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Water stress and salinity tolerance of *Mesembryanthemum crystallinum* L. in hydroponics

by

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DECLARATION

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

30 August 2023

Signed **Date**

Abstract

In their natural growing environment, halophytes such as ice plants are subjected to salt and drought stresses simultaneously, but our understanding of the impacts of combined stress on plants is restricted. This study evaluated the individual and combined impacts of salinity and drought stress on plant development, mineral content, proximate content, and phytochemical composition of *Mesembryanthemum crystallinum*. Treatments consisted of four different irrigation treatments of 1 = 100 mL once a day; 2 = 100 mL once every 2 days; 3 = 100 mL once every 4 days; 4 = 100 mL once every 8 days in conjunction with four different salt concentrations of (0, 200, 400 and 800 ppm) applied in each treatment. Salt concentrations were determined by gradually raising the concentration of NaCl in the nutrient solution, while the control treatment received daily irrigation without NaCl. The results showed that plants irrigated with 800 ppm salinity every four days had a substantial rise in leaf number, fresh and dry weight. During the growing weeks, the chlorophyll content was similar among treatments, except for week eight, when the combination of salinity and drought reduced the chlorophyll content among treatments. The highest yields of N, Mg, and Cu were consistently recorded in plants that were not subjected to saline treatment, whereas P, K, Ca, Na, Zn, and Fe were consistently documented in plants that were subjected to both drought and salinity. Plants subjected to drought stress alone had high levels of acid detergent fibre (ADF), crude fat, protein, and neutral detergent fibre (NDF), whereas plants subjected to a combination of drought and salinity had the greatest levels of ash and moisture. The same pattern was seen in phytochemical accumulation, with polyphenols and flavonols being abundant in plants only exposed to drought stress. Plants irrigated every eight days, on the other hand, consistently had the greatest antioxidant capacity (FRAP) regardless of drought or saline treatment. These results imply that *M. crystallinum* could provide an additional source of nutrients in areas prone to salinity and drought. As a result, domestication in South Africa, a country with a scarcity of water, is highly suggested.

Keywords: Aizoaceae; bio-saline agriculture; edible halophytes; functional foods; underexploited vegetables.

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DEDICATION

I dedicate this thesis to:

- God who created, directed, and strengthened me to persevere in the face of adversity.
- My ancestors who have given down DNA features that enable me to persevere through difficult times for the promising future of upcoming generation.
- My mother, Theodora Mndi, who has always been there for me since day one of my life and has never given up on me.
- My dear sisters, Oyama Mndi, Zimasa Fatyela, Sikhona Mndi, and Esona Mndi, who have always shown me unconditional love and support throughout my life.
- To my son, Lubanzi Sumeya Mndi.

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Chapter one: General introduction, structure of the thesis, problem statement, aims, hypothesis, and objectives

1.1 General introduction

Mesembryanthemum crystallinum L., commonly named ice plant under Aizoaceae family is a high valued nutritious facultative halophyte vegetable crop that has been widely distributed to the southern coastal regions of Africa and is widely distributed along the coastal areas of Europe, the USA, Mexico, Chile, the Caribbean, and western Australia (You *et al.,* 2021; He *et al*., 2022). This crop species succeeds drought and salinity stress by switching photosynthetic mechanisms from C_3 photosynthesis to crassulacean acid metabolism (CAM) (Guan *et al*., 2020). Drought and salinity are considered as the major environmental factors which the major environmental factors that consistently contribute directly towards food insecurity, countries economic growth, and the increasing population health and wellbeing crisis (Adetunji *et al*., 2021). High soil salinity and drought stress are twin factors which affects the plant development and metabolic activities such as water relations, photosynthetic assimilation, and nutrient uptake (Ali *et al*., 2019; Teklić *et al*., 2021). Water is crucial for plant growth and development, transporting mineral salts, photosynthates, and plant metabolic activities. Water availability to plants is hugely affected by physical soil water availability and soil water solutes concentration, which is most explained by water potential mechanism where an increase in salt levels reduce water energic movement and leads to defected water availability to plants (Yadav *et al*., 2020; Gul *et al*., 2023).

