Growth responses of *Mesembryanthemum crystallinum* to different nitrogen concentrations and selected growing media for hydroponics systems

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DECLARATION

I, Siphamandla Cebani, declare that the contents of this dissertation/thesis represent my independent work and the dissertation/thesis has not previously been submitted for academic examination towards any qualification. Moreover, it signifies my ideas and not necessarily those of the Cape Peninsula University of Technology.

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ABSTRACT

Mesembryanthemum crystallinum L. is a perennial halophyte that originated in southern and eastern Africa and is now widespread throughout the world. Recent studies have shown that the plant can grow in salty soils without losing its nutritional value. However, little has been reported about factors that modulate quantity of secondary metabolites in the plant. In this study, the twin effect of different concentrations of nitrogen (0, 0.36, 0.6, 0.8 g/L) and growing media (LECA clay, peat, vermiculite and silica sand) on the production of plant growth, mineral content, proximate and antioxidant metabolites in hydroponically cultivated *M. crystallinum* was investigated. The untreated plants (0 g/L of N) were used as the control. Findings from this study suggest that a combination of properly formulated nutrient solution and growing media can significantly improve plant development in *M. crystallinum*. Vermiculite-infused nutrient solutions of 0.8 g/L and 0.6 g/L of N fertilizer showed a considerable improvement in plant growth metrics when fed to *M. crystallinum*. It can therefore be concluded from this study that *M. crystallinum* can be grown hydroponically. As previous studies stated that *M. crystallinum* can only grow in coastal areas. In this study, the chlorophyll content of *M. crystallinum* treated with different nitrogen concentrations and grown in hydroponics with various growing media was examined. An instrument provided by Konica-Minolta called the Soil Plant Analysis Development (SPAD-502) meter was used to measure the chlorophyll concentration at the beginning of growing season (week 2) and just before harvesting (week 12). The LECA clay supplemented with 0.6 g/L of nitrogen concentration produced the highest chlorophyll during week two of the experiment which is statistically equivalent to values obtained in plants grown with vermiculite (0.6 g/L), peat (0.8 g/L), LECA clay (0.8 g/L) and peat (0.6 g/L).

The total phenols, flavonol, flavanol of the crude extract was determined while the antioxidant content was determined using the DPPH, FRAP and ABTS antioxidant assays. It was observed that leaves of *M. crystallinum* grown in LECA clay supplemented with N fertilizer (0.36 g/L) had the highest total phenolic content (6.4 mg GAE/g) than leaves from other treatments. The highest mean value (2.9 mg QE/g) of flavonols was found in LECA clay supplemented with 0.36 g/L of N fertilizer, followed by N fertilizer treated peat (2.7mg QE/g). Nutrients and proximate were done using AOAC while minerals were determined using inductively coupled plasma-optical emission spectrometer (AOAC).The highest ADF content was obtained from LECA clay supplemented with 0.8 g/L nitrogen concentration, highest ash content from vermiculite with no nitrogen concentration, highest fat content was obtained from silica sand with no supplementation, the highest moisture content was obtained from silica sand with 0.8 g/L nitrogen supplementation, whereas the highest NDF was contained from LECA clay with 0.36 g/L concentration. The findings of this study revealed that although, nutrients and proximate composition of *M. crystallinum* is intensely influenced by different growing media and various nitrogen concentration. Also, this study showed that the increased of nitrogen in the growing media cannot be useful in production of proximate composition of *M. crystallinum*. This study suggests that *M. crystallinum* can be grown in any type of growing media and can be irrigated with municipal water and require nitrogen fertilizer for optimum growth.

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DEDICATION

This dissertation is devoted to my late kids Liwa and Lomso Cebani, I appreciate your spiritual guidance and I will always love you.

STRUCTURE OF THE THESIS

The thesis is drafted differently to 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% masters studies which complies to 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 prior to 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 submitted for publication, 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 a potential cash crop for disturbed and saline lands in southern African, the common ice plant. It also highlights its use, distribution, the environmental effect on growth as well as potential cultivation methods, which could be adopted for commercial use.

Chapter Three: This chapter evaluated the vegetative growth of *Mesembryanthemum crystallinum* L*. i*n response to different nitrogen concentration and different soilless growing media in hydroponics systems. The research justification, materials and methods, results and discussions are presented.

Chapter Four: This chapter measured the chlorophyll content of *Mesembryanthemum crystallinum* L treated with different dosages of Nitrogen fertilizer and growth media under hydroponic systems. The research justification, materials and methods, results and discussions are presented.

Chapter Five: This chapter evaluated the impact of nitrogen concentrations and growing media on secondary metabolites and antioxidants content of *Mesembryanthemum crystallinum* L. grown in hydroponic systems. The research justification, materials and methods, results and discussions are presented.

Chapter Six: This chapter evaluated the effect of nutrients and their accumulation in *Mesembryanthemum crystallinum* L. grown in hydroponic systems with varying nitrogen concentrations and growth media. The research justification, materials and methods, results and discussions are presented.

Chapter Seven: General discussion, conclusions and recommendations.
This chapter deals with the general discussion, which connects the previous chapters and is followed by the conclusions of the study. Recommendations are made for further work; to introduce future research topics.

Chapter Eight: All references used.

Table of Contents

List of tables

List of figures

CHAPTER ONE

Research problem, aims, hypothesis, and objectives

1.1 Research problem

Food security has developed into a major problem as 800 million people are hungry, while many more millions are at further risk despite the progress that has been made in some parts of the world to reduce hunger (FAO, 2020). As Krumah (2018) points out, global climate change, including rising temperatures, water scarcity, and extreme weather changes, will continue to pose challenges in underdeveloped countries and already stressed agricultural ecosystems due to a continued population increase. Salt stress is a major physiological stress that affects plant production systems, it is estimated that 1.8 million ha of arable land in South Africa are affected by saline conditions (Yildiz *et al.,* 2020). Thus, it has become imperative to consider cultivation methods using hydroponics and salt-tolerant vegetables to increase production and support food security in communities. One of the immediate solutions working towards saving available water could be to investigate the cultivation of halophytes that have the potential to be directly irrigated with seawater (Garza-Torres *et al.,* 2020). *Mesembryanthemum crystallinum* is a halophyte that can be cultivated in high saline soils with high levels of sodium equivalent to those found in seawater. Loconsole *et al.,* (2019) documented that common ice can be used as food, a source of soap, and medicine. It is also harvested for both local and commercial purposes. In South Africa, wild edible plants are not freely used as edibles and there is little information on many of the species, growing these plants could provide local communities access to healthy nutritional diets. Additionally, cultivating new crops could also provide financial growth opportunities to disadvantaged communities through job creation. This study aims to determine the effects of different nitrogen concentrations and selected growth media on the growth of *M. crystallinum* to establish a growth protocol that will be optimal in hydroponics systems.

1.2 Aim

The study aims to investigate the effects of nitrogen and substrates on the vegetative growth, nutrient uptake, and antioxidant potential of *M. crystallinum*. To formulate a viable growth protocol for both home gardeners and potential commercial farmers.

1.3 Hypotheses

It is hypothesized that different nitrogen concentrations will increase nutrient uptake and plant growth of *M. crystallinum* and have a positive effect on the accumulation of metabolites. Additionally, selected growth substrates could positively influence root length and vegetative growth of *M. crystallinum*.

1.4 Objectives

1.4.1 Main objective

The purpose of this investigation is to determine the vegetative growth of *M. crystallinum* in response to various nitrogen concentrations and growth substrates to establish a suitable growth protocol.

1.4.2 Specific objectives

- To evaluate the rooting percentage, root length, number of roots, number of leaves, and plant height of *M. crystallinum* in response to different growth substrates.
- To evaluate the nutrient uptake by *M. crystallinum* in response to varying nitrogen concentrations.
- To evaluate the chlorophyll content in *M. crystallinum* leaves in response to varying nitrogen concentrations.
- To evaluate the fresh and dry weight of *M. crystallinum* in response to varying nitrogen concentrations.
- To evaluate the antioxidant potential of *M. crystallinum* in response to varying nitrogen concentrations.
- To evaluate optimal responses of *M. crystallinum* gathered under the specific objectives 1-5 for a suitable growth protocol.

CHAPTER TWO

A POTENTIAL CASH CROP FOR DISTURBED AND SALINE LANDS IN SOUTHERN AFRICAN, THE COMMON ICE PLANT

A potential cash crop for disturbed and saline lands in South Africa, the common ice plant

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2.1 Abstract

Climate change, increasing land scarcity and increasing freshwater scarcity are adversely affecting crop production in many countries, including South Africa. Current attempts to adapt to these conditions include the use of coastal plant species with potential economic value. Wild edible halophytes, commonly known as ice plants, may have uses as leafy greens. It is well adapted to saline dunes characterized by low soil fertility and minimal cultivation needs. However, it is not yet fully utilized as a leafy vegetable. In this review, we explored the potential of ice plant as a leafy vegetable and examined its uses, morphology, distribution, propagation, and possible cultivation methods. Furthermore, this review is expected to aid in further research and dissemination of this underutilized edible halophyte.

2.2 Introduction

Besides several mitigations taken to facilitate the world hunger problems, food security and nutrition persist as serious problems in many countries especially in developing countries (Pawlak & Kotodziejczak, 2020). As the population growth still strikes, the agricultural industry is expected to meet food demand. Studies anticipate strong growth of the population in the next 30 years (Frona *et al.,* 2019). As the population is expected to increase Giller *et al.,* (2021) stated that agriculture is required to increase food production by 70% to meet the food demand. Liliane and Charles (2020) also outlined that every 1% increase in agricultural yield translates into a 0.6-1.2% decrease in the number of absolute poor households.

As agriculture is expected to increase production, there are limiting factors such as drought, soil salinity, and climate change that pose a challenge to the industry (Ullah *et al.,* 2021). As the demand for water use is likely to escalate is expected to exceed the existing water supply capacity, a priority for horticultural researchers is to rework the improvement of water conservation methods by carefully determining and implementing water usage systems requiring less water, as 70% of water are continued to be used for irrigation agricultural industries (Worldbank, 2020). Botai *et al.* (2018) stated that compared to a mean global rainfall of 870 mm per annum, South Africa only receives 450 mm of rain per annum. Hence, its classification is the 30^{th} driest country in the world. This is not surprising as it is estimated that South Africa already exploits about 98% of its available water supply resources (Donnenfeld *et al.,* 2020). Hussaun *et al.* (2019) reported that salt stress is a major physiological stress that affects plant production systems as it is estimated that 1.8 million ha of arable land in South Africa are affected by saline conditions. Thus, it has become imperative to consider cultivation methods using salt-tolerant plants to increase production and support food security in communities. The demand for drought and salt tolerant plants to replace and build capacity to sustain agricultural production to fight the expected food scarcity in the future is exacerbated by food security concerns (Molotoks *et al.,* 2020).

Rodríguez-Hernández and Garmendia (2021) documented that *M. crystallinum* is a halophyte that can be cultivated in high saline soils with high levels of sodium equivalent to those found in seawater. Loconsole *et al.* (2019) reported, the plant is harvested for both local and commercial uses, (i.e., as food, medicine, and a source of soap). The species is also useful in soil desalinization projects and can be used as an ornamental plant grown in flower gardens (Ncaphayi, 2019). In South Africa, wild edible plants are not freely used as edible and there is also little information on many of the species. Notwithstanding the species not being used as food, Green *et al.,* (2018) reported that growing (cultivating) these plants could provide local communities access to healthy nutritional diets. Additionally, cultivating new crops could also provide financial growth opportunities to disadvantaged communities through job creation. Hydroponic cultivation is a common approach for ensuring optimum agricultural productivity while lowering irrigation demand.

As a result, it's critical to thoroughly research and investigate the potential of hydroponic techniques for growing salt-tolerant food crops. This study aims to determine the effects of different nitrogen concentrations and selected growth media on the growth of *M. crystallinum* to establish a growth protocol that will be optimal in hydroponics systems. Thus, the outcomes of this study will contribute to the existing information in terms of cultivation methodology for this species by stipulating necessary documentation and basis to further and/or future cultivation and research studies for this species.

2.3 Classification of *Aizoaceae*

Aizoaceae is one of the most important widespread succulent species family. The family consists of about 143 genera with approximately 2300 species (El Hawary *et al.,* 2020). *Aizoaceae* includes five groups, *Aizoon* group, *Mesembryanthemum* group, *Tetragonia* group, *Sesuvium* group, and *Mollogo* group primarily found in arid, coastal, and disturbed areas and they can grow in tropical and subtropical regions (Akinyede *et al.,* 2020; El-Rauof HSA, 2020). Two subfamilies, namely *Aizoodeae* and *Sesuvioideae,* are slightly succulent shrubs that are distributed worldwide (Kong et al., 2020). There are four subfamilies, two of which are slightly succulent shrubs that are distributed worldwide (*Aizoodeae* and *Sesuviodeae*) while the remaining two subfamilies, namely *Mesembryanthemoideae* and *Ruschioideae*, are commonly referred to as "mesembs" meaning succulent members of *Aizoaceae* (Kong *et al.,* 2020). According to Lombardi *et al.,* (2022*) Aizoaceae* are distinctive facultative halophytic because they are herbs, and rarely shrubs or trees, and they can either grow, perennially or annually. Members of the family could be a future food production sphere because challenges, such as climate change, global warming, soil salinity, and high population numbers, affect the current agricultural production are spanning out of control (El-Rauof HSA, 2021). Recently, many studies, on the cultivation of coastal wild plants were conducted to find a suitable cultivation methodology to address the issue of food security (WWF, 2021).

2.4 Mesembryanthemum crystallinum: the common ice plant

Mesembryanthemum crystallinum is an eminent facultative halophyte that can swing its photosynthetic carbon fixation passageway from C3 to CAM under a saline environment and other abiotic stress factors (Nogués *et al.,* 2020).

It is an annual, biennial or perennial prostrate plant that most commonly completes its cycle within a few months, depending on environmental conditions **(**He *et al.,* 2017). According to Nosek *et al.,* (2021), *M. crystallinum* is generally well-known as a common ice plant or carpet weed, but it is also known as vyies in South Africa. *Mesembryanthemum crystallinum* is a halophyte that resembles stones hence it is also known as mesembs (Amari *et al.,* 2020). It has succulent leaves and stems, growing to about 10 cm tall with stems 50 cm or more long trailing along the ground (Zhang *et al.,* 2021). The plant can be found on a wide range of soil types, from well-drained sandy soils including sand dunes to loamy and clay soils, hence, it can tolerate nutritionally poor or saline soils (Nogués *et al.,* 2020).

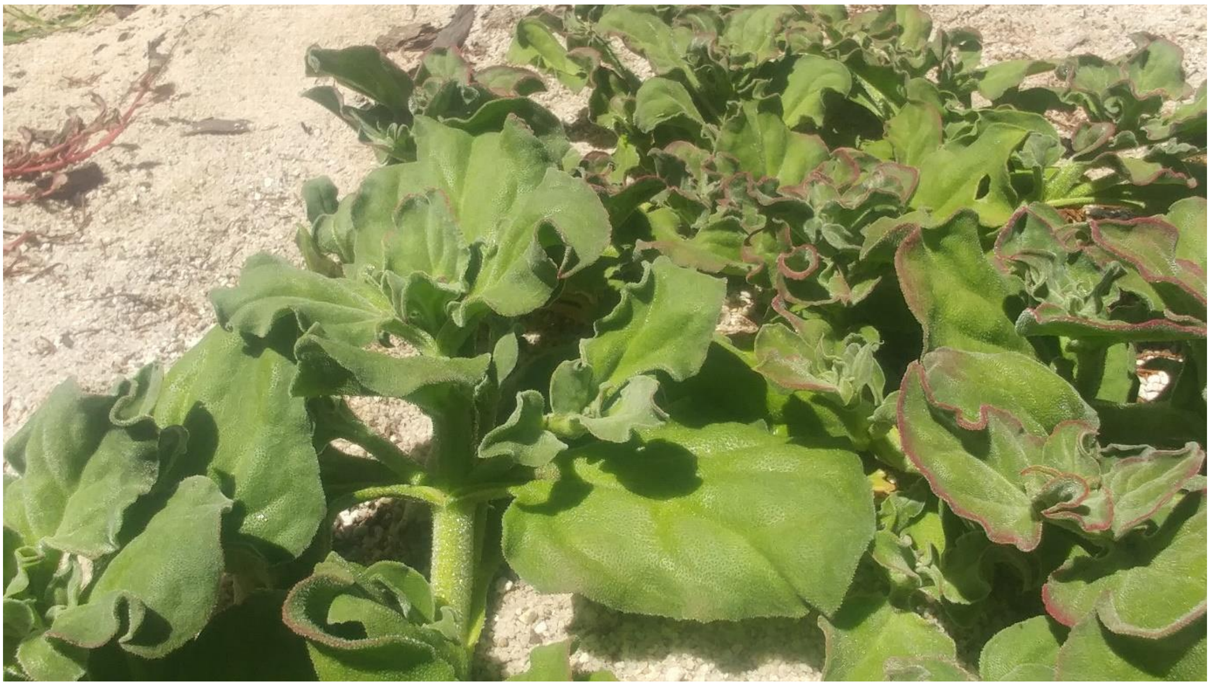

Figure 2.1 *Mesembryanthemum crystallinum* when grown in an open garden (Picture: Charles Laubscher).

