obtained in the interaction of 200mM+SS (19.02 µmol TE/g), while the lowest was recorded in the interaction of 400mM+PPV (9.39 µmol TE/g). There was no significant difference between 0mM and 100mM salinity levels in Leca clay medium however, 0mM and 200mM salinity levels in PPV had an equivalent effect on DPPH with 100mM salt in sand medium (Figure 4.5).

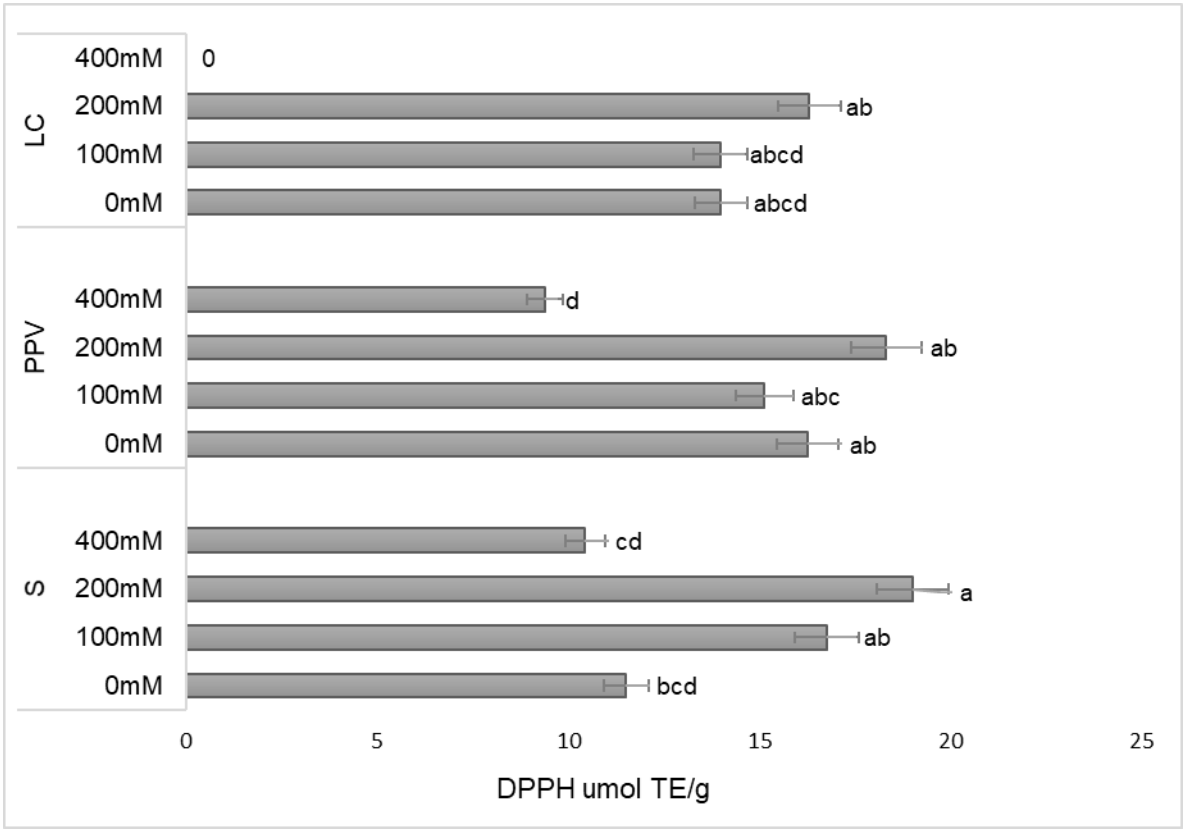

Figure 4.7: Influence of salinity and soilless media on DPPH. The mean values with different letters are significantly different at P ≤0.05.

Table 4.1: Mean squares from the analysis of variance for the effect of four varied salinity levels and different soil media on FRAP, ABTS, DPPH, Polyphenols and Flavonol content of hydroponically grown *T. ciliata*.