Approximately 20% and 50% of the world's cultivated areas and the world's irrigated lands, respectively, are affected by salinization, and these percentages are still expected to increase in the future (Zhang *et al.,* 2018; Shahid *et al.,* 2018). Recently, between 2015- 2017, Cape Town in the Western Cape Province of South Africa was hit by drought, which led to minimised food production, causing drastic decline in the country's economy due to reduced harvested food quantities, a significant drop in exports, poor fruit and vegetable quality, and loss of household's income due to minimised agricultural production (Trautmann, 2018). This has further prompted the need to consider cultivating underutilized edible crops such as *M. crystallinum* that could better withstand these environmental stresses and contribute to increased food security (Atzori, 2021).

1.2 Structure of the thesis

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

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

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

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

4. The number of articles included depends on the content and length of each article and takes full account of the university's requirements for the degree as well as the one article already published, or "ready-for-publication" expected for a master's degree in this discipline.

5. The thesis should be examined normally and according to the requirements 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:

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

Chapter Two: This chapter provides insight into food security status, freshwater scarcity, soil salinization impact, and the potential of *Mesembryanthemum crystallinum* (ice plant) as a leafy vegetable to contribute towards overcoming food, soil salinization and water crises. It also highlights *M. crystallinum* uses, distribution, the environmental effect on growth, and the effects of salinity and water stress on plant growth and physiology. It also details the importance or roles of antioxidants, secondary metabolites, and proximate composition of *M. crystallinum*.

Chapter Three: This chapter evaluated the interactive effect of different salinity concentrations and irrigation intervals on the vegetative growth of *M. crystallinum*. The research justification, materials and methods, results and discussions are presented.

Chapter four: This chapter evaluated the interactive effect of different salinity and irrigation intervals on the chlorophyll contents of *M. crystallinum*. The research justification, materials and methods, results, and discussions are presented with further recommendation for effective cultivation protocols.

Chapter Five: This chapter evaluated the effect of different salinity and irrigation intervals on antioxidants capacity of 2,2-Diphenyl-1-picrylhydrazyl (DPPH), Fluorescence recovery after photobleaching (FRAP), and Trolox equivalent antioxidant capacity (TAEC), and secondary metabolites contents of polyphenols, total alkaloids, and total flavonoids content of *M. crystallinum*. The research justification, materials and methods, results and discussions are presented.

Chapter Six: This chapter evaluated the effect of different salinity and irrigation intervals on full feed contents of *M. crystallinum* leaves. The research justification, materials and methods, results and discussions are presented.

Chapter Seven: This chapter presents 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: List of all references used in the thesis.

1.3 Problem statement

Gradual increase of human population growth results in increased demand for food while the global climate changes, mostly, water scarcity consistently results in less food production, thereby threatening the food security status worldwide (Dinar *et al*., 2019; Islam & Karim, 2019; Wang, 2019). The agricultural sector worldwide is facing a challenge to produce 70 per cent more food crops for an estimated additional 2.3 billion people by 2050 throughout the world (Rahneshan *et al.,* 2018; Gupta, 2019; Polash *et al.,* 2019; Searchinger *et al*., 2019), while global climate change is already having a significant negative effect on food production though the population has not doubled yet (Leisner, 2020). The Worldwide Fund-South Africa (2017) indicated in its report that agricultural industries use a considerable amount of water resources compared to other industries, and lack of water results in a massive decline in food production (D'Odorico *et al.,* 2019). There are very few vegetables available in the market already (Srinieng & Thapa, 2018; Polash *et al.,* 2019; Abbas *et al.,* 2020), suggesting the need to research underutilized edible vegetables in the wild and incorporate them into daily diets. *Mesembryanthemum crystallinum* has been recently abandoned while it is documented as an edible plant species that formerly served as a vegetable used as a substitute for crops such as spinach and cucumber (Herppich *et al*., 2012). There is little to no information about the interactive effect of water stress and salinity concentrations on the vegetative growth, chlorophyll contents, antioxidants capacity, phytochemical contents, and proximate compositions of *M. crystallinum* (Stavridou *et al.,* 2019; Lu *et al.*, 2021). It is therefore necessary to identify and utilize different strategies that can positively impact food security crises to ensure all nations are well-nourished throughout (Alaimo *et al*., 2020).

1.3 Aims

The study aimed at cultivating *M. crystallinum* under four different salt water concentrations of zero parts per million, 200 ppm, 400 ppm, and 800 ppm in conjunction with four different irrigation regimes of everyday irrigation, on every second day, on forth day, and on the eighth day respectively, to determine which treatment is optimal for cultivation of the plant in terms of vegetative growth, chlorophyll contents, antioxidants capacity, phytochemical contents, and proximate compositions.