2.4.1 Distribution and habitat

Mesembryanthemum crystallinum is widely distributed in dry areas, as well as along coastal areas where the soil is highly salty, due to spray from the sea. The plant can be cultivated in a wide range of habitats in Southern, North Africa and South Europe **(**He *et al.,* 2022).

Earlier Ncaphayi (2019) reported that in South Africa in particular, *M. crystallinum*, can be found growing in three provinces namely, the Eastern Cape, the Western Cape, and the Northern Cape, specifically on cliffs, dunes, roadsides, and even in disturbed habitats. Bueno and Cordovilla (2019) highlighted that as much as this plant withstands different habitats, it can also be extremely sensitive to frost.

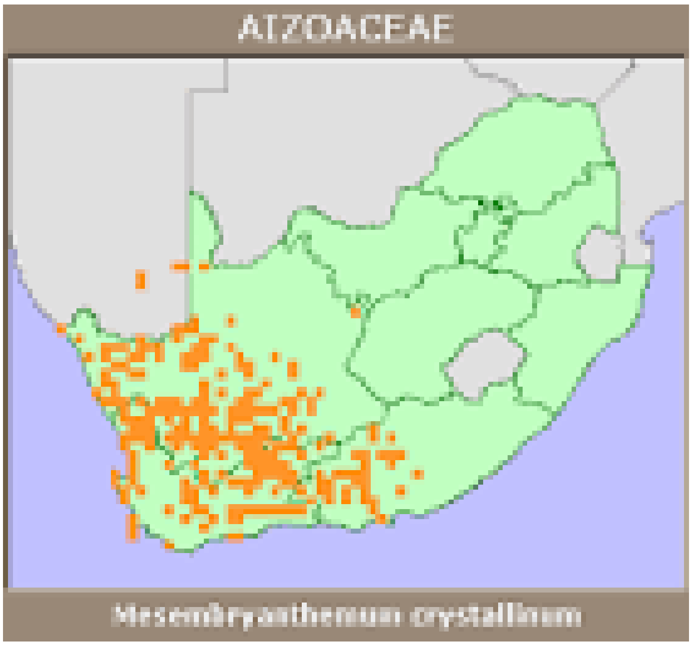

Figure 2.2 Geographical distribution map of Mesembryanthemum. crystallinum (adapted from **http://redlist.sanbi.org/species.php?species=148-18)**

2.4.2 Growing conditions of *M. crystallinum*

Mesembryanthemum crystallinum is best cultivated in full sun positions or conditions with fast fast-draining soil; sandy or gravelly soils (Tembo-Phiri, 2019). It thrives well under cool temperatures and prefers to be planted in autumn and hot climates (Loconsole, 2019). The young plants need water, while older ones require little or no water at all, as the roots restrict the absorption of water once the plant has accumulated enough water (Amin *et al.,* 2019).

During the heat summer, the plant requires irrigation after every two weeks to avoid more frequent watering (He and Qin, 2021). When planted in the field space plants should be spaced 15 to 18 cm apart and small amount of compost or slow release of fertilizer is recommended during cultivation (Zhang, 2018).

The species grows natively in severely salty soils along the south-western Cape coast, making it one of the contenders for introduction into the food market as a salt-tolerant food crop, especially as drought and salinity of agricultural lands continue to rise. As a result, studies on salt tolerance and nutrition, as well as research on this plant, should be carried out to introduce *M. crystallinum* as a salt-tolerant food crop. Coastal lands represent vast swaths of land that are now underutilized for traditional agriculture but might be utilised for halophytic food crops. Under saline conditions, halophytes are highly productive, which could be a solution to the problem of freshwater depletion, and their employment could ensure the productivity of saline soils (Ahmadi *et al.,* 2022).

2.5 Economic values of *M. crystallinum*

Mesembryanthemum crystallinum has a unique salty taste, with colourful deep green color and slightly coral-like shape leaves with a crisp taste (He *et al.,* 2021). Since *M. crystallinum* can directly tolerate saline soils and be irrigated with seawater, it can drastically reduce the chances of diseases affection (Loconsole *et al.,* 2019). Therefore, *M. crystallinum* can be grown without the use of pesticides and fertilizers (Śliwa-Cebula *et al.,* 2020). In addition, it can meet the requirements of organic vegetable production since it is domesticated from wild salt-tolerant plants without gene modification. The whole process is eco-safe and organic (Alamsyah & Othman, 2021). *M. crystallinum* is known edible vegetable, greater attention and research should be paid to the nutrient content of plants in this genus. Similarly, the water crisis that is threatening agricultural production must be addressed and investigated to find strategies that will boost production without increasing demand for freshwater (Dinar *et al.,* 2019).

Figure 2.3 Mesembryanthemum crystallinum salad dish (accessed at https://www.istockphoto.com/photo/ice-plant-salad)

2.5.1 Ethanobotanical uses of *M. crystallinum*

The leaves and fruits of *Mesembryanthemum crystallinum* are edible (Weeplian *et al.,* 2018). The leaves can be crushed and used as a soap substitute and have some medicinal uses (Kataoka *et al.,* 2021). The leaves can be boiled and can be eaten like spinach, although the only difference is that *M. crystallinum* has an acidic flavor with a salty taste (He *et al.,* 2017). According to Rusch, (2019) the leaves can be pickled or used as a garnish in dishes. *M. crystallinum* has been used as a model in many plants' physiology studies (Guan et al., 2020). It is being grown for its glistening leaves and striking flowers in gardens as a ground cover (Čermáková, 2019). Additionally, given its salt accumulation capability, may be useful in bioremediation (Śliwa-Cebula *et al.,* 2020).

2.5.2 Therapeutic potentials of *M. crystallinum*

Mesembryanthemum crystallinum is used around the world, to treat various health conditions, such as colds, fever, and even glaucoma (He *et al.,* 2017). Recently, research publications have shown that the ice plant consists of extremely high levels of isoflavones as well as flavonoids and isoflavoniods, and plant metabolites which have a fundamental effect on human metabolisms (Akinyede, *et al.,* 2020). Leconsole *et al.* (2019) had reported that the liquid juice extracted from *M. crystallinum* leaves can treat several diseases such as tuberculosis, obesity, diarrhoea, and infantile eczema states.

Since the common ice plant contains compounds that are very good at putting an end to inflammation, it is also used for promoting a healthier cardiovascular system. (He & Qin, 2017). In folklore medicine, the plant is used to treat minor skin problems while the sap of the herb may also be added to bathwater which is suggested for soothing large areas of the body that are irritated (Akinyede *et al.,* 2020). In addition, *M. crystallinum* can be used to relieve itching, swelling, and redness of the skin (Bernard, 2021). The plant leaves provide a safe trailside nibble that are sometimes used as food seasoning when pickled and as demulcent when applied to the skin and mucous membranes and can serve as cooking and healing poultice for treating sunburn or minor lesions (Leconsole *et al.,* 2019).

2.5.3 Nutrient content

FAO, (2021) outlined that understanding the nutrient content of various plant organs is essential to evaluating the role of nutrient elements in the physical process of the ecosystem. Nitrogen is essential in plants for the chemical process of proteins; hence nitrogen is important for the chemical processes of enzymes that control organic processes such as growth, photosynthesis, and respiration (Ghiasy-Oskoee *et al.,* 2020). The annual species of *M. crystallinum* concentrates excess Na, K, and Ca in the vegetative organs at the standing dead stage (He & Qin, 2017). Burana (2018), argue that *M. crystallinum* requires little or no nutrients at all. Research studies on salt tolerance nutrition should be carried out to introduce *M. crystallinum* as a salt-tolerant food crop with standard and analysed nutrient content.

2.6 Hydroculture

Hydroculture entails the growing of plants in a liquid form nutrient solution with or without the use of artificial media (Rubio-Asensio *et al.,* 2019). Commonly used media include expanded clay, coir, perlite, vermiculite, brick shards, polystyrene packing peanuts, and wood fiber (Alshrouf, 2017). Different techniques of soilless agriculture have been used and recorded over several civilizations with little information documented (Awad, 2017). However, the earliest published work on growing terrestrial plants without soil was published in 1627 book titled, *Sylva Sylvarum* by Sir Francis Bacon, the father of the scientific method, which he nominated "Water culture" (Butler, 2006).

Hydroculture was adopted as one of the solutions to food production problems such as water scarcity, soil salinity, and high population (FAO, 2015) and can be used in underdeveloped countries with limited space for food production purposes (Lagomarsino, 2019). The system of hydroculture makes it feasible to grow plants and vegetables in poor soil conditions such as deserts and that seawater can be used to mix nutrient solutions for hydroculture growing (Taghizadeh, 2021). The popularity of hydroculture has increased dramatically as water and salinity have become major issues affecting plant (Sharma *et al.,* 2018). Compared to traditional soil-grown crop production, hydroculture has the following advantages: up to 90 percent more water is used efficiently, production increase 3 to 10 times in the same amount of space, and many crops can be produced twice as fast in well-managed hydroculture system; additionally, soil-borne pests and diseases do not affect hydroculture plants, and plant growth is more vigorous (Gashgari *et al.,* 2018; Baiyin *et al.,* 2021).

It has been predicted that the world population will grow extensively in the coming years with at least 70% of the population residing in cities (Cheeseman, 2018). In a related development, the Worldwatch Institute reported that there is a need for the installation of hydroponics for rooftop farming in cities to meet the food supply and other demands. Being able to produce food within cities for urban populations eliminates the carbon footprint generated through the transport of food from rural areas to cities (Walters & Midden, 2018). Crops grown in hydroculture have a better possibility of achieving higher growth rates, higher quality, purity, and consistency (Zhao *et al.,* 2021). Techniques that have the potential to boost agricultural production while also ensuring food security must be thoroughly investigated to reduce the possibility of future food scarcity. South Africa is expected to suffer physical water scarcity by 2025 because of high population growths and declining freshwater reserves. The Western Cape is the province in South Africa that is most affected by water scarcity, and it is expected that this region will be unable to meet its agricultural needs soon (Botai *et al.,* 2018). Therefore, introduction of hydroculture growth protocol for halophyte plants such as *M. crystallinum* would be beneficial for the province, also saline lands dominate the province of the Western Cape.

Figure 2.4 Mesembryanthemum crystallinum growing in hydroponic system in greenhouse (Cebani, 2022).

Figure 2.5 Mesembryanthemum crystallinum growing on selected growing media in hydroponic system in greenhouse (Cebani, 2022).

2.6.1 Importance of nutrients

The challenge of food security must be met in agricultural production by increasing or improving production methods. The major contributors to the improvement of crop production are mineral nutrients (Schütz *et al.,* 2018).

Improving the nutritional status of plants by applying fertilizers has been a crucial step in increasing food production since the beginning of the “Green Revolution” (Reetsch *et al.,* 2020).

In the past, the increase in crop production was correspondingly associated with the increase in fertilizer consumption (Havlin & Heiniger, 2020), resulting in about 6.9-fold increase in N fertilization (Sun *et al.,* 2020). Fertilizer use for crop improvement has increased from 144 million tons in 1990 to 208 million tons in 2020 and is expected to rise to 300 million tons by 2030 to meet crop production demands (Cao *et al.,* 2018; Thomas, 2022). Thus, Nitrogen is essential for the synthesis of all proteins in plants as they are as well needed for the metabolic processes of synthesis of enzymes which control all the essential processes in any living organism such as photosynthesis, respiration, and growth (Rahimi *et al.,* 2021). However, the nitrogen deficiency can lead the plant to lose its original colour, this happens when a plant starts changing from pale green to a yellow color (Chaddy *et al.,* 2021).

2.6.2 Different growing media

Soil-based crop production is becoming a matter of the past because it is often associated with soil pests, salinity problems, and excessive application of pesticides (Altaf *et al.,* 2021). Due to pests and diseases associated with those plants, soil-based crops can be a danger to human health and lead to environmental pollution. As a result, many techniques have been put in place to overcome such problems with limited impact on the environment and health of human beings. Tzortzakis *et al.* (2020) identified soilless culture as one of the solutions for soil problems, given the benefits that it can offer, namely good results on the environment and its capacity in using water and fertilizer efficiency. Therefore, the use of desirable substrates is important to produce horticultural plants, as they directly and significantly affect the development and later maintenance of the plant. A suitable growing media provides sufficient support to the plant, serves as a reservoir for nutrients and water, and allows gaseous exchange and oxygen diffusion to the roots (Mahmoud *et al.,* 2019).

2.7 Conclusion

Halophytic crops such as *Mesembryathemum crystallinum* have been proposed as a potential solution for food production sustainability, it is critical to begin creating hydroponic growth methods for these plants to better understand their nutrient uptake, water requirement and growth protocol. Highly salty and coastal soils, which abound along the coast, are thought to be unsuitable for conventional agricultural development because of limited research and indigenous knowledge about edible coastal plants.

This land's potential can be studied to see if salt-tolerant crops can be used as an alternative for traditional vegetable crops.

This needs the development of more efficient technical advancements, such as hydroponics, to address the looming water shortage, global warming and high salt salinity. As a result of this perceptive, more research investigations on edible salt tolerant halophytic plants are needed. Due to their ability to resist high saline, dry environments and have potential to be irrigated with sea water, halophytic plants like *M. crystallinum* have the potential to become a significant vegetable crop during the expected dry seasons. The conclusions of this review are recommended as points of reference for government, researchers, food activists, entrepreneur, and policymakers based on scant information from literature that the plant was consumed by the Khoi-san people who lived along the western Cape coast before colonialization. The review also advises that more research be done on *M*. *crystallinum* hydroponic propagation and the development of a hydroponic growing technique to help address the issue of water shortage, food insecurity and youth unemployment as well advancement on this plant's breeding to generate types with excellent yields and nutrients.

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CHAPTER THREE

VEGETATIVE GROWTH OF *MESEMBRYANTHEMUM CRYSTALLINUM* IN RESPONSE TO DIFFERENT NITROGEN CONCENTRATION AND DIFFERENT SOILLESS GROWING MEDIA IN HYDROPONICS SYSTEMS

Vegetative growth of *Mesembryanthemum crystallinum* in response to different nitrogen concentration and different soilless growing media in hydroponics systems

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3.1. Abstract

Vermiculite-infused nutrient solutions of 40g and 30g of N fertilizer showed a considerable improvement in plant growth metrics when fed to *M. crystallinum*. This might be an option for potential growers of the plant in provinces suffering the negative effects of the drought. It can therefore be concluded from this study that *M. crystallinum* can be grown hydroponically. *Mesembryanthemum crystallinum* (common ice plant) is a facultative crassulacean acid metabolis (CAM) halophyte species that has adapted to extreme conditions thereby having the ability to recover from stress. The plant is salt-tolerant, edible and thrives in high-salt environments, such as seawater-irrigated soils, as well as extreme dry circumstances. Because the ice plant is an edible halophyte, it may be farmed commercially and used as a viable choice for saline soil phytoremediation. As a result, agricultural production can be maintained if soil remediation is applied A growing population, unsustainable resource use, food loss and waste, increased demand, the effects of climate change, a decline in biodiversity in places with arable land, increased hunger, and malnutrition are just a few of the unsettling concerns facing the agricultural industries all over the world. These issues jeopardize the capacity of the globe to produce enough food to meet demand both today and in the future. Findings from this study suggest that a combination of properly formulated nutrient solution and growing media can significantly improve plant development in *M. crystallinum*.

Therefore, it is crucial to introduce new crops, such as *M. crystallinum*, that can endure difficult conditions and boost the local economy by creating jobs while also giving rural residents access to nutritious and wholesome food.

3.2. Introduction

Mesembryanthemum crystallinum (common ice plant) is a facultative crassulacean acid metabolis (CAM) halophyte species that has adapted to extreme conditions thereby having the ability to recover from stress (Nosek *et al.,* 2021; Zhang *et al.,* 2022). The plant is salt-tolerant, edible and thrives in high-salt environments, such as seawater-irrigated soils, as well as extreme dry circumstances (Loconsole *et al.,* 2019). Because the ice plant is an edible halophyte, it may be farmed commercially and used as a viable choice for saline soil phytoremediation. As a result, agricultural production can be maintained if soil remediation is applied (Kotaoka *et al.,* 2021).

Cell division, elongation, and differentiation begin in a cluster of cells termed meristems, and result in plant development (Faber *et al.,* 2020). Both the shoot and root apical meristems are formed during embryogenesis and give rise to the shoot and root systems, respectively (Fuchs *et al.,* 2020). Plants may develop endlessly with specialized structures of the meristem, adding new organs provided they have access to materials they require such as fertilizer, water, sunlight and other conditions required for vegetative development (Hanlon *et al.,* 2019). The seeds of *M. crystallinum* germinate during the cooler months of the year and mature until late summer; when they dry out and die (Loconsole *et al.,* 2019). Some plants may be able to live another year if the circumstances are conducive. *Mesembryanthemum crystallinum* has four unique growth stages, namely germination, juvenile, adult, and flowering seed-set (Ncaphayi 2019). The juvenile stage lasts around six weeks, while th adult growth period might span several months. Flowering is accelerated by environmental stress, particularly salt stress. Six weeks later, seed set commences, followed by root, shoot, and leaf breakdown. Plants migrate from C3 to CAM in the adult phase (Ncaphayi 2019; Yu *et al.,* 2017).