Soil-less Medium	NaCl Conc.	FRAP	ABTS	DPPH	Polyphenols	Flavonols
Silica Sand	0.00	33.1±0.26de	34.13±0.55cd	13.02±0.78bcd	8.87±0.17abcd	3.94±0.34cde
	100mM	35.08±1.77cde	35.35±0.76c	16.79±0.46ab	8.98±0.55abcd	6.61±0.32ab
	200mM	40.78±0.46ab	37.79±0.94abc	19.02±0.46a	10.58±0.47ab	4.69±0.40cd
	400mM	31.71±1.74e	28.21±0.62e	10.44±1.55cd	6.99±0.58de	2.66±0.56de
PPV	0.00	36.97±0.18bcd	37.29±1.05abc	16.27±0.92ab	9.57±0.23abc	4.12±0.23cde
	100mM	39.84±0.52abc	34.9±0.91cd	15.14±1.41abc	8.06±0.10cde	8.01±0.49a
	200mM	44.5±0.52a	40.79±0.58a	18.33±1.28ab	11.07±0.78a	5.76±0.37bc
	400mM	35.59±0.52cde	31.0±1.38de	9.39±0.02d	6.06±0.05e	2.58±0.11de
LECA Clay	0.00	32.66±1.71de	36.38±0.68ebc	14.0±2.19abcd	8.49±0.51bcde	4.0±0.59cde
	100mM	34.7±0.91cde	37.17±0.49abc	13.98±0.59abcd	7.98±0.52cde	5.76±0.35bc
	200mM	42.62±1.07a	40.17±0.44ab	16.32±0.26ab	8.17±0.82bcde	2.38±0.14e
	400mM	0±0	0±0	0±0	0±0	0±0
Two-way ANOVA F-Statistics						
Soil-less Medium		139.42*	101.42*	16.87*	39.39*	32.76*
NaCl Conc.		210.93*	391.83*	64.76*	78.18*	93.58*
Soil-less Medium*NaCl Conc.		84.01*	133.03*	6.49*	11.95*	4.91*

Mean values ±SD are shown in columns. The mean values followed by different letters down the columns are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Fisher's least significant difference.

4.5 DISCUSSION

Growth medium plays an important role for successful cultivation as it plays a part of being an anchor and allows for gas exchange and holds water and nutrients for the plants (Fonteno, 1996; Pardossi *et al*., 2011; Hussain *et al*., 2014). Medium selection is amongst the most significant aspects influencing plant growth and productivity 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 are adapted to certain types of soils naturally (Teto *et al*., 2016; Faber *et al*., 2020). In this study, the results suggest 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 (Abad *et al*., 2002; Venter, 2010). 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 (Abad *et al*., 2002; Pardossi *et al*., 2011; Stefanov *et al*., 2016). 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.

From the results obtained in this experiment, salinity-induced oxidative stress is significantly ($P \leq 0.05$) effective for the accumulation of polyphenols. Generally, moderate salt concentration (200mM) showed a significantly positive effect on polyphenols. There was not much of a difference between 0mM and low salt concentration (100mM), while high salt concentration produced significantly lower means. These findings are in correspondence to the conclusion that was drawn by Rezazadeh *et al.*, (2012) that moderate salt concentration promoted the production of phenolic compounds within a halophytic plant, *Cynara scolymus* L. Abdallah *et al*. (2019) and Ksouri *et al*. (2007) found similar results and reported that high salinity levels restricted the accumulation of phenolic compounds in a halophytic plant, *Cakile maritima*. It has been reported that salinity stress reduces plant biomass and photosynthesis. As a result, plants divert the production of carbohydrates and produce secondary metabolites (Coley, 1986; Parvez *et al.*, 2020). It is 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 (Waring & Pitman, 1985; Shabala & Pottosin, 2014). Abdallah *et al*. (2016) and Wong *et al*., (2006) further stated that disturbances in enzyme

activity at high salt concentrations lead to reduced photosynthesis, leading to reduced growth and polyphenol content and capacity.

There was a different observation regarding flavonol content which was completely opposite to the trend that was observed in other parameters. The results just like 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 (100mM) 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. High salt levels (200 mM+) proved not to be effective for the accumulation of flavonols. These findings are in agreement with those of Ksouri *et al.* (2007) who reported that 100mM 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 (Waring and Pitman, 1985; Rezazadeh *et al*., 2012).

Ferric reducing antioxidant power (FRAP) was significantly affected ($P \leq 0.05$) by salinity and soilless media. The highest FRAP values were observed in moderate salt concentration (200 mM) across all soilless media, while the lowest mean value was surprisingly observed in the combination of 0 mM and Leca clay followed by high salt concentration in all other media. Low salt concentration (100 mM) recorded slightly higher FRAP values but significantly lower to those of moderate salinity. The results of this experiment agree with the correlation between FRAP and DPPH that was reported by (Sharma & Ramawat, 2014). These results may be supported by the fact that salinity-induced oxidative stress is associated with the increase in the production of secondary metabolites. Furthermore, they may be explained by the fact that high salinity can be toxic and lead to reduced photosynthesis, resulting in decreased synthesis of secondary metabolites (Ksouri *et al*., 2007; Sharma & Ramawat, 2014).