1.4 Hypothesis

Different irrigation intervals and salinity concentrations had varying effects on the vegetative growth, chlorophyll contents, nutrient contents, antioxidants capacity, and secondary metabolites contents of *M. crystallinum* resulting in high saline irrigation at moderate irrigation interval being the most optimal cultivation.

1.5 Objectives of the research

1.5.1 Main objective

This study aimed to determine the vegetative growth, antioxidants capacity, chlorophyll contents, secondary metabolite contents, and full-feed nutritive properties of *M. crystallinum* in response to different salinity concentrations and at different irrigation intervals to establish an appropriate growing protocol.

1.5.2 Specific objectives

1.5.2.1 To determine the vegetative growth of leaf number, stem height, and aerial fresh and dry weight of *M. crystallinum* in response to different irrigation intervals and salinity concentrations, to determine and establish an optimal growth protocol for this plant.

1.5.2.2 To determine chlorophyll contents of *M. crystallinum* in response to different irrigation intervals and salinity, to determine and establish an optimal growth protocol for this plant.

1.5.2.3 To determine the antioxidants capacity and secondary metabolites contents of *M. crystallinum* in response to different irrigation intervals and salinity concentrations, to establish its pharmacological potential.

1.5.2.4 To assess the nutritive contents of *M. crystallinum* leaves in response to different irrigation intervals and salinity, to determine and establish its nutritional composition and potential as a well-nourishing vegetable.

1.5.2.5 To determine the optimal response of *M. crystallinum* to different salinity concentrations and irrigation intervals using data obtained from the results to establish an appropriate growth protocol.

1.6 References

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Chapter two: The importance of *Mesembryanthemum crystallinum* L. and its potential as a salt and water-tolerant vegetable with high antioxidants capacity, secondary metabolites, and nutrition contents: A review

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2.1. Abstract

Water scarcity and soil salinization constantly reduce food production worldwide, while food demand is rapidly increasing. It has become crucial to consider growing wild coastal edible crops under saline conditions with lower quantities of irrigation water. *Mesembryanthemum crystallinum* is a member of the Aizoaceae family in the order Caryophyllales. Worldwide, soil salinisation and drought significantly negatively influence food production. Little is known about the water requirements and salt levels in cultivating *M. crystallinum* and what effect different salt concentrations and water amounts have on the vegetative growth, phytochemical yield, antioxidants capacity, and nutrition composition of this species. The result from this review contributes to closing the gap of information on the impact played by soil salinization and drought on food security and how the species *M. crystallinum* can address this crisis. Furthermore, the study reviews how plants respond to different water and salinity stresses which may encourage further studies on other edible coastal crops.

Key words: Ice-plant, halophytes, salinity, tolerance, coastal crop, drought, food security, water scarcity, irrigation.

2.2 Introduction

Soil salinization and water scarcity are the most considerable issues limiting modern agricultural production (Heydari, 2019; Ding *et al*., 2020). Globally, water scarcity has increased due to recent global climate changes, which enhance the need to review irrigation and the effects of soil salinization **(**Herppich *et al.,* 2012; Dinar *et al*., 2019). Approximately 20% and 50% of the world's cultivated areas and the world's irrigated lands, respectively, are affected by salinization, and these percentages are still expected to increase in the future (Zhang *et al.,* 2018; Shahid *et al.,* 2018). Most vegetable crops are extremely sensitive to environmental stresses such as salinity and water shortages as they are highly perishable during cultivation (Ullah *et al.,* 2016; Solankey *et al*., 2021). Trautmann (2018) reported that in the Western Cape province, South Africa, the agricultural production in the Ceres region, only 50% of onions and 80% of potatoes, was recorded due to water scarcity caused by recent droughts. This also affected seasonal workers to lose wages to the equivalent of 40 million rands. Hence, cultivating natural halophytic plants to become valuable vegetable crops could solve economic solutions in harsh global climates (Sakae *et al*., 2009; Abdallah *et al.,* 2016).