Despite efforts achieved in some regions of the world to alleviate hunger, food security has become a huge issue, with 800 million people going hungry and many more at risk (FAO, 2020). Continuous population growth, global climate change, including rising temperature, water scarcity, extremely weather variations, will continue to gravitate food insufficiency in developing countries and already stressed agricultural ecosystems (Krumah, 2018), although underdeveloped countries are believed to be affected by high population growth and accompanying risks such as land scarcity and soil salinity. Salt stress is a key physiological stress that impacts plant production systems and it was projected to affect 1.8 million acres of arable land I South Africa (Yildiz *et al.,* 2020). Therefore, it has become to think about using hydroponics and salt-tolerant vegetables to boost production and enhance community food security.

A growing population, unsustainable resource use, food loss and waste, increased demand, the effects of climate change, a decline in biodiversity in places with arable land, increased hunger, and malnutrition are just a few of the unsettling concerns facing the agricultural industries all over the world (Muluneh, 2021; Mbuli *et al.,* 2021). These issues jeopardize the capacity of the globe to produce enough food to meet demand both today and in the future (Calicioglu, 2019). Therefore, it is crucial to introduce new crops, such as *M. crystallinum*, that can endure difficult conditions and boost the local economy by creating jobs while also giving rural residents access to nutritious and wholesome food. Understanding the nutritional content of diverse plant organs is critical for analysing the role of nutrient components in ecosystem physical processes, according to FAO (2021). Because nitrogen is required for the chemical processes of proteins in plants, it is also required for the chemical processes of enzymes that control organic processes like growth, photosynthesis, and respiration (Ghiasy-Oskoee *et al.,* 2020). At the standing dead stage, the annual species *M. crystallinum* accumulates excess Na, K, and Ca in the vegetative organs (He & Qin, 2017). *M. crystallinum*, according to Burana (2018), requires very little or no nutrition. To establish M. crystallinum as a salt-tolerant food crop with standard and assessed nutrient content, research studies on salt tolerance nutrition should be conducted.

Plants are grown in a liquid nutrient solution with or without the use of artificial media in hydroculture (Rubio-Asensio *et al.,* 2019). Expanded clay, coir, perlite, vermiculite, brick shards, polystyrene packing peanuts, and wood fiber are also common media (Alshrouf, 2017).

Hydroculture has been adopted as one of the answers to food production issues such as water scarcity, soil salinity, and rising population (FAO, 2019), and it can be employed in developing nations with limited land for food production (Lagomarsino, 2019). Hydroculture allows plants and vegetables to be grown in poor soil conditions like deserts, and seawater can be used to combine nutrient solutions for hydroculture cultivation (Taghizadeh, 2021). As water and salinity have become key factors impacting plants, hydroculture has become increasingly popular (Sharma *et al.,* 2018). Hydroculture has the following advantages over typical soil-grown crop production: Water is used more efficiently by up to 90%. In a well-managed hydroculture system, productivity increases 3 to 10 times in the same amount of space, and many crops can be produced twice as quickly; also, soil-borne pests and diseases have no effect on hydroculture plants, and plant growth is more vigorous (Gashgari *et al.,* 2018; Baiyin *et al.,* 2021).

Growing wild edible plants could offer local populations with access to balanced nutritious diets. Wild edible plants are not freely used as edibles in South Africa, and there is little knowledge on many of the species. Furthermore, planting novel crops could give impoverished areas with financial opportunity by creating jobs. The goal of this study is to examine the effects of various nitrogen concentrations and growth media on the growth of *M. crystallinum* to develop an ideal growth regimen for hydroponics systems.

3.3 Materials and Methods

3.3.1 Experimental location

The experiment took place in the Department of Horticultural Sciences; research greenhouse at the Cape Peninsula University of Technology Bellville campus in Cape Town, South Africa, located at 33°55’56” S, 18°38’25” E. The study greenhouse had mist, a heating bed, and environmental control, with temperatures set to range from 21–26 C during the day and 12–18 C at night, with Relative Humidity averages of 60%. The greatest daily photosynthetic photon flux density (PPFD) was 1020 mol/m2/s, with an average of 420 mol/m^2/s.

3.3.2 Plant collection and nursery preparation

Viable seeds of *M. crystallinum* were collected on February 8th, 2021, from a selected plant population growing along the coast at the Granger Bay Campus of the Cape Peninsula University of Technology (33°53′58.2″ S, 18°24′41.4″ E). A total of 1,000 seeds were planted to ensure that at least 160 seedlings were available for the experiment. Seeds were exposed to freezer for twelve hours before sowing to disrupt dormancy, seeds were sprinkled onto damp paper towel and were wrapped. Sifting sand was done to eliminate foreign debris from a mixture that contained 70% sand and 30% peat. Sowing trays were filled with media, leveled, and watered. Seeds were planted, and then a thin layer of sand was put to cover. The trays were placed in a warm room with at least ten hours of light every day, and water was added again. Seed germination was aided by misting the trays with water twice a day, in the morning and afternoon; seeds sprouted in seven to twenty-one days. Seedlings were transplanted to pots (8.8 cm) after four weeks from trays to prevent competition, and plants were watered twice daily. After reaching two to three centimeters in height, plants were placed in a hydroponic system for two weeks to adapt, 160 seedlings of *M. crystallinum* of uniform size were transplanted into 12.5 cm pots with sterilized growth mix including silica sand, peat, vermiculite, and LECA clay. The plants that were picked grew the fastest and were left to grow. During this time, the plants were fed a nutrient solution three times per week. The nutrient solution was made by mixing Nutrifeed™ (produced by Starke Ayres Pty. Ltd. Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, South Africa, 1619) with municipal water at a rate of 10 g per 5L. The nutritional solution contained N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg), and Zn (240 mg/kg).

The raised seedlings were irrigated for 7 days with clean water after 14 days of growth to wash away any pollutants, and four treatments were organized by adding varying quantities of nitrogen fertilizer (0.36 g/L, 0.6 g/L, and 0.8 g/L) and 0 g/L as a control. Plants were fed with or without nitrogen treatment via spraying from a nutrient solution reservoir. Hydroponic systems were operated for 15 minutes in the morning and afternoon. The pH was maintained at 6.0 in all treatments.

All plants were harvested after 12 weeks of nitrogen-treated plant growth, and numerous postharvest measures were taken.

3.3.3 Hydroponic set-up

The primary purpose of this research was to examine the combined effect of different nitrogen concentrations and growing media on secondary metabolites and antioxidants content of *M. crystallinum* cultivated in hydroponic systems. The experiment used four similar hydroponic systems (Nutrient Film Technique). Each system was given the numbers S1 through S4. Tables were utilized as a surface for gutters. Sixteen gutters were placed on four different tables, with four gutters serving as plant supports on one of them. The gutters were held in place with cape ties. Ten pots having diverse substrates were placed in each gutter. Each table has its own water supply. Each reservoir has a submersible water pump that pumped fertilizer solution through a 20 ml pipe to each gutter. All gutters were sloped with bricks to allow the nutritional solution to circulate seamlessly. All gutters were covered with black plastic polyethylene sheets to keep the fertilizer solutions out of direct sunlight. Algae does not grow on polyethylene sheets. Each gutter was made to accommodate ten pots. Each gutter was filled with G1 (silica sand), G2 (vermiculite), G3 (LECA-clay), and G4 (peat) in the following order: G1 (silica sand), G2 (vermiculite), G3 (LECA-clay), and G4 (peat).

After germination, 160 turgid, robust, and disease-free seedlings (n=160) of the same height, leaf size, leaf color, branch number, and width were selected from propagated plants and placed in 12.5 cm pots with varied substrates each of which was prepared for 40 plants. Nitrogen was applied in four different doses (0g/L, 0.36g/L, 0.6 g/L, and 0.8 g/L) per reservoir and were fed to 40 plants (n=40) on each reservoir. Nitrogen was only applied by hand. Fertilizer was administered first thing in the morning to guarantee maximum effectiveness and it was ensured that plants were kept in a climate-controlled greenhouse. Every fortnight data collection was done and measurements were made with a calliper using centimetres (cm) as the measuring unit.

Table 3.1 Arrangement of each gutter on four NFT systems with different growing media and N fertilizer concentrations.

NFT/ Table	Gutter 1	Gutter 2	Gutter 3	Gutter 4
1	0.36 g/L Peat	0.36 g/L + Vermiculite	0.36 g/L LECA clay	0.36 g/L Silica sand
2	0.6 g/L+ Peat	0.6 g/L + Vermiculite	0.6 g/L + LECA clay	0.6 g/L + Silica sand
3	0 g + Peat	0 g + Vermiculite	0 g + LECA clay	0 g + Silica sand
4	0.8 g/L + Peat	0.8 g/L + Vermiculite	0.8 g/L + LECA clay	0.8 g/L + Silica sand

*NFT = Nutrient Filter Technique hydroponic system

3.4. Determination of Plant Growth

3.4.1. Plant Weight

To establish homogeneity within the samples, the weight of the plants was measured using a standard laboratory scale (RADWAG® Model PS 750.R2). Shoots, stems, and roots were measured after harvest, and the fresh weights of each sample were recorded. The plant material was subsequently room-dried to a consistent weight at 35 °C. The amount of water stored inside the plant tissues was compared to the difference between fresh and dry weight (Sogoni *et al*., 2021).

3.4.2. Number of leaves

The number of leaves was counted manually every two weeks to determine new growth in the treated plants.

3.4.3. Root length

To determine root length of each plant, roots were measured during the harvesting using measuring tape as to determine plants that produced the longest roots.

3.5 Results

3.5.1. Length of the roots

The root length of *M. crystallinum* was considerably impacted by different growth media and nitrogen concentrations. The vermiculite 40g concentration produced the longest root length, outperforming all other treatments. The shortest was attained at silica sand 0g concentrations, which was a control treatment.

3.5.2 The numbers of leaves

Mesembryanthumum crystallinum growth was shown to be varied in response to different growing mediums and nitrogen concentrations. Different nitrogen concentrations and growth mediums have an impact on the number of leaves. Other concentrations greatly outperformed the control, while control showed a large deal of variability in other weeks. In comparison to the control, the plants with the highest nitrogen concentration had the most leaves.

3.5.3 Total Fresh and Dry Weight

The findings showed that varied growing media and nitrogen concentrations had a substantial impact on *M. crystallinum* fresh and dry weight. Plants of *M. crystallinum* planted in various growing mediums and nitrogen concentrations produced fresh that weighed more than the control. The peat 30g treatment yielded the greatest fresh weight measurement, which was significantly higher than all other treatments, including control. The highest total dry weight was obtained with the Leca clay 40g treatment, whereas the lowest total dry weight was produced with the Leca clay 0g treatment.

Table 3.2 Effect of different growth media and nitrogen concentration on number of leaves of *Mesembryanthemum crystallinum*

Growth media	Nitrogen (g/L)	Week 2	Week 4	Week 6	Week 8	Week 10	Week 12
LECA clay	0.00	7.1±0.45bc	8.3±0.43c	5.4±0.3f	4.8±0.44f	3.2±0.60d	2.2±0.60d
	0.36	11.2±1.79ab	10.4±0.27ab	10.0±0.30abc	8.6±0.43abc	8.2±0.40ab	9.2±0.50a
	0.60	6.9 ±1.62bc	8.4±0.4bc	8.2±0.47b-e	7.9±0.50a-f	4.8±1.00a-d	4.4±1.15bcd
	0.80	7.1±0.28bc	8.4±0.22bc	8.1±0.43b-e	7.2±0.60a-f	7.2±7.20ab	7.4±0.50abc
Peat	0.00	7.8±0.36abc	9.4±0.43abc	6.4±0.27ef	5.9±0.40b	4.8±0.68bcd	3.8±0.70cd
	0.36	12.8±2.28a	8.6±0.6ab	10.2±0.36ab	9.0±0.40b	8.8±0.80a	9.2±0.80a
	0.60	3.2±0.49c	9.5±0.27abc	7.8±0.36cde	7.8±0.50a-e	5.0±0.60bcd	4.4±0.80bcd
	0.80	6.4±0.37bc	7.7±0.26c	6.4±0.26f	6.4±0.20b-f	3.0±0.60d	3.0±0.8a-d
Silica sand	0.00	7.5±0.4abc	8.7±0.30abc	6.5±0.5ef	5.2±0.76ef	4.4±0.83cd	3.6±0.88cd
	0.36	5.7±0.67abc	9.7±0.40abc	9.0±0.60a-d	8.2±0.42abc	5.6±0.60a-e	5.2±0.68a-d
	0.60	2.9±0.30c	9.0±0.30abc	8.8±0.68a-d	8.0±0.73a-e	5.4±0.99a-d	5.2±0.90a-d
	0.80	9.1±0.30ab	10.5±0.27a	10.6±0.40a	7.8±0.80a-e	6.6±0.67a-d	5.4±0.5a-d
Vermiculite	0.00	7.8±0.55abc	9.4±0.60abc	6.3±0.26ef	5.3±0.63def	3.4±0.73d	2.8±0.74d
	0.36	9.9±2.74ab	8.8±0.50ab	9.4±0.52a-d	7.9±0.95a-e	6.7±1.05	6.2±1.09d
	0.60	11.1±0.89ab	9.4±0.43abc	10.6±0.60a	9.3±0.37a	8.8±0.95a-d	8.2±1.28ab
	0.80	8.9±0.30ab	10.6±0.43a	10.6±0.52a	8.1±0.65a-d	7.8±0.76abc	7.4±1.27abc

Means that do not share a letter are significantly different at P ≤0.

Table 3.3 Effect of different growth media and nitrogen concentrations on root length, fresh weight, and dry weight of *M. crystallinun*

Growth media	Nitrogen (g/L)	Root length (cm)	Fresh weight (g)	Dry weight (g)
LECA clay	0.00	4.40±0.60cde	1.53±0.24d	0.42±0.99c
	0.36	11.20±1.79abc	7.11±1.90cd	1.73±0.33c
	0.60	6.90±1.62bcde	17.45±3.23bcd	8.00±1.46abc
	0.80	11.00±1.88a-d	29.23±5.23abc	12.65±2.46a
Peat	0.00	3.70±0.72de	7.92±2.67bcd	3.84±1.25abc
	0.36	12.80±2.28ab	7.38±2.28bcd	2.43±0.69c
	0.60	3.20±0.49e	50.40±12.80a	12.26±3.78ab
	0.80	5.00±2.02cde	12.09±8.63bcd	3.54±2.28abc
Silica sand	0.00	2.60±0.22e	3.31±0.74cd	1.25±0.37c
	0.36	5.70±0.67b-e	9.73±2.00bcd	3.31±1.02bc
	0.60	2.90±0.314e	10.74±1.56bcd	4.48±0.75abc
	0.80	4.70±0.67cde	15.24±3.5bcd	6.45±0.63abc
Vermiculite	0.00	6.00±0.58b-e	4.16±2.37cd	1.44±2.37c
	0.36	9.90±2.74a-e	33.35±5.24ab	4.22±1.27abc
	0.60	11.10±0.89a-d	19.86±6.52bcd	8.22±3.22abc
	0.80	14.60±2.73a	27.50±8.11a-d	9.18±3.12abc

Means that do not share a letter are significantly different at $P \leq 0.0$

3.6 Discussion

A growing population, unsustainable resource use, food loss and waste, increased demand, the effects of climate change, a decline in biodiversity in places with arable land, increased hunger, and malnutrition are just a few of the unsettling concerns facing the agricultural industries all over the world (Muluneh, 2021; Mbuli *et al.,* 2021). These issues jeopardize the capacity of the globe to produce enough food to meet demand both today and in the future (Calicioglu, 2019). Therefore, it is crucial to introduce new crops, such as *M. crystallinum*, that can endure difficult conditions and boost the local economy by creating jobs while also giving rural residents access to nutritious and wholesome food. Findings from this study suggest that *M. crystallinum* could survive the controlled environment and complete its life cycle, hence it can be grown hydroponically. This study also confirms that *M. crystallinum* does, require well-drained growing media or soils that are not too loose because it was discovered that leca clay had the shortest roots and fewest leaves. This is due to the inability of LECA clay to retain water (Libran *et al.,* 1995).

Nitrogen is one of the main factors restricting plant growth in both natural and agricultural environments since it is needed in the greatest amount of all critical elements for plant growth and development (Ghiasy-Oskoee *et al.,* 2020; Jimoh *et al.,* 2020). The roots of plants primarily take up nitrogen from the growth medium. Nitrogen fertilizer is mostly used in soilless culture to achieve maximum output. Both quality and productivity may be impacted by an inadequate or excessive supply (Nkcukankcuka *et al.,* 2022; Viljoen *et al.,* 2021). *Mesembryanthemum crystallinum*, a healthy, salty, succulent halophyte, is becoming more and well-known as a pre-made salad around the world (Agarie *et al.,* 2009; He & Qin, 2021). The current investigation disproved previous studies that *M. crystallinum* does not require fertilizer for growth Amari *et al*., (2020); Xia and Mattson, (2022) by finding that higher nitrogen concentrations resulted in more fresh weight, root length, and leaf number.

Although He and Qin (2021) claimed that *M. crystallinum* required saline conditions for optimal growth, this research study suggests otherwise research agrees with earlier findings by He *et al.,* (2017; 2020) that *M. crystallinum* does not necessarily need salinity to thrive. This study's findings indicated that growing *M. crystallinum* in a hydroponic system was impacted using municipal water and various nitrogen concentrations, as well as by the combination of various growing media and nitrogen concentrations. According to analysis of variance, growth media had a significance to root length. Nitrogen had a significant difference in root length, also combined analysis of variance of nitrogen and media had a significant difference.