Like other parameters, moderate salt concentration recorded significantly higher ABTS mean values compared to other concentrations. The highest mean value was recorded in the interaction of 200mM and PPV soilless mixture. There was not much of a difference between treatments of 0mM and low concentration in the sand and clay, while there was a significant difference in the PPV medium. The lowest mean value was observed in the interaction of 400mM and Leca clay just like in all other parameters. This phenomenon is associated with the fact that low to moderate salt concentrations do enhance the presence of polyphenolic compounds and antioxidants, while salt toxicity reduces the antioxidant capacity of halophytes (Ksouri *et al*., 2007). 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 (Machado & Serralheiro, 2017).

In this study, DPPH radical-scavenging activity was significantly affected (P≤ 0.05) by salinity and soilless media. The highest radical scavenging activity was observed in the moderate salt concentration (200mM) across all soilless media treatments, while the lowest was observed in the highest concentration (400mM). The trend was the same as in polyphenols regarding 0mM and 100mM concentrations. These findings agree with the results of Rezazadeh *et al*. (2012) 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 (Wong *et al*., 2006; Sharma & Ramawat, 2013). It is argued that not all plants respond to salinity the same way; others show a significant increase in production of radical-scavenging activity and others may show a significant decrease (Sharma & Ramawat, 2014).

4.6 CONCLUSION

Salinity-induced oxidative stress is useful for the production of antioxidants. These antioxidants are derived from plants and are more preferred compared to synthetic ones. It is therefore important to conduct screening of plants especially edible plants for antioxidant capacity. In this present study, it can be concluded that 200mM of NaCl was the most effective concentration for polyphenols while 100mM was most effective for flavonols. It can be reported that the 200mM concentration may not necessarily promote the vegetative growth of wild cabbage but is effective for the accumulation of antioxidants. PPV growth medium proved to work best when combined with 200mM salt level and can be used for the antioxidant production of this species. It can be concluded that Wild cabbage has the potential to be used as a source of nutritional antioxidants. Further studies are proposed to investigate the enzymatic activity involved in ensuring the production of antioxidants as salt concentration increases, in order to better understand the mechanisms involved.

4.7 ACKNOWLEDGEMENTS

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CHAPTER 5

RESULTS AND DISCUSSION

5.1 RESULTS AND DISCUSSION

Chapter 2 found that water scarcity and increasing salinization of agricultural lands are threatening food production in the future. Highly saline and coastal lands that are useless in conventional plant cultivation are covering a large surface in South Africa. It was further discovered that the growing of edible halophytic plants for food as substitutes for vegetables would be one of the ways to prevent food insecurity and mitigate the effects of water scarcity. It was found that *Trachyandra ciliata* has the potential of being cultivated under saline conditions for commercial purposes as a vegetable crop in hydroponics.

Chapter 3 focused on the effect of different salinity levels and soilless media on the vegetative growth of *T. ciliata* grown in hydroponics. Salinity significantly ($P \leq 0.05$) affected shoots wet weight, shoot dry weight, root wet weight, shoots wet weight, and both total wet and dry weight. The results showed that low levels of salinity (±100mM) do enhance the production of *T. ciliata* and that high levels of salinity reduce production. The highest mean values for shoots wet weight, root wet weight, and total wet weight were observed at 100mM salinity level, while high salinity levels recorded the lowest mean values. The control produced the highest mean values for shoot dry weight, root dry weight, and total dry weight although the margins were close to those of 100mM salinity treatment. This phenomenon is related to the decrease in water potential and osmotic potential as salinity decreases, leading to reduced photosynthesis and reduced production of carbohydrates. Soilless media had no significant effect on biomass. However, the medium with high water holding capacity (PPV) proved to be less productive compared to others with regards to biomass. This can be related to the fact that it holds excessive salts which may result in salt toxicity and decaying of plants in the root zone.

The number of leaves increased significantly at low salt levels and declined with increasing NaCl concentration as a result of salt toxicity. The most productive soil-less medium was a mixture of perlite: peat: vermiculite at 100mM salt level. Both salinity and soil-less media showed a significant effect on shoot height and interestingly, 0mM and 100mM concentrations showed no significant effect amongst each other in all media mixtures. PPV showed to be the most effective soil-less medium and recorded the highest mean values when in conjunction with the 0mM and low salinity levels (100mM).

The inflorescence is the most important aspect because it is the only part that is edible and can be used as a vegetable. Low salinity (±100mM) has significantly proven to enhance flower development and weight while high salinity prevents flower development. Treatments with high salt levels (20mM and 400mM) were not able to develop any flowers. This phenomenon may be related to the absence of hormones that are responsible for flower development due to salt toxicity. PPV soil mixture significantly proved to be the most

effective growth medium when compared to other soil-less media. Salinity had a significant effect on the accumulation of chlorophyll under different salinity levels although there was no difference between the control and low salt level (100mM). Chlorophyll content then decreased as salinity levels increased. The growth media had no effect on the chlorophyll content.