Food security and water scarcity worldwide are threatened by the increase in populations while the agricultural cultivated lands are becoming deteriorated due to the excessive use of agriculture chemicals, soil salinization, and soil erosion (Abdallah *et al*., 2016). It is predicted that South Africa alone will face physical water scarcity by 2025 due to the rapid population growth and decrease in fresh water supply (WWF, 2017; De Angelis *et al*., 2021). Agriculture sectors worldwide, are facing challenges to produce 70 percent more food crop for an estimated additional 2.3 billion people by 2050 throughout the world, while many rising factors continuously inhibits the increase in agricultural productivity (Rahneshan *et al.,* 2018). It is therefore important to maximize food availability by domesticating more edible species (Sakae *et al.,* 2009). Irrigating halophytes with saline water may stimulate growth and enhance Crassulacean acid metabolism (CAM), and may, thus, protect the harvested product by keeping its original quality (Choi *et al., 2*017).

Mesembryanthemum crystallinum (Ice plant) is an interesting edible plant species that are occasionally served as a cool green flavoured green salad and it can be used as a substitute for vegetables such as spinach and cucumber (Herppich, *et al.,* 2012). *Mesembryanthemum crystallinum* can be more tolerant to environmental stress as it is a C3 plant that can naturally switch to CAM to cope with environmental stress of a certain habitat (Choi *et al., 2*017; Bueno & Cordovilla, 2020), and is less perishable because of salt concentration contained (Ullah *et al.,* 2016). Furthermore, increased CAM potentially reduces transpiration water losses and carbon losses during storage (Hirpara *et al.,*2005).

Ice plants have been reported to improve the proliferation of keratinocytes, and lipolysis which contains a significant amount of D-pinitol, which has been reported to show or produce potent antioxidant effects (Choi *et al*., 2017). Historically, physicians used leaf juice to soothe inflammation of the mucous membranes of the respiratory or urinary system (Mohlakoana & Moteetee, 2021). In Europe, fresh juice has been used to treat water retention, and painful urination and soothe lung inflammation (Hirpara *et al.,* 2005).

The above-mentioned properties of *M. crystallinum* encouraged this study to be conducted as one of the alternative solutions to contribute toward confronting water scarcity, food insecurity, and soil salinization, while promoting irrigation with seawater and utilization of burdened salinized soils to produce more healthy, nutritive vegetable with prolonged shelf life. The objectives of this chapter were to review what is already known about this species, water crisis, food security and its economic impact. Furthermore, it includes but is not limited to the botanical description, habitats, historical uses, salt and water stress effects on this plant, its close relatives and other plants in general.

2.3 Botanical description of *M. crystallinum*

The *M. crystallinum* is a member of the Aizoaceae family in the order Caryophyllales (Choi *et al*., 2017; Zhang *et al*., 2018; Atzori, 2021). The species is an annual or pseudo-biennial prostrate plant that completes its life cycle within several months, depending on environmental conditions (Agarie *et al.,* 2009; Choi *et al*., 2017). It is a white flowering herbaceous plantsucculent leaves and stems with ovate, succulent leaves, and stems that are densely covered with bladder cells (Tembo-Phiri, 2019) (Figure 2.1). Flowers appear from spring to early summer and open in the morning and close at night (Loconsole *et al.,* 2019). These species are insect pollinated (Loconsole *et al.,* 2019). The leaves are ovate to spathulate, forming a tear-drop-shape, with wavy margins, and die from the apex when they are old. Their arrangement is simple, opposite or alternate, and they can be found in sparse or dense clusters. Lower leaves are extremely large and crowded (Adams *et al.,* 1997) (Figure 2.2).

Figure 2.1: Fully developed *Mesembryanthemum crystallinum* plant terrestrially, showing the bladder cells. Source: (Sinikeziwe Ncaphayi, 2019)

Figure 2.2: *Mesembryanthemum crystallinum* leaf shape and margins. Source: (Sinikeziwe Ncaphayi, 2019)

Mesembryanthemum crystallinum is a halophyte that can switch from the C3 photosynthesis mechanism to CAM which is accelerated by environmental stress such as high photon flux density, drought, and salinity development (Choi *et al*., 2017; Loconsole *et al.,* 2019). This plant can complete its life cycle on soils with high salinity levels equivalent to seawater (Agarie *et al.,* 2009; Li *et al*., 2020). The leaves are covered with epidermal bladder cells, which appear to be ice-like crystals covering the plant (Choi *et al*., 2017; Atzori, 2021). The plant germinates and becomes established after a short rainy season (winter), followed by progressive drought stress coupled with increasing salinity (Adams *et al.,* 1997; Nogués *et al*., 2020). Further research on edibility, phytochemicals composition, antioxidant potential, nutrition composition, and plant physiological response to the environment must be well understood. All benefits must be utilised to contribute towards food security, pharmaceutics industries, and adding to society's knowledge.