At 40g of vermiculite, the root length was the longest; at 0g of silica sand, it was the shortest. Due to the nature of silica sand, which causes minute particles to become stuck in root hairs, these two growing mediums differed from one another. The shortest roots were produced in the silica sand 0g treatment because these particles reduced gaseous exchange and nutrient uptake. Vermiculite nevertheless displayed the longest root length. Vermiculite improves water, nutrient, and aeration retention, leading to stronger, healthier plants. Vermiculite developed the longest root length in this investigation because a well-aerated growing medium allowed for unfettered root growth and nutrient uptake. The number of leaves has demonstrated the study's considerable significance.

Peat 18g and silica sand 30g had the most leaves on the second week, while vermiculite 40g and silica sand 40g had the most leaves on the fourth week, while peat 40g and Leca clay 0g had the fewest leaves. Leca clay 0g had the fewest leaves on week six compared to vermiculite 40g, silica sand 40g, and vermiculite 30g. In week eight, vermiculite 30g had the highest number of leaves, while Leca clay 0g had the fewest. Peat 18g for week ten and Leca clay 18g had the highest number of leaves, while peat 40g, vermiculite 0g and Leca clay 0g had the lowest number of leaves.

3.7 Conclusion

Findings from this study suggest that a combination of properly formulated nutrient solution and growing media can significantly improve plant development in *M. crystallinum*. Vermiculite-infused nutrient solutions of 40g and 30g of N fertilizer showed a considerable improvement in plant growth metrics when fed to *M. crystallinum*. This might be an option for potential growers of the plant in provinces suffering the negative effects of the drought. It can therefore be concluded from this study that *M. crystallinum* can be grown hydroponically.

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CHAPTER FOUR

MEASURING THE CHLOROPHYLL CONTENT OF *MESEMBRYANTHEMUM CRYSTALLINUM* L TREATED WITH DIFFERENT DOSAGES OF NITROGEN FERTILIZER AND GROWTH MEDIA UNDER HYDROPONIC SYSTEMS

Measuring the chlorophyll content of *Mesembryanthemum crystallinum* L treated with different dosages of Nitrogen fertilizer and growth media under hydroponic systems

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4.1 Abstract

Mesembryanthemum crystallinum is a halophytic coastal plant that can withstand harsh conditions like drought and highly salinized soils. It thrives along the coast and in disturbed areas and requires little maintenance. In this study, the chlorophyll content of *M. crystallinum* treated with different nitrogen concentrations and grown in hydroponics with various growing media was examined. An instrument provided by Konica-Minolta called the Soil Plant Analysis Development (SPAD-502) meter was used to measure the chlorophyll concentration at the beginning of growing season (week 2) and just before harvesting (week 12). The readings were taken during weeks 2 and 12 of the trial, between 11 am and mid-day. The chlorophyll content of *M. crystallinum* was significantly impacted by different growth media and nitrogen concentrations. The LECA clay supplemented with 0.6 g/L of nitrogen concentration produced the highest chlorophyll during week two of the experiment which is statistically equivalent to values obtained in plants grown with vermiculite (0.6 g/L), peat (0.8 g/L), LECA clay (0.8 g/L) and peat (0.6 g/L). The lowest chlorophyll content was obtained in samples grown under peat with no nitrogen (0 g) supplementation. The second data collection for chlorophyll content was done before harvesting. The highest chlorophyll content was obtained from plants grown under LECA clay supplemented with 0.8 g/L of nitrogen concentration, while the lowest chlorophyll content was recorded in plants grown under peat that was not supplemented with nitrogen (control). However, other treatments in LECA clay, silica sand and vermiculite had equivalent on chlorophyll yield. It was concluded that N fertilizer significantly contributes to the production of *M. crystallinum* chlorophyll content.

Keywords: Chlorophyll, statistically, halophyte, optimum and yield

4.2 Introduction

Mesembryanthemum crystallinum is a halophytic coastal plant that is used frequently in salads as a spinach alternative. The plant can withstand extreme conditions like drought and highly salinized soil, and it grows along the coast and in disturbed regions and does not need particular care (Everingham *et al.,* 2019). The edibility of *M. crystallinum* and its alternative use as dietary supplement can increase household income, ensure food security, and create jobs. Since seasonal rainfall and various meteorological phenomena have an impact on crop health, biochemical processes, and physical growth, their reliance on rainfall poses a danger to crop yields (Mkonda *et al.,* 2017). However, *M. crystallinum* can complete its cycle to full potential in overcoming seasonal barriers and could maximize productivity under rain-fed conditions (Hinojosa *et al.,* 2018).

Small-scale farming makes a substantial contribution to the food security, socioeconomic development, and agricultural production of developing countries (Filippini *et al.,* 2018). Due to soil salinity, global warming, and climate variability, small-scale farmers in South Africa face a loss in output viability. Setshedi and Modirwa (2020) outlined that small-scale farmers often struggle with low yields that are far below the potential of the land and lack the knowledge necessary to optimize their potential. Therefore, it is crucial to offer small-scale farmers creative, cost-effective, and efficient solutions and ideas to maximize their production and generate optimum yields. Therefore, a deeper understanding of crop health markers and agricultural dynamics could help small-scale farmers spot crop health problems early on and put into practice the necessary solution to assure good production (Mwale *et al.,* 2021).

Numerous research has outlined various crop health markers (Kay *et al.,* 2008; Liliane & Charles, 2020). For instance, soil quality, microorganisms, crop yield, nitrogen levels, and crop yield were identified as indicators of the health and productivity of crops (Stefan *et al.,* 2021). Of recent, chlorophyll has been noted as one of the most significant, reliable indicators of crop health and productivity, thus, chlorophyll content is used as a crop health indicator (Wibowo, 2019). This is because of the biochemical photosynthetic processes and biophysical leaf pigment that indicate plant productivity (Liu *et al.,* 2019). Therefore, keeping an eye on plant diversity and chlorophyll content could help assess crop production over time, which is crucial for examining minor crop changes and maximizing healthy harvests (Fahad *et al.,* 2017).

Crop chlorophyll concentration has been estimated and monitored using different objective tools like chlorophyll meters. As a regular indicator of chloroplast content, photosynthetic function, and plant metabolism, leaf chlorophyll concentration is a significant parameter (Fahad *et al.,* 2017). Chlorophyll is an antioxidant component that is found in the chloroplast of green plants and is mostly found in the green parts of leaves, stems, flowers, and roots (Casa *et al.,* 2014). However, the formation of chlorophyll, which is the primary source of plant energy, is mostly dependent on sunlight penetration (Lin *et al.,* 2022). Due to the existence of diverse pigments like chlorophyll, anthocyanins and carotenoid, which together make up the spectral characteristics of a plant body, green plants have unique characteristics. Chlorophyll has a significant impact on plant physiology as well, being a crucial macromolecule that helps determine the efficiency of photosynthesis and the pace at which energy is used is chlorophyll (Voitsekhovskaja and Tyutereva, 2015). Additionally, it provides us with energy in the form of food or plant matter. Antioxidant properties of chlorophyll can be employed to find new medicines (Pérez-Gálvez, 2020).

The amount of chlorophyll in the leaves reflects the health of the plant and can be used to gauge when additional fertilizer is required. Shibaeva et al., (2020) outlined that the spad-502 is a small, portable meter that measures the quantity of chlorophyll in plant leaves. This will assist to gauge crop productivity and make plans for improvement of crop quality.

In this study, the concentration of chlorophyll in leaves of *M. crystallinum* grown in hydroponics with different growing media and varying nitrogen concentrations was examined with a view to determine the optimal conditions for chlorophyll production in the species. The results of this study will help potential commercial growers of *M. crystallinum* to determine the exact dosage of nitrogen fertilizer and growth media type needed to improve chlorophyll yield in the species and its close relatives.

4.3 Materials and Methods

4.3.1 Experimental location

The experiment took place in the Department of Horticultural Sciences; research greenhouse at the Cape Peninsula University of Technology Bellville campus in Cape Town, South Africa, located at 33°55’56” S, 18°38’25” E. The study greenhouse had mist, a heating bed, and environmental control, with temperatures set to range from 21–26 C during the day and 12–18 C at night, with relative humidity averages of 60%. The greatest daily photosynthetic photon flux density (PPFD) was 1020 mol/m^2/s, with an average of 420 mol/m^2/s.

4.3.2 Plant collection and nursery preparation

Viable seeds of *M. crystallinum* were collected on February 8th, 2021, from a selected plant population growing along the coast at the Granger Bay Campus of the Cape Peninsula University of Technology (33°53′58.2″ S, 18°24′41.4″ E). A total of 1,000 seeds were planted to ensure that at least 160 seedlings were available for the experiment. Seeds were frozen for twelve hours before sowing to disrupt dormancy. Sifting sand was done to eliminate foreign debris from a mixture that contained 70% sand and 30% peat. Sowing trays were filled with media, leveled, and watered. Seeds were planted, and then a thin layer of sand was put to cover. The trays were placed in a warm room with at least ten hours of light every day, and water was added again. Seed germination was aided by misting the trays with water twice a day, in the morning and afternoon; seeds sprouted in seven to twenty-one days. Seedlings were transplanted to pots (8.8 cm) after four weeks from trays to prevent competition, and plants were watered twice daily. After reaching two to three centimeters in height, plants were placed in a hydroponic system for two weeks to adapt, 160 seedlings of *M. crystallinum* of uniform size were transplanted into 12.5 cm pots with sterilized growth mix including silica sand, peat, vermiculite, and LECA clay.

The plants that were picked grew the fastest and were left to grow. During this time, the plants were fed a nutrient solution three times per week. The nutrient solution was made by mixing Nutrifeed^{TM} (produced by Starke Ayres Pty. Ltd. Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, South Africa, 1619) with municipal water at a rate of 10 g per 5L.

The nutritional solution contained N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg), and Zn (240 mg/kg).The raised seedlings were irrigated for 7 days with clean water after 14 days of growth to wash away any pollutants, and four treatments were organized by adding varying quantities of nitrogen fertilizer (18, 30, and 40 g) and 0 as a control. Plants were fed with or without nitrogen treatment via spraying from a nutrient solution reservoir. Hydroponic systems were operated for 15 minutes in the morning and afternoon. The pH was maintained at 6.0 in all treatments. All plants were harvested after 12 weeks of nitrogen-treated plant growth, and numerous postharvest measures were taken.

4.2.3 Hydroponic set up

The primary purpose of this research was to examine the combined effect of different nitrogen concentrations and growing media on chlorophyll content of *M. crystallinum* cultivated in hydroponic systems. The experiment used four similar hydroponic systems (Nutrient Film Technique). Each system was given the numbers S1 through S4. Tables were utilized as a surface for gutters. Sixteen gutters were placed on four different tables, with four gutters serving as plant supports on one of them. The gutters were held in place with cape ties. Ten pots having diverse substrates were placed in each gutter. Each table has its own water supply. Each reservoir has a submersible water pump that pumped fertilizer solution through a 20 ml pipe to each gutter. All gutters were sloped with bricks to allow the nutritional solution to circulate seamlessly. All gutters were covered with black plastic polyethylene sheets to keep the fertilizer solutions out of direct sunlight. Algae does not grow on polyethylene sheets. Each gutter was made to accommodate ten pots. Each gutter was filled with G1 (silica sand), G2 (vermiculite), G3 (LECA-clay), and G4 (peat) in the following order: G1 (silica sand), G2 (vermiculite), G3 (LECA-clay), and G4 (peat).

After germination, 160 turgid, robust, and disease-free seedlings (n=160) of the same height, leaf size, leaf color, branch number, and width were selected from propagated plants and placed in 12.5 cm pots with varied substrates each of which was prepared for 40 plants. Nitrogen was applied in four different doses (0g/L, 0.36g/L, 0.6 g/L, and 0.8 g/L) per reservoir and were fed to 40 plants (n=40) on each reservoir.

Nitrogen was only applied by hand. Fertilizer was administered first thing in the morning to guarantee maximum effectiveness and it was ensured that plants were kept in a climate-controlled greenhouse. Every fortnight data collection was done, and measurements were made with a calliper using centimetres (cm) as the measuring unit.

Table 4.1 Arrangement of each gutter on four NFT systems with different growing media and N fertilizer concentrations (g/L).

NFT/ Table	Gutter 1	Gutter 2	Gutter 3	Gutter 4
1	0.36 g/L + Peat	0.36 g/L + Vermiculite	0.36 g/L + LECA clay	0.36 g/L +Silica sand
2	0.6 g/L + Peat	0.6 g/L + Vermiculite	0.6 g/L + LECA clay	0.6 g/L + Silica sand
3	0 g/L + Peat	0 g/L + Vermiculite	0 g/L + LECA clay	0 g + Silica sand
4	0.8 g/L + Peat	0.8 g/L + Vermiculite	0.6 g/L + LECA clay	0.8 g/L + Silica sand

*NFT = Nutrient Filter Technique hydroponic system

4.4 Chlorophyll content measurement

The Soil Plant Analysis Development (SPAD-502) meter supplied by Konica-Minolta (Milnerton, Cape Town, South Africa) was used to measure the chlorophyll concentration both before and during the growing season. This instrument measures red light transmission at 650 nm, the wavelength at which chlorophyll absorbs light, and infrared light transmission at 940 nm, the wavelength at which there is no absorption (Nkcukankcuka et al., 2021).

The device determines a SPAD level using these two transmission values, which serves as a proxy for chlorophyll content. The chlorophyll content was measured in two completely developed leaves 7 from each treatment, and the SPAD-502 meter averaged the results to provide a final result. The readings were taken during weeks 2 and 12 of the trial, between 11 am and midday.

4.5 Results

The chlorophyll content of *M. crystallinum* was significantly impacted by different growth media and nitrogen concentrations at week 2 (start) and week 12 (end) of the experiment. At week 2 the highest chlorophyll concentrations were recorded in *M. crystallinum* supplemented with 0.6 g/L and 0.8 g/L nitrogen in LECA clay and peat although vermiculite treated with 0.6 g/L N fertilizer had equivalent effect.

The LECA clay supplemented with 0.6 g/L nitrogen concentration produced the highest chlorophyll during week two of the experiment, which is statistically equivalent to values obtained in plants grown with vermiculite (0.6 g/L), peat (0.8 g/L), LECA clay (0.8 g/L) and peat (0.6 g/L). The lowest content of chlorophyll was obtained in samples grown under peat with no nitrogen (0 g/L) supplementation. The second data collection for chlorophyll content was done before harvesting, the highest chlorophyll content was obtained from plants grown under LECA clay supplemented with 0.6 g/L of nitrogen concentration, while the lowest chlorophyll content was recorded in plants grown under peat that is not supplemented with nitrogen concentration which is control 0 g/L. However, other treatments in LECA clay, peat and vermiculite had equivalent on chlorophyll yield.

Table 4.2 Effect of different growth media and nitrogen concentrations on chlorophyll content of *Mesembryanthemum crystallinum.*

Growth media	Nitrogen (g/L)	Week 2	Week 12
LECA clay	0.00	0.71±0.25cd	1.12±0.02bcde
	0.36	0.80±0.26abcd	1.19±0.02abcde
	0.60	0.91±0.53a	1.27±0.06a
	0.80	0.90±0.34a	1.13±0.02bcde
Peat	0.00	0.77±0.03abcd	1.09±0.01e
	0.36	0.86±0.03abc	1.19±0.02abcde
	0.60	0.90±0.03a	1.19±0.09abcde
	0.80	0.89±0.05a	1.17±0.03abcde
Silica sand	0.00	0.72±0.04bcd	1.14±0.02abcde
	0.36	0.82±0.03abcd	1.23±0.02ab
	0.60	0.88±0.40ab	1.10±0.03de
	0.80	0.88±0.20ab	1.19±0.02abcde
Vermiculite	0.00	0.68±0.03d	1.1±0.02cde
	0.36	0.76±0.03abcd	1.22±0.01abc
	0.60	0.91±0.03a	1.21±0.01abcd
	0.80	0.87±0.3abc	1.1±0.02cde

Means that do not share a letter are significantly different at $P \leq 0.05$

4.6 Discussion

Planning nutrient management techniques requires improving the accuracy of plant output predictions to guarantee a balance between demand under climate change and nutrient supply (Elbasiouny *et al*., 2022). Plant growing models frequently oversimplify the prerequisites for plant growth. These models' architecture has some unknowns, including crop parameters, which leads to biased plant growth.

This study employed spad to quantify the amount of chlorophyll to determine the health of the *M. crystallinum*. According to reports, the most important factor for *M. crystallinum* is leaf photosynthesis. On the other hand, there are not many studies that have looked at the connection between nitrogen application and chlorophyll content of

M. crystallinum. As a crucial component of the Calvin-Benson cycle, chlorophyll helps to drive the production of nicotinamide adenine dinucleotide phosphate and chemical energy in the form of adenosine triphosphate (Miller *et al.*, 2020). Chlorophyll is responsible for harvesting light during photosynthesis. Chlorophyll serves as a reliable parameter to estimate nitrogen fertilizer because it also indicates nutritional status because of nitrogen fertilizer (Kubar *et al.,* 2022).