Chapter 4 focused on the effect of salinity and different soilless media on the antioxidant capacity of *T. ciliata* grown in hydroponics. salinity-induced oxidative stress is significantly ($P \leq 0.05$) effective for the accumulation of polyphenols. Moderate salt concentration (200mM) showed a significantly positive effect on the accumulation of polyphenols while high and 0mM salt concentration produced significantly lower means. DPPH radical-scavenging activity and FRAP were significantly affected ($P \leq 0.05$) by salinity and soilless media. The highest mean values were observed in the moderate salt concentration (200mM) across all soilless media treatments, while the lowest was observed in the highest concentration (400mM). Similar to other parameters, moderate salt concentration recorded significantly higher ($P \leq 0.05$) ABTS mean values compared to other concentrations. The highest mean value was recorded in the interaction of 200mM and PPV soilless mixture. This phenomenon is associated with the fact low to moderate salt concentration does enhance the presence of polyphenolic compounds and antioxidants, while salt toxicity reduces the antioxidant capacity of halophytes. 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.

There was a contradictory observation regarding flavonol content, which was opposite to the trend that was observed in other parameters. The results just like in all other parameters proved that salinity as well as soilless media have a significant effect ($P \leq 0.05$) on the accumulation of flavonols. However, in this case, low salt concentration (100mM) recorded significantly higher mean values in all soil mixtures. High salt levels (200mM+) were proven not to be effective for the accumulation of flavonols. These results are also in agreement with the reports that state that low to moderate salt concentrations positively contributes to the accumulation of antioxidants (flavonols).

CHAPTER 6

CONCLUSION AND RECOMMENDATIO

6.1 CONCLUSION

Highly saline and coastal lands that are useless in conventional plant cultivation are covering a large surface in South Africa. This then provokes the need to explore the potential of Salt tolerant crops to substitute the normal vegetable crops that are prone to a decrease in production. Hydroponics in this study has proven to be the favorite cultivation method in the future because of its efficiency in water usage and it has been reported that it uses 10 times less water than conventional cultivation. It is therefore important to put more focus and resources on hydroponic cultivation protocols of important crops because of the threat that is posed by water scarcity in the future. This phenomenon further calls for more research studies to be conducted on the salt tolerance of halophytic plants that are edible and have potential as possible food or medicinal plants.

There are a lot of studies that have been conducted on the Asphodelaceae family in the past based on the medicinal properties of its members. *T. ciliata*, also belonging to the same family has no records in the literature, but little information state that it is edible and was used by Khoi-San people who lived in the Cape floristic region in the past. This study contributes to the rediscovering of knowledge about this plant and provides its hydroponic growth protocol. This current study reveals that low salinity levels (100 mM) may be used successfully in hydroponics in conjunction with a mixture of perlite as well as peat and vermiculite (1:1:1) for purposes of biomass, compactness, and production of the edible inflorescence. However, further research may still be conducted and smaller NaCl levels should be used such as 0 mM, 50 mM, 100 mM, and 150 mM in order to determine the exact level that is effective for inflorescence production.

This study is in agreement with many other authors regarding the fact that salinity-induced oxidative stress is positively effective for the accumulation of antioxidants. Antioxidants that are derived from plants are more preferred compared to synthetic ones. It is therefore important to conduct screening of plants especially edible plants for antioxidant capacity. In this present study, it can be concluded that 200mM of NaCl is the most effective concentration in the production of antioxidants followed by 100mM for the production of flavonols. It can be reported that the 200 mM concentration may not necessarily promote the yield of wild cabbage but is effective for the accumulation of antioxidants. PPV growth medium proved to work best when combined with 200 mM salt level and can be used for the antioxidant production of this species. It can be concluded that Wild cabbage has the potential to be used as a source of nutritional antioxidants. Further studies are proposed to investigate the enzymatic activity involved in ensuring the production of antioxidants as salt increases, in order to better understand the mechanisms involved.

6.2 RECOMMENDATIONS

Drought, climate change, and soil salinization are major environmental factors threatening crop production in the future. Therefore, the use of drought and salt-tolerant crops is recommended in order to prevent the effect of water scarcity that is threatening the country. The cultivation of wild cabbage on a large scale should be taken into consideration by farmers. It is also recommended there should be a serious consideration of using diluted seawater for the irrigation of salt-tolerant crops to mitigate the effect of these threats. Furthermore, hydroponics and greenhouse production of food and medicinal crops should be seriously considered in order to save freshwater for the eminent future crisis. The introduction of this plant to the market will help increase the variety of vegetable crops and will assist in job creation and the economy of the country. It is recommended that this plant be re-introduced in vegetable gardens in rural areas because it is easy to grow and that will assist in ensuring food security and sustainability of underprivileged areas.