2.4 Distribution and edaphic adaptation of *M. crystallinum*

In terms of distribution, *M. crystallinum* is native to southern and eastern Africa and is widely distributed and naturalized along the coasts of the southwestern United States, the Pacific coast of Mexico, Western Australia around the Mediterranean and Chile (Adams *et al.,* 1997; Agarie *et al.,* 2009; Olckers *et al*., 2021). In South Africa, *M. crystallinum* is found in the Eastern Cape, Northern Cape, and Western Cape, mostly on the coast (Agarie *et al.,* 2009; Olckers *et al*., 2021) (Figures 2.3 and 2.4). *Mesembryanthemum crystallinum* is found on a wide range of soil types, from well-drained sandy to loamy and clay soils and tolerate nutritionally poor or saline conditions (Adams *et al.,* 1997; Alshalmani *et al*., 2020; Atzori, 2021). Like many other introduced species, it also grows in disturbed areas such as roadsides, rubbish dumps and homestead yards (Alshalmani *et al*., 2020). It is important to consider tender crops like *M. crystallinum* that can adapt to severe environmental conditions requiring minimal attention to maximise food availability internationally (Atzori, 2021).

Figure 2.3: Flowering *Mesembryanthemum crystallinum* plants growing along the coast. Source: Ladislav Hoskovec (2009)

Figure 2.4: Dying back *Mesembryanthemum crystallinum* plants along the coastal area after seed maturation on coast at Granger Bay, Cape Town. Source: Mndi Okuhle (2021)

2.5 Folkloric uses of *M. crystallinum*

Mesembryanthemum crystallinum has been used as a vegetable in several European countries and cultivated as a food crop in China (Agarie *et al.,* 2009 & You *et al*., 2021). With its succulent, mellow, slightly salt-tasting leaves and young shoots, this species is a delicious flavouring as a green salad (He *et al*., 2020; Carmen & López, 2021). In Europe, it is also known as a cooked tender vegetable, which can be served raw or cooked as both stems and leaves are edible (Tembo-Phiri, 2019) (Figures 2.5 and 2.6). The species *M. crystallinum* is characterized by the presence of antioxidant enzymes such as ascorbate peroxidase, superoxide dismutase and catalase (Choi *et al*., 2017; Poór *et al*., 2018). Historically, physicians utilized leaf juice to enhance water retention and soothe inflammation of the mucous membranes of the respiratory or urinary system (Loconsole *et al*., 2019; Mohlakoana, 2020; Mohlakoana & Moteetee, 2021). Further research on *M. crystallinum* benefits and utilisation of such benefits is crucial in improving the wellbeing and pharmaceutical standards.

Figure 2.5: Freshly harvested *Mesembryanthemum crystallinum* to be prepared for food. Source: https://image.app.goo.gl/8LA3L2q

Figure 2.6: *Mesembryanthemum crystallinum* salad with Soy Sesame dressing. Source: http://images.app.goo.gl/CjotD6ivFx5erUPn6

2.6 Plant responses to water stress and salinity effect

When exposed to high salt content, plants tend to lower the osmotic potential of soil water and, consequently, the availability of soil water to plants (Hirpara *et al*., 2005; Abdallah *et al*., 2016). Salinity and drought also affect a set of sugar-alcohol-compatible solutes (Adams *et al.,* 1997; Hirpara *et al*., 2005). Adams *et al.* (1997) suggested that stress in the early development stage prevents the plant's progression to the maturity form, a phase in which the plants advance to mature growth, flowering, and seed development. The salts may also enter the plant and permanently damage the plant cells (Abdallah *et al*., 2016; Tsai *et al*., 2019).

In response to osmotic stresses caused by drought and salinity, other plants generally accumulate compatible solutes such as sugars, amino acids, polyols, betaines, and ecotones in cytoplasmic compartments (Agarie *et al.,* 2009; Abdallah *et al*., 2016). Some halophytic experience changes from C3 photosynthesis to CAM in response to high photon flux density, drought, and salinity (Loconsole *et al*., 2019). It is therefore important to consider species that can also tolerate salinity and drought stress to maximize food availability while minimizing water usage and maximizing the use of abandoned saline-affected lands and unusable salt water (Atzori, 2021).