Findings from this study agrees with other studies that the higher the nitrogen fertilizer application the higher the chlorophyll content (Zhang *et al.,* 2022; El-Sorady *et al.,* 2022; Muhammad *et al.,* 2022). The LECA clay supplemented with 30 g nitrogen concentration yield the highest chlorophyll content, whereas the lowest chlorophyll content was obtained in samples grown under peat with no nitrogen concentration, the parameters were measured during the first two weeks of experiment. However, results from this study agrees with Bojovic and Markovic (2009) who asserted that NPK fertilization was the most beneficial type of fertilization, and unfertilized soil was the least beneficial.

The chlorophyll content of *M. crystallinum* was significantly impacted by different growth media and nitrogen concentrations. The LECA clay supplemented with 0.6 g/L of nitrogen concentration had equivalent effect on chlorophyll content with vermiculite (0.6 g/L), peat (0.8 g/L), LECA clay (0.8 g/L) and peat (0.6 g/L) as these treatments produced highest chlorophyll content during week two of the experiment. This corroborates findings of Trelka et al., (2010) reported that growing medium had no impact on the amount of chlorophyll in leaves. Nevertheless, Muhammad *et al.* (2022) agrees with the current study the amount of chlorophyll in leaves increased with increasing N application. The lowest content of chlorophyll was obtained in samples grown under peat with no nitrogen (0 g/L) supplementation. The second data collection for chlorophyll content was just done before harvesting, the highest chlorophyll content was obtained from plants grown under LECA clay supplemented with 0.6 g/L of nitrogen concentration.

The critical importance of applying high N fertilizer was earlier confirmed by a significant increase in the chlorophyll content of maize leaves (Ramzan *et al*., 2023). Whereas, in this study, the lowest chlorophyll content was recorded in plants grown under peat that is not supplemented with nitrogen fertilizer which is control 0 g/L. In agreement with the present study Salehi (2014) reported that lowest levels of chlorophyll b and carotenoids were found in the 100% peat treatment.

He and Qin (2021) reported that *M. crystallinum* transferred from full N to zero N, resulted to leaf reduction within two days which leads to lowest chlorophyll content. The researchers further stated that a reduction in nitrogen supply reduced leaf expansion of young *M. crystallinum*. In addition, Li *et al*. (2018) stated that nitrogen deprivation reduces root cell hydraulic conductivity and the over plant hydraulic conductivity, leading to the decrease of plant leaf, thus leading to stunt and unhealthy plants.

4.7 Conclusion

The chlorophyll status of *Mesembryanthemum crystallinum* grown in hydroponic systems was significantly influenced by various growing media and nitrogen concentrations. This study measured the amount of chlorophyll in two distinct stages. The LECA clay supplemented with 0.6 g/L nitrogen concentration had equivalent effect on chlorophyll content with vermiculite (0.6 g/L), peat (0.8 g/L), LECA clay (0.8 g/L) and peat (0.6 g/L) as these treatments produced highest chlorophyll content during week two of the experiment, while the lowest chlorophyll content was obtained from peat without N fertilizer. As a result, it was determined that N fertilizer significantly contributes to the production of *M. crystallinum* chlorophyll content. To comprehend chlorophyll content percentage in all weeks, more research is necessary.

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CHAPTER FIVE

THE IMPACT OF NITROGEN CONCENTRATIONS AND GROWING MEDIA ON SECONDARY METABOLITES AND ANTIOXIDANTS CONTENT OF *MESEMBRYANTHEMUM CRYSTALLINUM* L. GROWN IN HYDROPONIC SYSTEMS

The impact of nitrogen concentrations and growing media on secondary metabolites and antioxidants content of *Mesembryanthemum crystallinum* L. grown in hydroponic systems

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5.1 Abstract

Mesembryanthemum crystallinum L. is a perennial halophyte that originated in southern and eastern Africa and is now widespread throughout the world. Recent studies have shown that the plant can grow in salty soils without losing its nutritional value. However, little has been reported about factors that modulate quantity of secondary metabolites in the plant. In this study, the twin effect of different concentrations of nitrogen (0, 0.36, 0.6, 0.8 g/L) and growing media (LECA clay, peat, vermiculite and silica sand) on the production of antioxidant metabolites in hydroponically cultivated *M. crystallinum* was investigated. The untreated plants (0 g/L of N) were taken as the control. Eight weeks after cultivation, healthy leaves of *M. crystallinum* were harvested from different treatments, washed, air-dried, oven-dried, pulverised and extracted with ethanol. The total phenols and flavonols of the crude extract was determined while the antioxidant content was determined using the DPPH, FRAP and ABTS antioxidant assays. It was observed that leaves of *M. crystallinum* grown in LECA clay supplemented with N fertilizer (0.36 g/L) had the highest total phenolic content (6.4 mg GAE/g) than leaves from other treatments. The highest mean value (2.9 mg QE/g) of flavonols was found in LECA clay supplemented with 0.36 g/L of N fertilizer, followed by N fertilizer treated peat (2.7 mg QE/g). The vermiculite that was supplemented with N fertilizer had the lowest flavonol content (1.8 mg QE/g), while the peat that had no N fertilizer had the second lowest flavonol content (0 g/L). This study found no connection between high phytochemical and antioxidant content and fertilizer use. Because vermiculite consistently had the lowest mean values, the study also shows that growing media with a high-water holding capacity have no beneficial effects on the accumulation of phytochemicals and antioxidants.

Keywords Antioxidants, phytochemicals, perennial, and phenols

5.2 Introduction

Cancer, heart disease, diabetes, stroke, and arthritis are just few of the chronic conditions that cause disability and death (Schmidt, 2016). The economy and lifestyle are a major social determinant of health that is negatively impacted by all these chronic diseases (Cockrham *et al.,* 2017). Recent research has highlighted the crucial part that antioxidants play in preventing chronic diseases. Antioxidants have been shown to have beneficial effects in the fight against oxidative stress (Sharifi-Rad *et al.,* 2020). Within plants secondary metabolism, polyphenols make up the largest category of compounds. They come from the shikimate pathway, which is activated in response to unfavourable environmental factors that lead to an increase in the production of radical oxygen species (Hasanuzzam *et al.,* 2020). As a result of growing in highly salinized soils, halophyte plants have been noted to have a high polyphenol content (Pungin *et al*., 2022).

The availability of nitrogen (N) fertilizer plays a crucial part in controlling the accumulation of antioxidants (Anas *et al*., 2020), but it is unclear how different N fertilizers affect antioxidant properties of *M. crystallinum.* Nitrogen availability has an impact on the antioxidant quality of horticultural crops (Ahmad *et al*., 2022). Lack of nitrogen causes levels of ascorbic acid, flavonoids, and flavonols to rise. While Yadav *et al*, (2023) noted that nitrogen limitation also tends to impede crop growth, the overall effectiveness of this approach is still unknown. Antioxidants are extremely beneficial to human health, and *M. crystallinum* accumulates them depending on the environment (Calvo *et al.,* 2022). Therefore, it is crucial to investigate the variables that control the antioxidant *M. crystallinum* content especially, with regards to nitrogen supplementation.

Mesembryanthemum crystallinum L. commonly known as the "ice plant," is a perennial succulent plant, not an annual herbaceous one. It belongs to the family Aizoaceae (Kang *et al.,* 2023). The plant is a halophilic plant that originated in southern and eastern Africa and is now widespread throughout the world (Loconsole *et al.,* 2019).

The name ice plant, which is used in a broad sense to describe how the stems and leaves resemble ice crystals, is frequently used to refer to the plant. The common ice plant can grow in salty soils and can withstand cold temperatures without losing any of its nutritional value (Agarie *et al.,* 2022). Recent studies have used the common ice plant and described its potential uses in phytoremediation, human health, and as food since it is widely consumed in some regions (Siliwa-Cebula *et al.,* 2020). Its succulent texture leaves behind a taste that is mildly salty and evocative of the ocean when chewed (Xia *et al.,* 2022). Because of this, the common ice plant is highly prized in haute cuisine, and lots of other customers want to try it now (Calvo *et al.,* 2022).

The mechanisms underlying the biological activity and phytochemistry of common ice plants are poorly understood due to paucity of research on factors that may enhance accumulation of secondary metabolites in the plant (Kang *et al.,* 2023). Since halophytes contain a lot of polyphenols and they have a protective effect against diseases that are very common, it is possible to obtain healthy secondary metabolites and antioxidant extracts from these abundant and underutilized coastal plants (Pungin *et al.,* 2022). It became imperative to promote its cultivation, as the common ice plant has been shown to have antiseptic and neuroprotective properties in addition to its many health benefits. The main goal of this study was therefore to determine how different nitrogen concentrations and growing conditions affected the secondary metabolites and antioxidants in *M. crystallinum*. This is important to provide the dosage of N fertilizer that will optimize the biosynthesis of secondary metabolites and antioxidants in common ice plant grown.

5.3 Materials and Methods

5.3.1 Experimental location

The experiment took place in the Department of Horticultural Sciences; research greenhouse at the Cape Peninsula University of Technology Bellville campus in Cape

Town, South Africa, located at 33°55'56" S, 18°38'25" E. The study greenhouse had mist, a heating bed, and environmental control, with temperatures set to range from 21–26 C during the day and 12–18 C at night, with Relative Humidity averages of 60%. The greatest daily photosynthetic photon flux density (PPFD) was 1020 mol/m^2/s, with an average of 420 mol/m^2/s.

5.3.2 Plant collection and nursery preparation

Viable seeds of *M. crystallinum* were collected on February 8th, 2021, from a selected plant population growing along the coast at the Granger Bay Campus of the Cape Peninsula University of Technology (33°53′58.2″ S, 18°24′41.4″ E). A total of 1,000 seeds were planted to ensure that at least 160 seedlings were available for the experiment. Seeds were frozen for twelve hours before sowing to disrupt dormancy. Sifting sand was done to eliminate foreign debris from a mixture that contained 70% sand and 30% peat. Sowing trays were filled with media, leveled, and watered. Seeds were planted, and then a thin layer of sand was put to cover. The trays were placed in a warm room with at least ten hours of light every day, and water was added again. Seed germination was aided by misting the trays with water twice a day, in the morning and afternoon; seeds sprouted in seven to twenty-one days. Seedlings were transplanted to pots (8.8 cm) after four weeks from trays to prevent competition, and plants were watered twice daily. After reaching two to three centimeters in height, plants were placed in a hydroponic system for two weeks to adapt, 160 seedlings of *M. crystallinum* of uniform size were transplanted into 12.5 cm pots with sterilized growth mix including silica sand, peat, vermiculite, and LECA clay. The plants that were picked grew the fastest and were left to grow. During this time, the plants were fed a nutrient solution three times per week. The nutrient solution was made by mixing Nutrifeed^{tm} (produced by Starke Ayres Pty. Ltd. Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, South Africa, 1619) with municipal water at a rate of 10 g per 5L. The nutritional solution contained N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg), and Zn (240 mg/kg).

The raised seedlings were irrigated for 7 days with clean water after 14 days of growth to wash away any pollutants, and four treatments were organized by adding varying quantities of nitrogen fertilizer (0.36 g/L, 0.6 g/L, and 0.8 g/L) and 0 g/L as a control. Plants were fed with or without nitrogen treatment via spraying from a nutrient solution reservoir. Hydroponic systems were operated for 15 minutes in the morning and afternoon. The pH was maintained at 6.0 in all treatments. All plants were harvested after 12 weeks of nitrogen-treated plant growth, and numerous postharvest measures were taken.

5.3.3 Hydroponic set up

The primary purpose of this research was to examine the combined effect of different nitrogen concentrations and growing media on secondary metabolites and antioxidants content of *M. crystallinum* cultivated in hydroponic systems. The experiment used four similar hydroponic systems (Nutrient Film Technique). Each system was given the numbers S1 through S4. Tables were utilized as a surface for gutters. Sixteen gutters were placed on four different tables, with four gutters serving as plant supports on one of them. The gutters were held in place with cape ties. Ten pots having diverse substrates were placed in each gutter. Each table has its own water supply. Each reservoir has a submersible water pump that pumped fertilizer solution through a 20 mm pipe to each gutter. All gutters were sloped with bricks to allow the nutritional solution to circulate seamlessly. All gutters were covered with black plastic polyethylene sheets to keep the fertilizer solutions out of direct sunlight. Algae does not grow on polyethylene sheets. Each gutter was made to accommodate ten pots. Each gutter was filled with G1 (silica sand), G2 (vermiculite), G3 (LECA-clay), and G4 (peat) in the following order: G1 (silica sand), G2 (vermiculite), G3 (LECA-clay), and G4 (peat).

After germination, 160 turgid, robust, and disease-free seedlings (n=160) of the same height, leaf size, leaf color, branch number, and width were selected from propagated plants and placed in 12.5 cm pots with varied substrates each of which was prepared for 40 plants. Nitrogen was applied in four different doses (0g/L, 0.36g/L, 0.6 g/L, and 0.8 g/L) per reservoir and were fed to 40 plants (n=40) on each reservoir. Nitrogen was only applied by hand.

Fertilizer was administered first thing in the morning to guarantee maximum effectiveness and it was ensured that plants were kept in a climate-controlled greenhouse. Every fortnight data collection was done, and measurements were made with a calliper using centimetres (cm) as the measuring unit.

Table 5.1 Arrangement of each gutter on four NFT systems with different growing media and N fertilizer concentrations.

NFT/ Table	Gutter 1	Gutter 2	Gutter 3	Gutter 4
1	0.36 g/L Peat	0.36 g/L + Vermiculite	0.36 g/L LECA clay	0.36 g/L Silica sand
2	0.6 g/L+ Peat	0.6 g/L + Vermiculite	0.6 g/L + LECA clay	0.6 g/L + Silica sand
3	0 g + Peat	0 g + Vermiculite	0 g + LECA clay	0 g + Silica sand
4	0.8 g/L + Peat	0.8 g/L + Vermiculite	0.8 g/L + LECA clay	0.8 g/L + Silica sand

*NFT = Nutrient Filter Technique hydroponic system

5.4. The antioxidant analysis

5.4.1 Sample Preparation

The harvested shoot material was air-dried in a well-ventilated room for 7-14 days. Plants were milled to a fine powder in a Junkel and Kunkel model A 10 mill. One hundred milligram (100 mg) of the dried powdered material was macerated in 25 mL of 70% (v/v) ethanol (Merck, Modderfontein, South Africa) for 1 hour to extract the shoot material. The mixture was filtered, and the supernatant was used for all analyses after centrifugation at 4000 rpm for 5 minutes.

5.4.2 Determination of antioxidant capacity and content

Antioxidant activity and secondary metabolite accumulated in leaves were evaluated by assays for DPPH (diphenylpicrylhydrazyl), ABTS/TEAC (total antioxidant capacity), and reducing antioxidant power of iron (FRAP), total polyphenols and flavonols.

5.4.3 Polyphenol Assay

The assay for total polyphenols (Folin assay) was performed as described by Sogoni *et al.,* (2021). The phenol reagent of Folin and Ciocalteu (2 N, Sigma, Gauteng, South Africa) was diluted 10-fold with distilled water and a 7.5% sodium carbonate solution (Sigma-Aldrich, Gauteng, South Africa) was prepared. In a 96-well plate, 25 µL of the crude extract was mixed with 125 µL of the phenol reagent of Folin and Ciocalteu and 100 µL of sodium carbonate. The plate was incubated for 2 hours at room temperature.

The absorbance was then measured at 765 nm in a Multiskan Spectrum plate reader (Thermo Electron Corporation, USA).

Polyphenol levels of the samples were calculated using a gallic acid standard curve (Sigma-Aldrich, Gauteng, South Africa), the concentration of which varied from 0 to 500 mg/L. Results were expressed as mg gallic acid equivalents (GAE) per g dry weight (mg GAE /g DW).

5.4.4 Ferric Reducing Antioxidant Power (FRAP) Assay

The FRAP assay was performed according to the method of Ngxabi *et al.,* (2021). The FRAP reagent was prepared by mixing 30 mL of acetate buffer (0.3 M, pH 3.6) (Merck, Modderfontein, South Africa) with 3 mL of 2,4,6- tripyridyl-s-triazine (10 mM in 0.1 M hydrochloric acid) (Sigma-Aldrich, Gauteng, South Africa), 3 mL iron (III) chloride hexahydrate (FeCl3-6H2O) (Sigma-Aldrich, Gauteng, South Africa), and 6 mL distilled water. In a 96-well plate, 10 μL of the crude sample extract was mixed with 300 μL of the FRAP reagent and incubated for 30 minutes at room temperature. The absorbance was then measured at 593 nm in a Multiskan Spectrum plate reader (Thermo Electron Corporation, USA). The FRAP values of the samples were calculated using an L-ascorbic acid (Sigma-Aldrich, Gauteng, South Africa) standard curve with concentrations ranging from 0 to 1000 μM. Results were expressed as μM ascorbic acid equivalents (AAE) per g dry weight (μM AAE/g DW)

5.4.5. Estimation of flavonol content

The flavonol content of the extracts was determined using quercetin as a standard of 0, 5, 10, 20, 40, and 80 mg/L in 95% dissolved in ethanol as described by (Bulawa et al., 2023). For each sample, 12.5 μL of crude sample extracts were mixed with 12.5 μL 0.1% HCl (Merck, South Africa) in 95% ethanol and 225 μL 2% HCl. The extracts were then incubated at room temperature for 30 minutes. At a temperature of 25 degrees Celsius, the absorbance was measured at 360 nm. The results were estimated in milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW).