CHAPTER 7
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7.1 REFERENCES

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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.

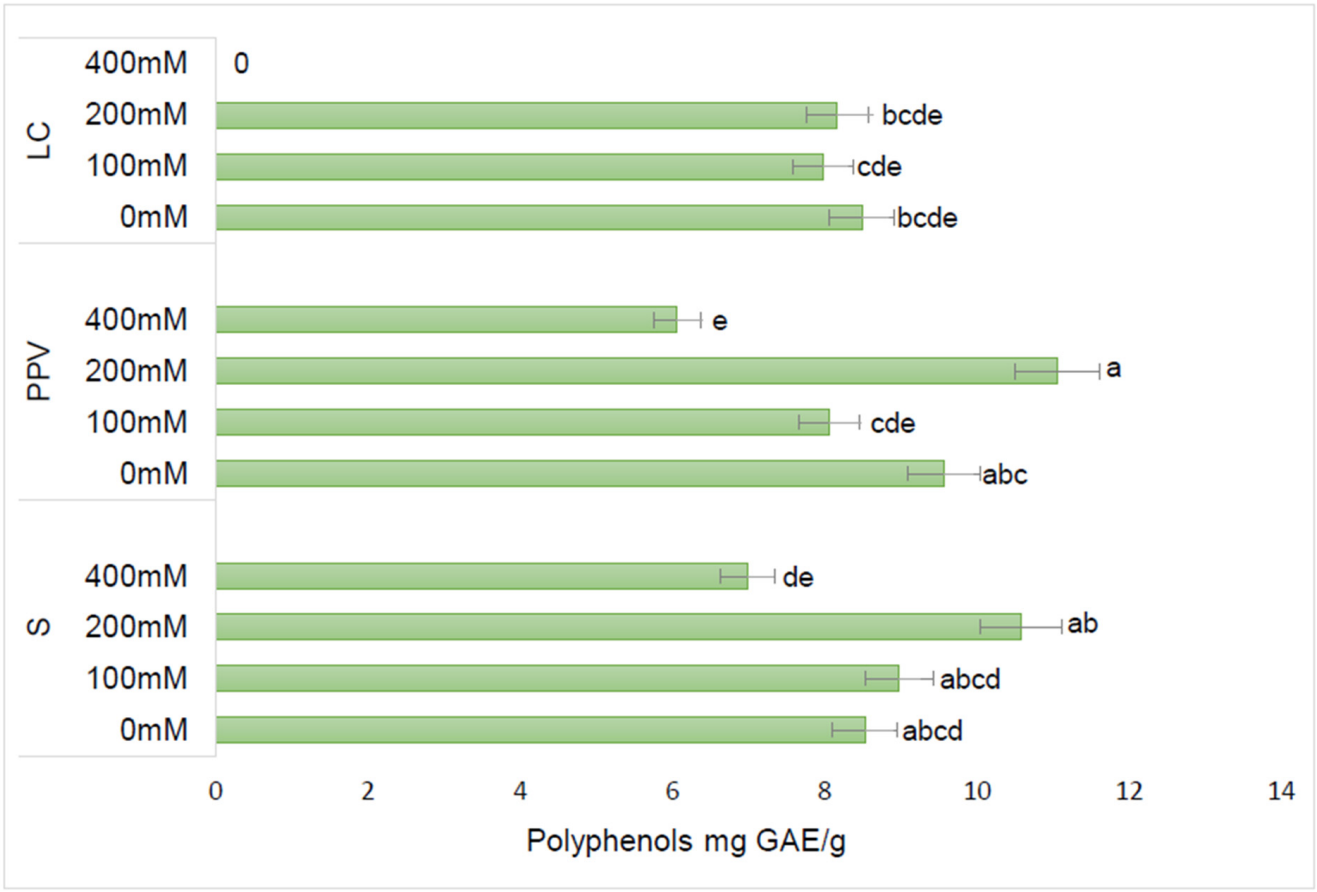

Figure 3. Influence of salinity (NaCl) and growth medium on the polyphenol content of *T. ciliata.* The mean values with different letters are significantly different at $p \leq 0.05$. $n = 10$ replicates.

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).