2.7 Economic and nutritive values of vegetables

Vegetables are essential for daily meals because they serve as a joyful portion for the main dish and improve the palatability of starchy staple foods (Misselhorn, 2005; Vandebroek & Voeks, 2018). In tropical Africa, micronutrient deficiency is the major cause of health problemsthat can potentially lead to high mortality rates and low economic productivity (Grubben *et al*., 2014; Singh *et al*., 2019). Nutritionists of WHO-FAO stated that a daily portion of different vegetables is crucial for a well-balanced diet, especially for children and pregnant women. Vegetables are the most affordable and accessible source of micronutrients, and it is expected that countries should pay more attention to food security projects that promote fresh green vegetables for health (Singh, *et al*., 2019; Ryckman *et.al*., 2021). The most reported widespread micronutrient deficiencies worldwide are zinc, iron, vitamin A, folate, iodine, vitamin B12 and other B vitamins (Misra *et al.,* 2008). The WHO-FAO recommends a daily global average intake of fruit and vegetables at 400 g per person to obtain a balanced diet. Vegetables are richer in micronutrients than fruits (Grubben *et al*., 2014). The nutritional value of traditional leafy vegetables is higher than that of several commonly known vegetables (Misselhon, 2005). Although many traditional leafy vegetables have the potential for income generation, they still fail to compete with more commonly known exotic vegetables due to a lack of awareness and knowledge of their cultivation and use (Misra *et al.,* 2008). Consumption of traditional diets is known to many societies who reported to have many beneficial effects such as preventing some age-related degenerative diseases such as arteriosclerosis, stroke, etc. (Misra *et al.,* 2008). It is therefore crucial to maximise consideration of domesticating and utilising all vegetables in exposure to minimise the effects of malnutrition and other deficiency disorders in societies while minimising expenses (Leakey *et al*., 2021).

2.8 Effects of drought and soil salinisation on food production, economy, and food security status

Drought and soil salinization significantly affect food production quantities and the economy that continuously supports food security worldwide (Wen *et al*., 2020; Gomez-Zavaglia *et al.,* 2020). Whenever food production drops, the supply becomes less, leading to significant increases in food prices and eventually disadvantaging lower-income people in affording and maintaining their needs (Dinar *et al.,* 2019; Lokuruka, 2020). South Africa’s Western Cape has been significantly hit by drought from 2015 to 2018 (Otto *et al.,* 2018 & Meza *et al*., 2021). These water crises affected the province’s many green industries directly and indirectly, resulting in the unemployment rate and the economy declining (Williams, 2019; Pienaar & Boonzaaier, 2018).

Table 1: Economic impact of the drought on the Western Cape province, South Africa agricultural sector in 2016 and 2017.

Industry	Total Production 2016/17	Estimated Total Production 2017/18	Drop in Production (%)	GVA Shock 2016/17 vs 2017/18 (R million)	Employment losses
Wine Grapes	1 599 728	1 279 782	-20.0	-591.21	-2 809
Table Grapes	186 772	153 000	-18.1	-787.36	-4 019
Pome Fruit	1 376 279	1 256 773	-8.7	-898.26	-9 635
Stone Fruit	319 424	293 288	-8.2	-458.26	-2 070
Citrus	311 955	287 887	-7.7	-259.24	-1 280
Alternative Fruit*	7 693	7 037	-8.5	-36.35	-220
Major Vegetables**	1 104 580	881 280	-20.2	-78.73	-2 716
Grains***	1 558 200	986 928	-36.7	-2 812.97	-7 482
Total	6 464 630	5 145 975	-20.4	-5 922.37	-30 230

Drought Policy Brief, (Source: Pienaar & Boonzaaier, 2018).

Gardening sector and ornamental plant retailers in Cape Town generally experienced a decrease in plant sales volume from 2015 to 2018, while in the Ceres region for agriculture crops, 50% fewer onions and 80% fewer potatoes were planted due to lack of water availability (Williams, 2019). Reduction in crops grown in affected areas resulted in a revenue loss of over R40 million regarding the employment of seasonal workers (Zwane, 2019 & Ziervogel, 2019).