5.4.6 Antioxidant Capacity of DPPH radicals

A solution of 0.135 mM DPPH prepared in a dark bottle was used to generate the DPPH radicals (Sogoni et al., 2021). About 300 µL of DPPH solution was reacted with Trolox standard (6-Hydrox-2,5,7,8-tetramethylchroman-2- 20 carboxylic acid) solution prepared in graded concentrations (0 - 500 M) and 25 µL of crude extract. After 30 minutes of incubation, the absorbance was measured at 517 nM. The results were expressed as M/Trolox equivalent per gram dry weight (M TE/g DW).

5.4.7 Determination of ABTS/ TEAC antioxidant capacity

The antioxidant capacity of ABTS was measured using a method described by (Jimoh et al., 2019) with minor modifications. A 7 mM ABTS solution and a 140 mM potassiumperoxodisulphate (K2S2O8) solution (Merck, South Africa) were used as stock solutions. The experiment solution was then prepared by adding 88 µL K2S2O8 to 5 mL ABTS solution. These two solutions were combined and allowed to react in the dark for 24 hours at room temperature. Trolox (6-Hydrox-2,5,7,8-tetramethylchroman-2- 20 carboxylic acid) was used as the standard, and concentrations ranging from 0 to 500 M were used. Crude sample extracts (25 µL) were allowed to react with 300 µL ABTS in the dark at room temperature for 5 minutes before the absorbance was measured in a microplate reader at 734 nm at 25 °C.

Table 5.2 The effect of different nitrogen concentrations and media on *M. crystallinum* secondary metabolites and antioxidants

Media	Nitrogen (g/L)	ABTS/ TEAC (µmol TE/g)	DPPH (µmol TE/g)	FRAP (µmol TE/g)	Flavonols (QE/g)	Polyphenols (mg GAE/g)
LECA clay	0.00	12.9±0.31a	10.5±0.29abcd	28.0±1.01a	2.7±0.08ab	5.6±0.16ab
	0.36	8.0±0.32de	8.0±0.24ef	21.0±0.35bcd	1.8±0.08def	3.8±0.08fg
	0.60	9.8±0.43b-e	8.9±0.08de	22.4±0.25bc	1.9±0.013ef	4.4±0.10def
	0.80	9.0±0.49cde	10.9±0.17abc	21.0±0.8bcd	2.4±0.13bcd	4.9±0.21bcd

Peat	0.00	9.0±0.33cde	9±0.3cde	21.6±0.75bcd	2.0±0.05cdef	3.8±0.15fg
	0.36	9.0±0.24cde	8.0±0.29ef	18.0±0.77d	1.8±0.07f	3.5±0.1gh
	0.60	7.9±0.64e	6.8±0.07f	19.0±0.9cd	2.1±0.1cdef	3.0±0.09h
	0.80	9.0±2cde	9.0±0.3cde	18.0±0.93d	1.9±0.0.9def	3.5±0.03gh
Silica sand	0.00	12.0±0.43ab	10.0±0.45a-d	22.5±0.61b	2.5±0.09abc	5.1±0.12bcd
	0.36	12.0±0.1ab	11.5±0.42a	26.0±0.09a	2.2±0.08bcdef	4.7±0.17cde
	0.60	11.5±0.61abc	11.2±0.38ab	28.0±0.26a	2.9±0.03a	5.3±0.02bc
	0.80	8.7±1.37de	9±0.41b-e	21.5±0.27bcd	2.1±0.11cdef	5.2±0.15bc
Vermiculite	0.00	11.0±0.34abc	8.2±0.35ef	19.0±0.59bcd	2.3±0.07bcde	5.6±0.22ab
	0.36	10.0±0.29b-e	7.8±0.38ef	22.0±0.95bc	2.3±0.1bcde	5.3±0.15bc
	0.60	10.7±0.16a-d	10±0.53a-d	22.0±0.31bc	2.3±0.06bcd	6.3±0.17a
	0.80	11.0±0.16abc	11±0.56ab	21.0±0.46bcd	1.9±0.09def	4.3±0.18ef

Means that do not share a letter are significantly different at P ≤0.0

5.5 Results

5.5.1 Polyphenol content

The effect of different growing media and nitrogen concentrations on polyphenols. The polyphenol content was expressed as mg/g of the sample in gallic acid equivalent (GAE) as presented on the table below. Polyphenols extracts showed a vary significantly at $P \leq 0.05$ when different growing media and nitrogen concentrations were compared with each other and with the control. Plants that were subjected to Leca clay supplemented by 0.36 g/L of N fertilizer had the highest mean value of polyphenol content (6.4 mg GAE/g) in their leaves as compared to other treatments. The lowest mean value of polyphenol content was yield from extract that were grown under vermiculite supplemented by 0.36 g/L N fertilizer.

5.5.2 Flavonols content

The flavonols content in the leaves vary significantly at $P \leq 0.05$, when different growing media supplemented with different N fertilizer concentrations compared with each other (Table 2). Plants that were subjected to LECA clay supplemented by 18 g N fertilizer had the highest mean value of flavonols content (2.9 mg QE/g) in their leaves as compared to other treatments. The lowest mean value of flavonols content (1.8 QE/g) was yield from extract that were grown under vermiculite supplemented by 0.36 g/L of N fertilizer.

5.5.3 FRAP content

Nitrogen fertilizer with different growing media had a significant influence on the FRAP capacity of the leaves of *M. crystallinum*. The three treatments namely peat supplemented with N fertilizer (0.6 g/L) with mean value of (28.1 umol AAE/g), LECA clay supplemented with N fertilizer (0.36 g/L) with mean value of (28 umol AAE/g) and LECA clay supplemented with N fertilizer (0 g/L) with mean value of (26.2 umol AAE/g) had the highest mean value when compared to all other treatments. Whereas the lowest value was obtained from vermiculite supplemented with N fertilizer (0.8 g/L) with mean value of (18.5 umol AAE/g) and vermiculite supplemented with N fertilizer (0 g/L) with mean value of (18.3 umol AAE/g).

5.5.4 DPPH content

The DPPH antioxidants content in the leaves of *M. crystallinum* was significantly influenced by the N fertilizer supplemented with different growing media. The LECA clay that was not supplemented with N fertilizer (control - 0 g/L) had the highest mean value (11.5 umol TE/g) compared to other treatments. While the lowest DPPH content of control with the mean value of (6.8 umol TE/g) was found in extracts of plants that were grown on vermiculite supplemented with N fertilizer (0.36 g/L).

5.5.5 TEAC content

The TEAC content in the tested leaf extract of *M. crystallinum* was significantly influenced by the N fertilizer supplemented with different growing media. The peat treated N fertilizer (0.6 g/L) had the highest mean value (13 umol TE/g) compared to other treatments. The lowest mean value (7.9 umol TE/g) was found in plants grown on vermiculite supplemented with N fertilizer (0.36 g/L).

5.6 Discussion

Therapeutic activities in plants are an indication of their phytochemical diversity (Jimoh *et al.,* 2019). The antioxidant activities of plants are influenced by the phytochemicals they contain. Flavonoids, phenolic acids, and alkaloids are among the phytochemicals that play overlapping roles in plant defense mechanisms, pollinator attraction, singlet oxygen scavengers, high energy radiation absorbers, reducing agents, and allelopathy (Tungmunnithum *et al.,* 2018). Allelopathy is used by *M. crystallinum* as part of a successful strategy to eliminate competition from neighboring plants. Allelopathy has been implicated as the cause of an *M. crystallinum* success dominance in its environment (Cheng & Cheng, 2015).

Mesembryanthemum crystallinum possesses salts during its life cycle, so it prefers coastal, unoccupied, or sparsely occupied areas (He *et al.,* 2022). This salt is released after the plant dies due to rain leaching (Wungrampha *et al*., 2020; Ding *et al.,* 2010; and Vivrette & Muller, 1977).

Although the salt does not appear to have a direct toxic influence on grassland species, it does produce a detrimental osmotic environment that prevents the growth of nontolerant species. Many plant species are sensitive to salts, especially during the germination stage, which is when *M. crystallinum* salt is responsible for competing plant growth suppression (Kamran *et al.,* 2019*).*

The impact of various growing media supplemented with varying N fertilizer concentrations on the quantity and relative activity of these phytochemicals cannot be overstated (Zhao *et al.,* 2021). A combination of mineral resources provided by the soil and internal nutrient trade-off determine the carbon-nutrient balance of the plant, which influences the synthesis and retention of defensive chemicals in the plant. It is justified to assess phytochemical variations in *M. crystallinum* cultivated on different growing media in order to document the media with the highest phytochemical yield, given that various reports indicate fertilizer application reduces the production of phenolics and other secondary metabolites Ibrahim *et al.,* (2011); Waterman and Mole, (2019), a trend which may be reversed by a growth medium depending on its physicochemical content.

Consuming fruits and vegetables are thought to be an excellent source of polyphenols and antioxidants, which are helpful in defending the body from reactive species damage. Phenolic compounds are particularly interesting among these phytochemicals due to their significance in food flavor and taste as well as their health-promoting qualities (Shahidi & Ambigaipalan 2015). The total polyphenols content (6.4 mg GAE/g) in *M. crystallinum* leaves that were grown under LECA clay supplemented with N fertilizer (0.36 g/L) was significantly higher than other treatments. The findings of this study demonstrated that the accumulation of phytochemicals and antioxidants was significantly influenced by light soilless media coupled with moderate N fertilization. The LECA clay was the most consistent growing medium and that produced the greatest mean value of polyphenols across the board. Since secondary metabolites often form on solid media, it is not surprising that LECA clay has an impact on the accumulation of phytochemicals and antioxidants (Ngxabi *et al.,* 2021).

LECA clay can improve compaction resistance, drainage, retain water, insulate roots, and give roots with increased amounts of oxygen supporting vigorous growth, it is unclear whether this impact is caused by the solid matrix itself or by faster depletion or drainage of nutrients (Nielsen *et al.,* 2004; Hamid *et al.,* 2022). LECA clay functions as a growth medium for the generation of antioxidants and phytochemicals (Kabra *et al.,* 2019). Likewise, LECA clay supplemented with0.36 g/L dosage of N fertilizer had the highest mean value (2.9 mg QE/g) of flavonols followed by peat supplemented with N fertilizer with the mean value of 2.7 mg QE/g. The lowest flavonols content was recorded from vermiculite supplemented with N fertilizer with the mean value (1.8 mg QE/g), whereas the second lowest content of flavonols was obtained from peat with no N fertilizer (0 g/L).

However, this study does not agree with previous studies which stipulate that vermiculite enhances the production of flavonols (Synowiec & Krajewska, 2020; Nakabayashi *et al.,* 2013). The FRAP content presented three concentrations that statistically shared grouping, which is peat supplemented with N fertilizer (0.6 g/L), LECA clay supplemented with N fertilizer (0.36 g/L) and LECA clay with no N fertilizer (0 g/L). The lowest mean value of 18.2 umol AAE/g of FRAP content was recorded from 0 g/L vermiculite. The highest content of DPPH was obtained from *M. crystallinum* leaves that were grown under vermiculite. The highest mean value of TEAC was obtained from peat supplemented with N fertilizer (0.6 g/L), while the lowest was obtained from vermiculite supplemented 0.36 g/L of N fertilizer. The results of this study showed that there is no relation between high fertilizer and high content of phytochemicals and antioxidants. Also, the study proves that growing media that have high water holding capacity does not positively affect the accumulation of phytochemicals and antioxidants, as the lowest mean values were obtained from vermiculite, and this was consistent throughout.

5.7 Conclusion

Plants grown in LECA clay supplemented with 0.36 g/L consistently produced the highest levels of metabolites. According to this study, there is no correlation between high fertilizer use and high phytochemical and antioxidant content.

The study also demonstrates that growing media with a high-water holding capacity have no beneficial effects on the accumulation of phytochemicals and antioxidants because vermiculite consistently had the lowest mean values.

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CHAPTER SIX

THE NUTRIENTS AND THEIR ACCUMULATION IN MESEMBRYANTHEMUM CRYSTALLINUM L. GROWN IN HYDROPONIC SYSTEMS WITH VARYING NITROGEN CONCENTRATIONS AND GROWTH MEDIA

The nutrients and their accumulation in *Mesembryanthemum crystallinum* L. grown in hydroponic systems with varying nitrogen concentrations and growth media

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6.1 Abstract

The circulation of nutrients in plants is significant throughout the stages of plant growth, resulting to modifications during different life cycle such as vegetative, flowering and senescence. Rich source of nutrients are plants, and there has been recent global interest in the research of the nutritional composition of halophytes plants to address food security, prevent some diseases, and tackle malnutrition. The aim of this research study was to develop a growth protocol to produce mineral and nearby compositions in *Mesembryanthemum crystallinum*, it is necessary to study the effects of various nitrogen concentrations and growing media on *M. crystallinum* when it is cultivated in a hydroponic system. Four different growing media and different nitrogen concentration: Peat, vermiculite, LECA clay and silica sand and 0 g/L, 0.36 g/L, 0.6 g/L and 0.8 g/L. Nutrients and proximate were done using AOAC while minerals were determined using inductively coupled plasma-optical emission spectrometer (AOAC).The highest ADF content was obtained from LECA clay supplemented with 0.8 g/L nitrogen concentration, highest ash content from vermiculite with no nitrogen concentration, highest fat content was obtained from silica sand with no supplementation, the highest moisture content was obtained from silica sand with 0.8 g/L nitrogen supplementation, whereas the highest NDF was contained from LECA clay with 0.36 g/L concentration. The findings of this study revealed that although, nutrients and proximate composition of *M. crystallinum* is intensely influenced by different growing media and various nitrogen concentration.

Also, this study showed that the increased of nitrogen in the growing media cannot be useful in production of proximate composition of *M. crystallinum*. However, this study also suggested that growing media with high retention are suitable to produce minerals supplemented with any nitrogen concentration including control.

Keywords: Proximate, composition, malnutrition, and moisture content

6.2 Introduction

Global warming has a significant impact on the agricultural sector, which is reliant on the environment and natural resources (Davies, 2014). Agricultural systems are under additional stress because of climate change-related issues like elevated temperature and an increase in the frequency of extreme weather events like drought, which also affect crop nutrition and potential production (FAO, 2007; Du Preez, 2017; Zwane, 2019). The agriculture industry has been utilizing a lot of fertilizer to increase crop nutrition and yield throughout time to sustain food supply (Sedlacek *et al.,* 2020). Nitrogen is now more important than ever (Good, 2018) being the most widely used fertilizer since it is crucial to the growth and development of plants (Galloway *et al*., 2017). However, increased fertilizer use has been shown to have detrimental environmental effects. The ecology suffers because more than 40 to 70 percent of nitrogen fertilizers are lost to the environment. The availability of nitrogen is a crucial environmental component for plant growth (Erisman *et al*., 2015) as it directly influences plant metabolism, material movement, and nutrient distribution. It was discovered that nitrogen addition improved nutrient cycling, balanced plant nutritional composition, and increased enzyme activity (Aczel, 2019).

Proper planting and nitrogen application patterns enable optimal use of water, air, and heat, which is crucial for boosting plant development and water-nitrogen usage efficiency (Lv *et al.,* 2023). As a result, hydroponic systems are becoming more and more popular. The hydroponic system is a productive culture for producing crops in assisted agriculture (Velazquez-Gonzalez *et al.,* 2022). For ornamental plants, vegetable gardens, and field crops, hydroponic systems are where fertilizers are most frequently employed.

It is common knowledge that plants produced in hydroponic systems can expand up to 50% faster than those grown in soil. Hydroponics can benefit from automatic irrigation and fertilization management since it creates a safe environment for culture and frees up room for multi-layer vertical production (De Clercq *et al.,* 2018). To keep up with the demand for food, crop output must increase in a sustainable way, according to the growing world population (Hirel *et al.,* 2011).

Mesembryanthemum crystallinum L., a succulent edible plant, is becoming popular as a new salad ingredient. Due to the high concentration of phenolic and other antioxidants in ice plants, they have a high nutritional value for humans (Xia *et al*., 2022; He & Qin, 2021). The ice plant is consumed as food, as a nutraceutical, and in therapeutic cosmetics (Varzakas *et al*., 2016). Food diversity has a direct impact on people's wellbeing and is a crucial component of household food security (Sibhatu *et al.,* 2022). Intake of micronutrients can be effectively increased by including salads and raw vegetables in diets in the United States. In addition to increasing the likelihood that daily nutrient needs will be met, *M. crystallinum* is a unique and highly valuable salad ingredient (Beal *et al.,* 2022). Niu and Masabni (2018) stated that growers in greenhouses are looking to diversify their crops as hydroponics and controlled environment technologies are increasingly used to produce high-quality, fresh vegetables.

Despite its edibility and pharmacological values, there is dearth of information on *M. crystallinum* particularly on the formation of essential minerals under various growing conditions supplemented with varied nitrogen concentrations. As a result, knowledge on the possibilities and advantages of nutrition is lacking or incomplete. To develop a growth protocol to produce mineral and proximate compositions, it is necessary to study the effects of various nitrogen concentrations and growing media on *M. crystallinum* when it is cultivated in a hydroponic system.