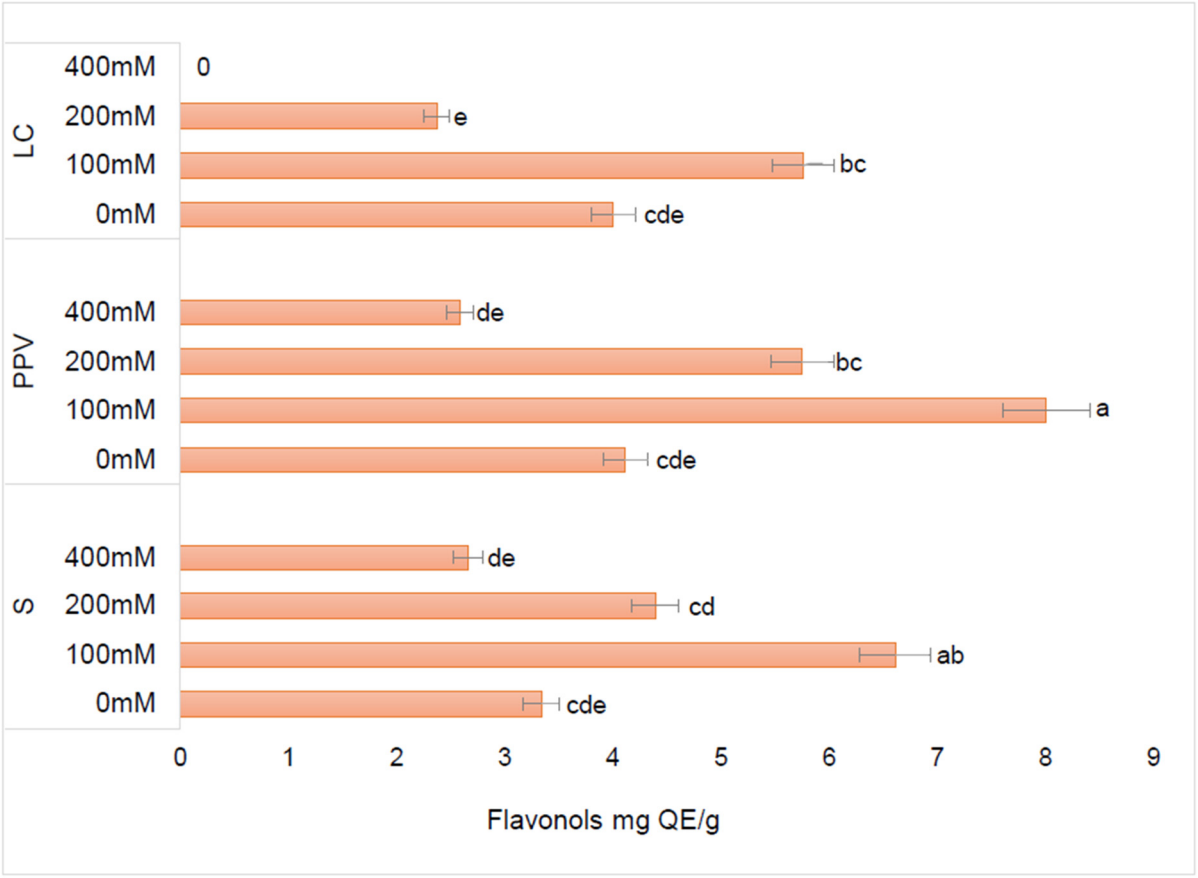

Figure 4. Influence of salinity (NaCl) and soilless media on flavonol content. The mean values with different letters are significantly different at $p \leq 0.05$. $n = 10$ replicates.

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).