6.3 Materials and Methods

6.3.1 Experimental location

The experiment took place in the Department of Horticultural Sciences research greenhouse at the Cape Peninsula University of Technology Bellville campus in Cape Town, South Africa, located at 33°55’56” S, 18°38’25” E.

The study greenhouse had mist, a heating bed, and environmental control, with temperatures set to range from 21- 26 °C during the day and 12–18 °C at night, with Relative Humidity averages of 60%. The greatest daily photosynthetic photon flux density (PPFD) was 1020 mol/m^2/s, with an average of 420 mol/m^2/s.

6.3.2 Plant preparation, treatments and irrigation

The primary purpose of this research is to look at how *M. crystallinum* responds to different nitrogen concentrations and different growing media in hydroponic systems in to develop a growth protocol to produce minerals and proximate. The experiment used four similar hydroponic systems (Nutrient Film Technique). Each system was given the numbers S1 through S4. Tables were utilized as a surface for gutters. Sixteen gutters were placed on four different tables, with four gutters serving as plant supports on one of them. The gutters were held in place with cape ties. Ten pots having diverse substrates were placed in each gutter. Each table has its own water supply. Each reservoir has a submersible water pump that pumped fertilizer solution through a 20 ml pipe to each gutter. All gutters were sloped to allow the nutritional solution to circulate. All gutters were covered with black plastic polyethylene sheets to keep the fertilizer solutions out of direct sunlight. Algae does not grow on polyethylene sheets. Each gutter was made to accommodate ten pots. Each gutter was filled with G1 (silica sand), G2 (vermiculite), G3 (LECA-clay), and G4 (LECA-clay) in the following order: G1 (silica sand), G2 (vermiculite), G3 (LECA-clay), and G4 (LECA-clay) (coco-peat).

The first task was to collect and sow viable seedlings from Granger Bay Campus. The next step was to choose turgid, robust, and disease-free plants from the propagated plants once the seed germinated. As a result, the experiment was carried out with disease-free plants of the same height, leaf size, leaf color, branch number, and width. 160 plants (n=160) were chosen from propagated plants and placed in 12.5 cm pots with varied substrates. Different nitrogen concentrations were fed to 40 plants (n=40). Nitrogen was applied in four different doses per reservoir: 18g, 300g, 0g, and 40g. Nitrogen was only applied by hand. Fertilizer was administered first thing in the morning to guarantee maximum effectiveness. The plants were kept in a climate-controlled greenhouse.

Mesembryanthemum crystallinum seeds were collected on February 8th, 2021, from a selected plant population growing along the coast at the CPUT Granger Bay campus (33°53′58.2″ S, 18°24′41.4″ E). A total of 1,000 seeds were planted to ensure that at least 160 seedlings were accessible for the experiment. Seeds were frozen for twelve hours before sowing to disrupt dormancy. Sifting sand was done to eliminate foreign debris from a mixture that contained 70% sand and 30% peat. Sowing trays were filled with media, levelled, and watered. Seeds were planted, and then a thin layer of sand was put to cover. The trays were placed in a warm room with at least ten hours of light every day, and water was added again. Seed germination was aided by misting the trays with water twice a day, in the morning and afternoon; seeds sprouted in seven to twenty-one days. Seedlings were transplanted to pots (8.8 cm) after four weeks from trays to prevent competition, and plants were watered twice daily. After reaching two to three centimeters in height, plants were placed in a hydroponic system for two weeks to adapt, 160 *M. crystallinum* seedlings of uniform size were transplanted into 12.5 cm pots with sterilized growth mix including silica sand, peat, vermiculite, and LECA-clay. The plants that were picked grew the fastest and were left to grow. During this time, the plants were fed a nutrient solution three times per week. The nutrient solution was made by mixing Nutrifeed^{tm} (produced by Starke Ayres Pty. Ltd. Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, South Africa, 1619) with municipal water at a rate of 10 g per 5L. The nutritional solution contained N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg), and Zn (240 mg/kg). The planted plants were given 7 days of clean water after 14 days of growth to wash away any pollutants, and four treatments were organized by adding varying quantities of nitrogen fertilizer (18, 30, and 40 g) and 0 as a control. Plants were fed with or without nitrogen treatment via spraying from a nutrient solution reservoir. Hydroponic systems were operated for 15 minutes in the morning and afternoon. The pH was maintained at 6.0 in all treatments. All plants were harvested after 12 weeks of nitrogen-treated plant growth, and numerous postharvest measures were taken.

Table 6.1 Arrangement of each gutter on four NFT systems with different growing media and N fertilizer concentrations.

NFT/ Table	Gutter 1	Gutter 2	Gutter 3	Gutter 4
1	0.36 g/L Peat	0.36 g/L + Vermiculite	0.36 g/L LECA clay	0.36 g/L Silica sand
2	0.6 g/L+ Peat	0.6 g/L + Vermiculite	0.6 g/L + LECA clay	0.6 g/L + Silica sand
3	0 g + Peat	0 g + Vermiculite	0 g + LECA clay	0 g + Silica sand
4	0.8 g/L + Peat	0.8 g/L + Vermiculite	0.8 g/L + LECA clay	0.8 g/L + Silica sand

*NFT = Nutrient Filter Technique hydroponic system

6.3.3 Nutritional analysis

Leaves and stems of *M. crystallinum* cultivated in different media treated with various nitrogen concentrations were harvested near flowering. The harvested plant samples were kept in a labelled brown paper bag (16 x 24 cm). The marked brown paper bags containing the leaves and stem were laid on a table in a cool room to air dry after they were oven-dried at 40 degrees Celsius. The crispy dried plant materials were powdered using a capacity standard coffee grinder (Mellerware- Aromatic Coffee Mill & Grinder) and sent to the analytical lab of the Department of Agriculture and Rural Development in KwaZulu Natal Province for full feed analysis.

6.3.4 Mineral analysis

The elemental analysis and the mineral composition of each set of plant harvested from replicated treatments in the experiment were determined using the Inductively Coupled Plasma-Optical Emission Spectrometer in the analytical laboratory of the Department of Agriculture and Rural Development, KwaZulu Natal Province as described by Jimoh *et al.,* (2020).

6.4 Proximate Analysis

6.4.1 Moisture content

The procedure described by Jimoh *et al.,* (2020) was slightly modified to determine the moisture content. Empty porcelain containers were dried in an oven at 105 °C for one hour, allowed to cool, and weighed at W1.

M. crystallinum samples that had been ground into about 1 g each were put in a vessel and dried in an oven at 105 °C to a constant weight. Before being reweighed, the container and its contents were cooled in a desiccator (W3). The equation below was used to calculate the percentage moisture content.

Equation 1: % Moisture content = $\frac{W2-W3}{W2-W1} \times 100$

6.4.2 Crude Fibre content

This crude fibre content was assessed with a few minor modifications from Miechowska and Dmowski (2006) protocol. About 2 g of the powdered samples were boiled for 30 minutes with a digestion tablet in 100 mL of 1.25 percent concentrated H_2SO_4 before being filtered under pressure. The digested residue was rinsed with 100 mL of 1.25% NaOH solution after being repeatedly washed with boiling water until a clear mixture was obtained. Once the residue had been created, it was dried at 100 °C, cooled in a desiccator, and weighed (F1). The residues were then cooled in a desiccator, incinerated for 5 hours at 550 °C in a muffle furnace, and then reweighed (F2). The estimated crude fibre percentage was.

Equation 2: % Crude fibre = $\frac{F1-F2}{\text{Original weight of the pulverised sample}} \times 100$

6.4.3 Crude fat content

The crude fat content was calculated following the laboratory procedures from the Association of Official Analytical Chemists (AOAC), (2016). A pulverized sample weighing about 1 g was extracted in 100 mL of diethyl ether and shaken for 24 hours on an orbital shaker. The mixture was then filtered, and the filtrate was collected in clean beakers that had previously been weighed (W1). After shaking the ether extract for an additional 24 hours on an orbital shaker, it was diluted with 100 mL of diethyl ether to equilibrate, and the filtrate was collected in a beaker (W1). Before being reweighed in the beaker, the ether filtrate was concentrated to dryness in a steam bath and oven-dried at 55 C. (W2). Thus, the crude fat content was determined to be.

Equation 3: % Crude fat content = $\frac{W2-W1}{\text{original weight of the pulverised sample}} \times 100$

6.4.4 Ash content

The protocol described by AOAC (2016) protocol was used to determine the percentage ash content of the treated plant samples. After being marked with sample codes using a heat-resistant marker, porcelain crucibles were oven-dried at 105 °C for an hour. The crucibles were weighed after desiccating to cool them (W1).

Afterwards, 1 g of ground samples was measured and placed into porcelain crucibles that had already been weighed (W2). To ash the samples absolutely, the crucibles with their contents were put in a muffle furnace set to 250 °C for an hour and then 550 °C for 5h. After cooling in a desiccator, the samples were weighed (W3). The samples' ash content was calculated as

Equation 4: % Ash content = $\frac{\mathrm{W2-W3}}{\mathrm{W2-W1}} \times 100$

6.4.5 Crude protein

This was determined by heating 2 g of ground samples with 20 mL of concentrated H_2SO_4 to a clear mixture while using a digestion tablet as a catalyst as reported by Adegbaju *et al.*, (2019). After being filtered and digested, the extracts were distilled and then dissolved in 250 mL. In a 500 mL round-bottomed flask, the aliquot containing 50 mL of 45% NaOH underwent further distillation, and 150 mL of the distillate was transferred into a flask containing 100 mL of 0.1 M HCl. Methyl orange was then used to titrate this against 2.0 mol/L of NaOH. A yellow color change signaled the end of the titration, and the equation below was used to calculate the percentage nitrogen content.

Equation 6: $=\frac{[(\text{ml std acid} \times \text{N of acid})-(\text{ml bank} \times \text{N of base})]-(\text{ml std base} \times \text{N of base})\times 1.4007}{\text{original weight of the pulverised sample}}$

Where N = normality and the percentage crude protein were obtained by multiplying the nitrogen value by a constant factor of 6.25 (USDA, 2018)

6.4.6 Neutral detergent fibre (NDF)

The NDF composition of the samples was determined using the equation below, as described by Idris *et al.,* (2019).

Equation 7: % NDF = $\frac{(\mathrm{W1+W2})-\mathrm{W1}}{\text{Weight of the sample}} \times 100$

6.4.7 Non-Fibre carbohydrate (NFC)

The below formula was used to calculate the sample's non-fiber carbohydrate content.

Equation 8: % NFC = $100 - (\%\ \text{Ash} + \%\ \text{Crude fat} + \%\ \text{Crude protein} + \%\ \text{NDF})$

6.4.8 Energy content

By multiplying the values for total carbohydrate, crude lipid (excluding fiber), and crude protein by factors (17 KJ, 37 KJ, and 17 KJ) using the FAO (2003) conversion factor, the energy content of each sample of M. crystallinum from various treatments was calculated.

Energy content (KJ/100 mg) = (CHO17) + (Crude fat37) + (Crude protein17), where CHO stands for total carbohydrate in the equation below; (Tylutki *et al.,* 2008).

Equation 9: CHO = NFC + N

6.5 Results

This study showed significant differences in proximate contents of M. crystallinum grown from different growing media and various nitrogen concentrations with regard fat, ash, ADF, protein, and moisture.

6.5.1 Effects of soil types on mineral elements

According to this study, several mineral elements including phosphorus (P), calcium (Ca), potassium (K), magnesium (Mg), and sodium (Na) were evaluated. The peat media treated with 0.6 g/L concentration produced the maximum yield of Ca. While silica sand that had been treated with 0.8 g/L of concentration had the lowest Ca content. Different growing mediums and nitrogen concentrations were used, and while vermiculite supplemented with 0 g/L, 0.8 g/L, and 0.6 g/L had the highest Mg content, LECA clay with 0.36 g/L and 0.8 g/L, as well as silica sand with 0.8 g/L nitrogen concentration, had the lowest Mg content values.

The silica sand supplemented with 0.8 g/L concentration had the maximum sodium (Na) level, while the silica sand supplemented with 0 g/L concentration had the lowest content. Different growing media had an impact on N levels; the peat supplemented with 0.8 g/L nitrogen concentrations had the highest content, while vermiculite with a 0 g/L control had the lowest. Vermiculite treated with 0.6 g/L nitrogen concentration had the maximum potassium content. The silica sand with the highest K/Ca+Mg value had a nitrogen content of 0 g/L, while the peat with the lowest nitrogen content had 0.8 g/L of nitrogen added. The maximum content value for Zn was achieved from silica sand mixed with 0.36 g/L nitrogen concentration. Zn was affected by different growing media and different nitrogen concentrations. The vermiculite added 0.36 nitrogen concentration yielded the lowest concentration.

Table 6.2 Effect of different growing media and different nitrogen concentration on proximate content of *M. crystallinum* leaves

Media	Nitrogen (g/L)	ADF %	Moisture %	Ash%	Fat %	Protein %	NDF %	NFC %	Carbohydrate %
LECA Clay	0.00	11.93±0.07gh	8.47±0.15defg	35.25±0.63d	2.33±0.23ab	20.44±0.55h	23.72±0.29fg	18.26±0.44bc	41.98±0.37c
	0.36	20.92±0.69ab	11.20±0.38g	31.15±0.59f	2.05±0.07aabc	23.62±0.62de	30.83±0.88a	12.35±0.54e	43.18±0.71abc
	0.60	20.39±0.59ab	8.72±0.38cdef	32.94±0.57ef	2.04±0.06abc	28.26±0.34bc	27.07±0.57bcd	9.69±0.39f	36.76±0.48e
	0.80	21.68±0.32a	8.01±0.03fg	38.08±0.88c	2.06±0.06abc	27.17±0.37c	27.24±1.24bc	5.45±0.64g	32.69±0.94f
Peat	0.00	11.39±0.39h	8.77±0.15bcdef	32.23±0.73f	1.89±0.25bcd	21.34±0.65gh	21.62±0.48h	22.62±0.53a	44.24±0.51ab
	0.36	14.36±0.38ef	8.90±0.08efg	29.00±0.70g	2.07±0.07abc	28.98±0.29b	22.74±0.74gh	17.21±0.45c	39.95±0.60cd
	0.60	12.99±0.39eg	9.09±0.2abcd	32.00±1.11f	1.66±0.16cd	23.67±0.33de	22.89±0.15gh	19.78±0.44b	42.67±0.30bc
	0.80	11.69±0.41hg	9.04±0.26abcd	31.75±0.88f	2.06±0.06d	34.04±0.06a	20.11±0.52i	12.04±0.38e	32.15±0.45f
Silica Sand	0.00	20.26±0.75b	9.46±0.38abc	33.02±0.37ef	2.38±0.13a	19.65±0.70h	28.36±0.41b	16.59±0.40cd	44.95±0.41a
	0.36	16.96±0.34c	8.69±0.61cdefg	27.89±0.25g	2.17±0.07ab	24.96±0.59d	27.03±0.14bcd	17.95±0.26c	44.98±0.20a
	0.60	14.76±0.54de	9.74±0.37ab	36.10±1.00cd	1.91±0.13bcd	23.87±0.33de	25.50±0.54de	12.62±0.50e	38.12±0.52d
	0.80	11.99±0.69gh	9.83±0.37a	38.89±0.79b	1.88±0.2bcd	23.55±0.55de	20.55±0.46i	15.13±0.50cde	35.68±0.48ef
Vermiculite	0.00	11.70±0.6gh	8.99±0.01abcde	42.02±0.49a	2.08±0.10abc	19.94±0.07h	24.95±0.06ef	11.01±0.18ef	35.96±0.08ef
	0.36	15.79±0.21cd	6.80±0.07abcdef	32.65±0.65ef	1.95±0.06abc	23.06±0.43ef	26.31±0.33cde	16.03±0.37cd	42.34±0.35bc
	0.60	14.83±0.37de	8.83±0.30bcdef	33.11±0.29ef	2.31±0.19cdab	21.95±0.75eg	25.81±0.41cde	16.82±0.41cd	42.63±0.41bc
	0.80	15.06±0.25de	8.39±0.29defg	34.42±0.57de	0.99±0.05def	22.89±0.59de	26.27±0.29cde	15.43±0.38cde	41.70±0.34c

Means that do not share a letter are significantly different at $P \leq 0.05$. * ADF = acid detergent fibre, NDF = neutral detergent fibre, NFC = non fibre carbohydrate

Table 6.3 Macronutrients and micronutrients of *M. crystallinum*

Media	Nitrogen (g/L)	Macronutrients (mg/100g)						Micronutrients (mg/100g)			
		K	Ca	Mg	N	P	Na	Mn	Fe	Zn	Cu
LECA Clay	0.00	10288.5±1.5e	1420.5±0.5h	1249.4±0.6f	3361.5±1.5m	529.3±1.0e	2861.5±1.5c	17.45±0.10d	65.09±0.11h	9.36±0.04e	0.93±0.03d
	0.36	862.0±2.0p	1549.5±0.5g	790.5±0.5l	3881.5±1.5f	662.0±2.8b	978.5±1.5n	8.42±0.00l	49.60±0.50j	8.40±0.10gh	0.11±0.01g
	0.60	9422.5±2.5i	1630.5±0.5e	891.6±1.6j	4471.5±1.5c	660.9±1.3b	1020.5±0.5m	10.70±0.30i	50.90±0.10i	7.85±0.15i	0.15±0.05fg
	0.80	7379.0±1.0m	1390.5±0.5i	761.8±1.8m	4397.0±3.0d	550.6±0.9d	2171.0±1.0f	10.85±0.15i	79.60±0.40f	8.00±0.10hi	0.23±0.03ef
Peat	0.00	9919.0±1.0f	1569.0±1.0f	917.5±2.5i	3519.0±1.0l	549.5±0.7d	3774.5±4.0b	9.75±0.05j	23.15±0.50m	8.75±0.05fg	0.00±0.00h
	0.36	9338.5±1.5j	2168.5±1.5b	919.0±1.0i	4677.5±2.5b	529.7±0.4e	1158.5±1.5k	11.30±0.01h	20.77±0.03o	15.39±0.41c	0.15±0.05fg
	0.60	10371.5±1.5d	3039.0±1.0a	959.1±0.9g	3728.5±1.5j	549.3±1.1d	1678.5±1.5g	6.15±0.05m	24.04±0.06l	8.95±0.050ef	0.10±0.00gh
	0.80	7083.0±3.0n	2070.5±0.5d	960.5±0.5g	5439.0±1.0a	489.0±1.4f	2509.0±1.0b	15.95±0.06e	32.55±0.05k	15.84±0.06b	0.00±0.00h
Silica Sand	0.00	8958.5±1.5k	1210.5±0.5k	868.5±1.5k	3257.0±3.0n	49.8±0.3i	188.5±1.5p	9.30±0.00k	67.80±0.09g	7.90±0.00i	0.24±0.04ef
	0.36	8611.0±1.0l	2099.0±1.0c	939.0±1.0h	3997.5±2.5e	839.8±0.3a	1029.0±1.0l	35.80±0.30a	22.05±0.25n	17.95±0.15a	0.95±0.05d
	0.60	10849.0±1.5b	1180.5±0.5l	1578.5±1.5d	3768.0±2.0h	589.5±0.7c	1429.0±1.0h	17.30±0.20d	139.05±0.45c	9.15±0.15ef	2.05±0.06c
	0.80	4062.0±2.0o	770.5±0.5p	480.9±0.9n	3848.0±2.0g	339.8±0.3g	7762.0±2.0a	17.95±0.05c	22.38±0.08n	8.2±0.21hi	0.31±0.01e
Vermiculite	0.00	9494.0±6.0h	1359.0±1.0j	2048.0±2.0a	3178.5±1.5o	489.5±2.1f	409.0±1.0o	9.66±0.05jk	384.75±0.15a	9.32±0.12e	2.15±0.05c
	0.36	10662.0±1.5c	1109.5±0.5m	1450.5±1.5e	3762.5±2.5hi	60.8±1.3h	1272.0±2.0j	13.75±0.25g	81.95±0.15e	6.35±0.50j	2.15±0.05c
	0.60	11231.0±0.5a	1049.5±0.5n	1589.5±0.5c	3631.5±1.5k	588.9±1.6c	1332.0±2.0i	15.17±0.07f	107.35±0.05d	6.75±0.60j	2.45±0.05b
	0.80	9859.0±1.0g	988.5±1.5o	1718.9±1.1b	3759.5±0.5i	50.1±0.2i	2421.5±1.5e	20.48±0.08b	169.47±0.17b	10.54±0.05d	3.11±0.01a

Means that do not share a letter are significantly different at P ≤0.05.

6.6 Discussion

This study found substantial differences in the macro and microelements nutrients, and proximate uptake in *M. crystallinum* plants grown on varied growing conditions and nitrogen concentrations. The movement of nutrients throughout plants is important at all stages of plant development, leading to changes during various life cycles like vegetative, blooming, and senescence (Perez-Llorca & Munne-Bosch, 2021). Plants are a rich source of nutrients, and there has recently been interest on a global scale in the study of the nutritional makeup of halophytes plants to address food security, prevent some diseases, and combat malnutrition (Talabi *et al*., 2022).

The relative production of plants depends on the abundance of leaves, plant height, and quality yield depend on the environmental sources of mineral nutrition in plants (Kathpalia & Bhatla, 2018). Plants need to be exposed to a proper environment that is suitable for the acquisition of mineral nutrients, transport and utilization to provide the ideal growth output (Delhaize *et al.,* 2015; Seleiman *et al.,* 2021). Therefore, developing a plant growth protocol requires establishing an appropriate growing medium and nitrogen. The concentration of chemicals that are readily available is also impacted by the growing medium; these compounds are taken up by the root hairs and distributed to the remainder of the plant. Based on findings from this study and earlier research by Masclaux-Daubresse *et al.,* (2010), the uptake of minerals by plants is influenced by the growing medium, as evidenced in the nutritional value of *M. crystallinum* cultivated on various growing media.

Dietary components such as potassium and sodium are essential for a healthy and balanced diet in humans. These minerals help a variety of body processes, including bettering brain and heart function, constructing and maintaining strong teeth and bones, and preserving healthy muscles (Foirentini *et al.,* 2021). This study showed that *M. crystallinum* grown on vermiculite with 0.6 g/L nitrogen supplementation possesses a high potassium content. Given the significant impact of holding capacity of the growing medium on plant mineral uptake, the high potassium content cultivated in vermiculite supplemented 0.6 g/L may be attributed to bioretention of minerals in vermiculite (Pisa *et al.,* 2020). Potassium is a vital mineral that the body needs for healthy fluid and nerve transmission, controlling blood pressure, relating waste, and contraction (Udensi, 2017).

A balanced diet should have plenty of potassium. As intracellular and extracellular cations required for the maintenance of muscle contractility, blood pressure, and condition of nerve impulses, potassium is of enormous functional significance (Gerlinger *et al.,* 2019). According to the minerals that were analysed in this study, *M. crystallinum* may be a source of these minerals. Different growing media had an impact on *M. crystallinum*'s mineral composition in the current investigation.

The highest Ca level was found in *M. crystallinum* samples grown in peat with 0.6 g/L nitrogen addition. This agrees with the findings of Jama-Rodzeńska *et al.* (2022) who reported that fertilized peat substrate improve the calcium content in *Lactuca sativa* L. This study also shows that the highest nitrogen concentration does not result to the highest Ca level. The primary functions of calcium in the human body include the activation of specific enzymes, cell signalling, nerve function, and support of the skeletal system via strong bone tissue (Waheed *et al.,* 2019). It has long been understood that Ca is a crucial ingredient for plants and that different plant species have different requirements for Ca as well as tolerance levels for Ca in the rhizosphere. These variations among plant species not only affect the flora of calcareous soils naturally, but also have an impact on breeding crops to increase the delivery of calcium to the human body (White & Broadly, 2003).

The moisture, ash, crude fat, crude protein, carbohydrate, and energy contents of the leaves of *M. crystallinum* were determined. The amount of moisture in many foods is a measure of their water activity, which aids in preserving the protoplasmic material that gives leaves their distinctive texture and cells (Sudha *et al.,* 2015). The plant samples grown with silica sand had the greatest reported moisture content on all nitrogen concentrations, also, LECA clay had a significantly low moisture content was observed this is due to poor water retention (Hamouz *et al.,* 2018). The plant samples that were exposed to vermiculite without any nitrogen supplementation had the greatest ash content values. In general, a high ash content implies that the plant is rich in dietary fibres, which shelters digesting organisms in the gastrointestinal tract. Vegetables should have fibre since it improves insulin and glucose tolerance. The measurements of neutral detergent fibre and acid detergent fibre indicate the digestibility and palatability of the fibres found in plant cell walls, including cellulose (Kaneko *et al.,* 2023). Plant samples made from LECA clay and treated with 0.36 g/L nitrogen concentration yielded the greatest value of ADF.

The LECA clay that had 0.8 g/L of nitrogen added to it had the greatest ADF value. However, Low-fat diets focused on plants are beneficial for your health. However, unsaturated fatty acids rule in real plant-based diets. Dietary lipids increase the palatability of plants by absorbing and preserving their flavour (Mouritsen, 2016). The present study's measurements of the fat composition in *M. crystallinum* (2.37 - 0.99%) are lower than those from prior research that Jimoh *et al.* (2020) has reported for *Amaranthus caudatus* L. The silica sand with nitrogen concentration augmentation had the highest content value of fat. In comparison to Jimoh *et al*. (2020), the results for protein content reported in this study were much greater. This study shows the ideal protein value from samples grown in peat growing media with a 0.8 g/L concentration supplement.

6.7 Conclusion

The results of this investigation showed that although *M. crystallinum* nutrients and nearby composition are strongly influenced by its growing medium and nitrogen content. Additionally, this study demonstrated that adding more nitrogen to the growing medium is ineffective for producing *M. crystallinum* near-complete composition (proximate content). However, this study also made the case that high retention growing media are appropriate to produce minerals supplemented with any level of nitrogen, even the control. Therefore, this result concurs with earlier findings indicating the mineral uptake is influenced by the growing medium. Further research is advised to determine the minerals and nearby compositions of *M. crystallinum* at various growth stages to determine which growth stage yields the best minerals and nearby under which growing medium and nitrogen concentration.

6.8 References

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CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Chapter seven: General discussion, conclusions and recommendations

7.1 General discussion

Food security has developed into a major problem as 800 million people are hungry, while many more millions are at further risks despite the progress that has been made in some parts of the world to reduce hunger, particularly in Africa where the hungry population is growing (FAO, 2015). Global climate change, including rising temperatures, water scarcity, and extreme weather changes, will continue to pose challenges in already stressed agricultural ecosystems due to a continued population increase (Krumah, 2018). It is estimated that over the next thirty years, the rate of earth's population will increase more than the way it is currently increasing (FAO, 2015) resulting in increasing demand for food. As the demand for food supply is escalating and expected to exceed the existing production capacity, a priority for horticultural researchers is to rework the improvement of production methods by determining and implementing efficient systems.

In South Africa, wild edible plants are not freely used as edible and there is also little information on many of the species (Shai *et al.,* 2020). Notwithstanding the species not being used as food in South Africa, Abd El-Gawad and Shehata (2014) reported that growing these plants could provide local communities access to healthy nutritional diets. Additionally, cultivating new crops could also provide financial growth opportunities to disadvantaged communities through job creation. *Niederle (2012*) documented that *M. crystallinum* is a halophyte that can be cultivated in high saline soils with high levels of sodium equivalent to those found in seawater. As Xia *et al*. (2022) stated, the plant is harvested for both local and commercial uses, as food, medicine, and a source of soap suggesting that *M. crystallinum* is a plant with diverse medicinal and food values. Therefore, this study will close the information gap on cultivation, pharmacological potential and nutritional benefits of *M. crystallinum* under different growing media and nitrogen treatments.

The effects of different growing media and nitrogen concentrations on vegetative growth of *M. crystallinum* were reported in chapter 3. Growth parameters such as leaf number, leaf length, root length, fresh and dry weight were examined. Plant growth results for *M. crystallinum* plants that were treated with nutrient solutions containing 40 and 30 grams of nitrogen fertilizer combined with vermiculite were the highest.

Findings from this study are supported by a recent study by Hassan *et al*. (2023), which found that vermiculite combined with a high nitrogen treatment produced the highest values for the most vegetative growth. According to Pisa *et al*. (2020), vermiculite increases water retention which results in a high total nitrogen content. Compared to other growing media, the vermiculite material has greater interaction with aeration and water-holding ability which may be responsible for this outcome.

Additionally, this study demonstrated that peat provided the maximum fresh weight when supplemented with 30g nitrogen, outperforming LECA-clay, and silica sand. This is a result of key function of peat in nutrient retention and high water-holding capacity. The high humus content of peat allows it to store nutrients and always avail nutrients to plants (Kitir *et al*., 2018). Plants treated with LECA-clay but not supplemented with nitrogen had the lowest results. Thus, it has been demonstrated in this work that *M. crystallinum* needs nitrogen for optimum growth. This contravenes the finding by Xia *et al.* (2022) that *M. crystallinum* does not require fertilizer for growth. Finding from this study might be an option for potential growers of the plant in provinces suffering the negative effects of the drought.

The effects of various nitrogen treatments and selected growing media on the chlorophyll content of *M. crystallinum* was examined in chapter 4. The results of this investigation showed that nitrogen application had a positive effect on chlorophyll production in *M. crystallinum*. The nitrogen concentration in LECA clay supplemented with 0.6 g/L had a comparable effect on chlorophyll content to vermiculite (0.6 g/L), peat (0.8 g/L), LECA clay (0.8 g/L), and peat (0.6 g/L) treatments produced the highest chlorophyll content. This corroborates findings of Trelka *et al*., (2010) who reported that growing medium had no impact on the amount of chlorophyll in leaves. Nevertheless, Muhammad *et al*. (2022) agrees with the current study that the amount of chlorophyll in leaves increased with increasing N application. These results indicate that, *M. crystallinum* can be cultivated in most growing media and nitrogen treatments for the generation of chlorophyll. The results of this study also suggest that *M. crystallinum* needs nitrogen to increase chlorophyll production. Growing media is not always necessary for chlorophyll formation (Ngxabi *et al*., 2021). Findings from this study agrees with other studies that the higher the nitrogen fertilizer application the higher the chlorophyll content (Muhammad *et al*., 2022; El-Sorady *et al*., 2022; Zhang *et al*., 2022).

Chapter 5 measured how the secondary metabolites and antioxidant capacity of *M. crystallinum* were modulated by various nitrogen treatments and selected growing media. Polyphenols and antioxidants, which aid in protecting the organism from reactive species damage are abundant in fruits and vegetables (Rudrapal *et al.,* 2022). The diversity of phytochemicals in plants is reveals their therapeutic effects (Jimoh *et al*., 2019). The phytochemicals that plants contain have an impact on their antioxidant properties. Nitrogen availability and the right growing medium are two of the most crucial factors that control the accumulation of antioxidants (Song *et al*., 2019). The total polyphenol content of *M. crystallinum* leaves cultivated in LECA clay supplemented with N fertilizer (0.36 g/L) was significantly higher (6.4 mg GAE/g). The findings of this study demonstrated that the accumulation of phytochemicals and antioxidants was significantly influenced using light soilless medium and moderate N fertilizer. This work contradicts earlier research by Rahim Doust *et al*. (2023), which claimed that only growing media with a high capacity for retaining water, such as peat and vermiculite can produce significant levels of secondary metabolites. Three concentrations that statistically share the rankings in the FRAP content: peat with added nitrogen fertilizer (0.6 g/L), LECA clay with additional nitrogen fertilizer (0.36 g/L), and LECA clay without added nitrogen fertilizer (0 g/L). To increase the amount of FRAP produced, large nitrogen (N) external inputs are frequently used, but this work shows that 0 g/L can result in significant FRAP content. This study proves that *M. crystallinum* plants may be the source of antioxidants. The vermiculite without the supplementation of N fertilizer had the lowest flavonol content (1.8 mg QE/g), while the peat that had no N fertilizer treatment had the second lowest flavonol content (0 g/L). This study found no connection between high phytochemical and antioxidant content and fertilizer use. Since vermiculite consistently had the lowest mean values of phytochemicals, this study shows that growing media with a high water-holding capacity have no beneficial effects on the accumulation of phytochemicals and antioxidants.

The impact of different growth media and nitrogen treatments on the nutritional and proximate content of *M. crystallinum* was investigated in Chapter 6. The study demonstrated that vermiculite supplemented with 0.6 g/L nitrogen concentration had a positive effect on *M. crystallinum* plants, hence, a high potassium content was recorded from this treatment.

Whereas *M. crystallinum* samples grown in peat with 0.6 g/L nitrogen input had the highest calcium content. The highest Ca level was found in *M. crystallinum* samples grown in peat with 0.6 g/L nitrogen addition. This agrees with the findings of Jama-Rodzeńska *et al*. (2022) who reported that fertilized peat substrate improves the calcium content in *Lactuca sativa* L. The amount of moisture in many foods is a measure of their water activity, which aids in preserving the protoplasmic material that gives leaves their distinctive texture and cells (Sudha *et al*., 2015). Plant samples harvested from silica sand had the greatest reported moisture content. Due to poor water retention in LECA clays, a significantly low moisture content was observed (Hamouz *et al*., 2018). The plant samples that were cultivated with vermiculite without any nitrogen supplementation had the greatest ash content values. The fat compositions in *M. crystallinum* (2.37 - 0.99%) reported in the present study are lower than those from prior research that Jimoh *et al*., (2020) had reported.

7.2 Conclusion and recommendations

According to this study, *M. crystallinum* grew significantly better when different nitrogen concentrations and growth material were used in hydroponic systems. Findings from the study suggest that *M. crystallinum* can be easily cultivated in commercial quantities without the use of pricey or expensive equipment. The impacts of diverse nitrogen concentrations and growth media, however, on plant development, chlorophyll content, nutrient uptake and translocation, and antioxidant capacity, on the other hand, also demonstrated a substantial effect to support healthy plant growth. These findings suggest that *M. crystallinum* may be grown in hydroponic systems using varied nitrogen concentrations and growth media as a viable leafy green produce.

To support its economic viability as a leafy green vegetable, it is advised that additional research be conducted on the effect of sea water when applied directly to plants as well as its antibacterial and antifungal activities. It can therefore be concluded from this study that *M. crystallinum* can be grown hydroponically under different growing media and various nitrogen treatments to improve its yield and to produce nutrients, phytochemicals and antioxidants.

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