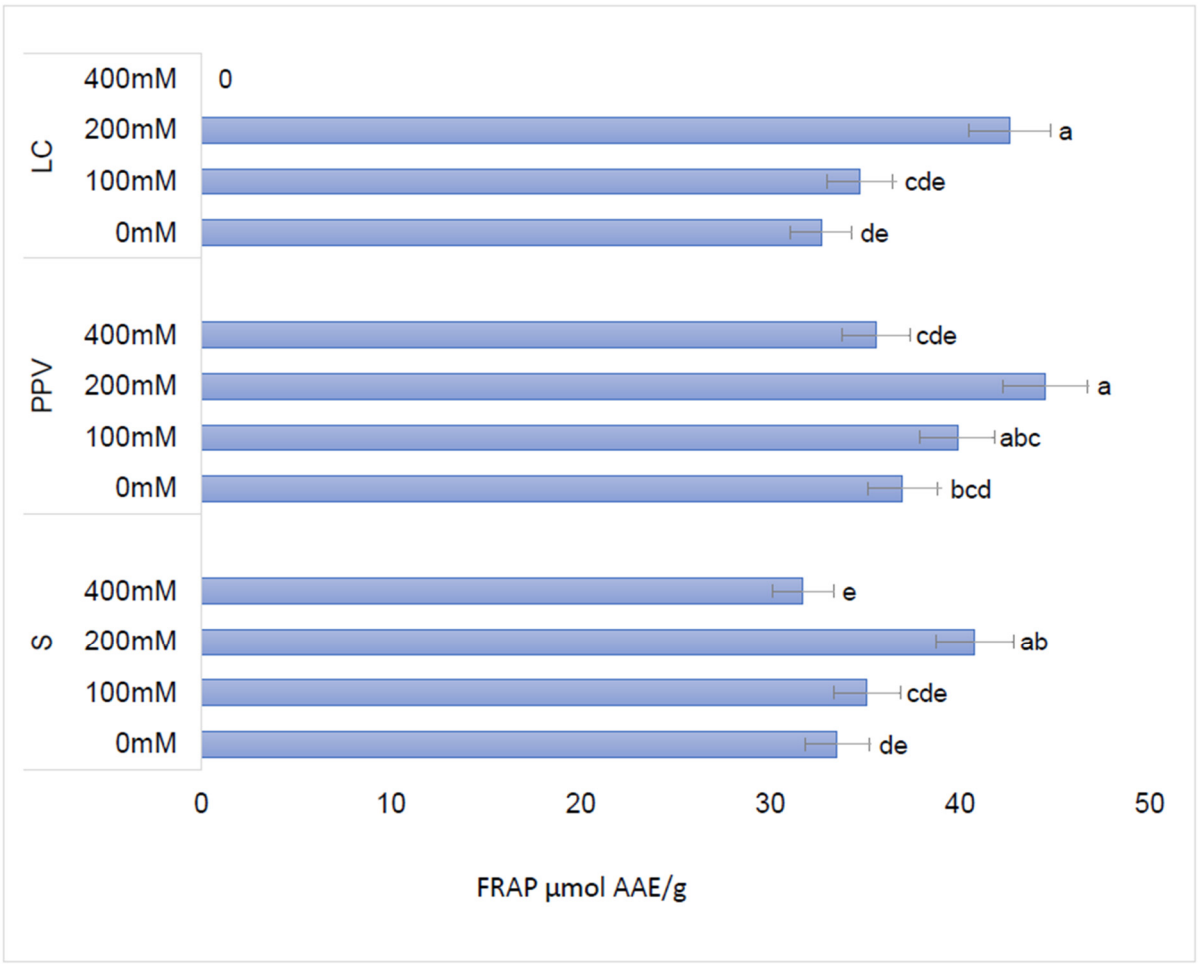

Figure 5. Influence of salinity (NaCl) and soilless media of FRAP. The mean values with different letters are significantly different at $p \leq 0.05$. $n = 10$ replicates.

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.

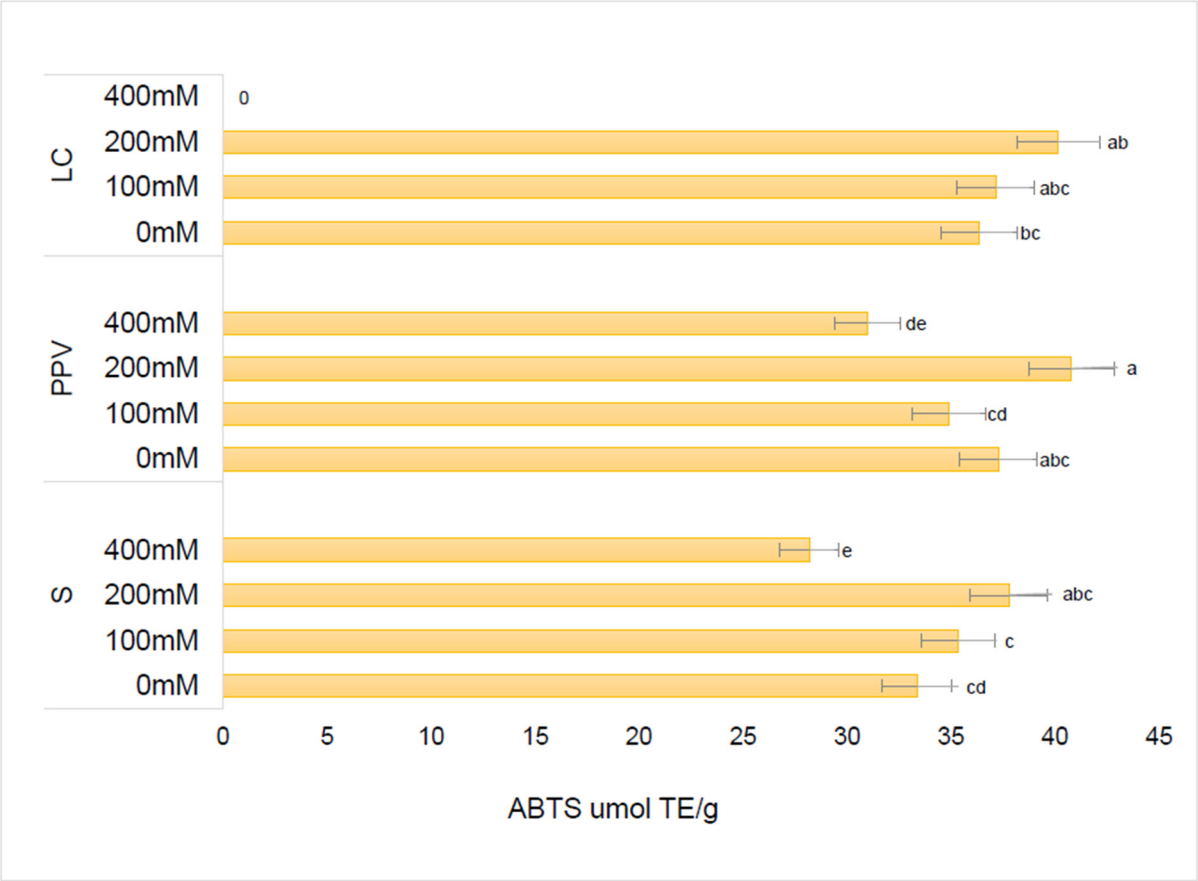

Figure 6. The influence of salinity (NaCl) and soilless media on ABTS. The mean values with different letters are significantly different at $p \leq 0.05$. $n = 10$ replicates.

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).

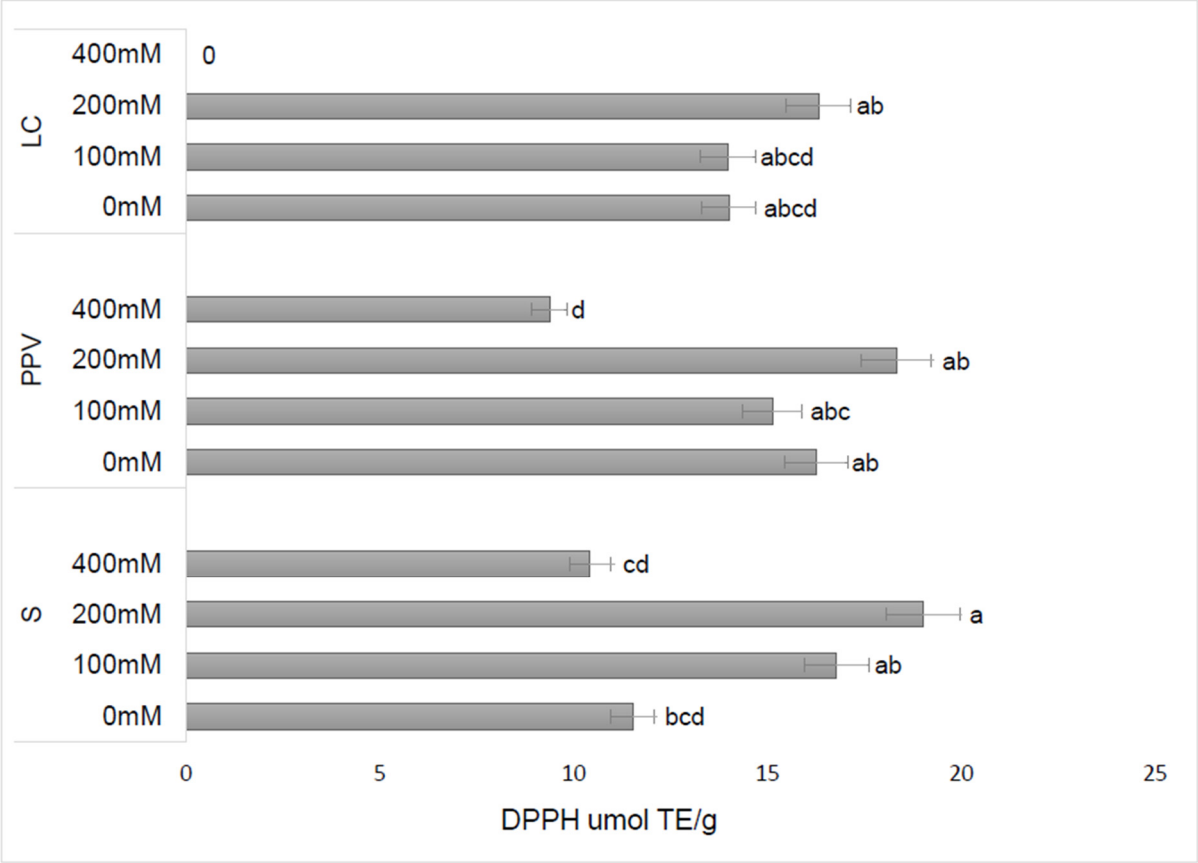

Figure 7. Influence of salinity (NaCl) and soilless media on DPPH. The mean values with different letters are significantly different at $p \leq 0.05$. $n = 10$ replicates.

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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