The irrigation of crops in the Western Cape province is a crucial driver for economic growth and jobs in the region (Trautmann, 2018). The WWF (2018) estimated an economic loss of R5.9 billion in agriculture in this Cape province alone, with 30,000 job losses and exports dropping to 13-20%. According to Pienaar & Boonzaaier (2018), the income losses are valued at around R259 million. It was stated by the WWF (2018) that this is due to reduced farming outputs and additional income losses as export volumes decline.

Due to the lack of available water many hectares of productive fruit trees and vineyards were removed ahead of the normal replanting schedule as well as to prevent disease and pests from spreading (WWF, 2018). As a result, about 6% of all farmers indicated that they would not be able to continue farming if water allocations were cut by 60% (Pienaar & Boonzaaier, 2018). Considering these extreme measures during seasonal drought patterns, drought-tolerant halophytic crops such as *Mesembryanthemum crystallinum* could potentially struggle through these drought periods with minimal water availability (Atzori, 2021). Therefore, research on salt tolerance and watering requirements is important to develop an appropriate growth protocol for domestication, which is necessary to maximize future production and alleviate food shortages. Similarly promoting the utilization of saline arable land and minimal water use in conjunction with sea water could benefit in saving scarce freshwater resources.

2.9 Conclusion

Food insecurity and freshwater scarcity review revealed that coastal edible plants need to be considered to maximise food availability while minimising water usage. *M. crystallinum* properties reported indicates that this species has potential to be considered as a potential edible coastal species that can adapt to grow in saline conditions with minimum irrigation to yield at its maximum. Even though Ice plant shows promising potential, information on its responses to treatments of salinity levels and irrigation intervals is lacking and if investigated could determine the interaction between these two treatments in developing a more precise growth protocol of this plant.

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Chapter three: Vegetative growth of *Mesembryanthemum crystallinum* L. in response to different irrigation intervals and salinity levels

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3.1 Abstract

The shortage of fresh water and increasing soil salinization significantly affect food production worldwide and this consistently increases poverty. It has therefore become crucial that various strategies are adopted to overcome these crises by endorsing salt-tolerant edible crops such as *Mesembryanthemum crystallinum* to contribute to food security issues while minimizing water over-use. This study evaluated the tolerance of *M. crystallinum* to salinity in combination with different irrigation intervals to formulate an appropriate vegetative growth protocol for this species while minimizing water overuse. The *M. crystallinum* was treated with different irrigation intervals of daily irrigation (control), every second day, on the fourth day, on the eighth day in conjunction with different salt concentrations of 0 ppm (control), 200 ppm, 400 ppm, and 800 ppm achieved by adding sodium chloride to reverse osmosis (RO) water with 0 ppm. The combination of different salinity and irrigation intervals resulted in a total of 16 different treatments. The growth parameters measured were the number of leaves and stem length, while post-harvest parameters were fresh and dry weights. The results indicated that stem length growth was most promoted by treatment “0 ppm” salt level on daily irrigation while number of leaves development were promoted by “800 ppm every 4th day irrigation” treatment. For fresh and dry weight, “800 ppm 4th day irrigation” treatment yielded the highest masses, providing evidence that this species can successfully be cultivated in saline environments with moderate application of water for increased harvest yields.

Keywords: Drought, food security, irrigation intervals, salinity, vegetative growth, water stress.

3.2 Introduction

Increasing agricultural production has become an urgent requirement for the expanding population (Jiang *et al.*, 2012). Sustainable development of agriculture is impacted by the shortage of quality fresh water in arid and semi-arid areas urges the implementation of regulated irrigation and the use of saline water to sustain crop yields (Jiang *et al.*, 2012; Min *et al.*, 2014). There is significant interest in using saline and brackish irrigation water in agriculture to overcome drought and increase crop yields, (Jiang *et al.,* 2012; Min *et al*., 2014) even though soil salinity is one of the most important abiotic stresses limiting crop production worldwide (Min *et al.*, 2014). Most studies indicate that both crop biomass and yield quality decline as soil salinity and water stress increase (Mounzer *et al.*, 2013; Min *et al.*, 2014). Likewise, excess soil salt can reduce osmotic potentials to the point that crops cannot take up enough water (Min *et al.*, 2014). It is, therefore, crucial to schedule recommended water applications to benefit crops to eliminate water stress in plants and reduce production (Badr *et al.*, 2012). Inappropriate scheduling of water and fertilizer application led to salinity buildup under long-term use (Mounzer *et al.*, 2013).

To mitigate these agricultural issues, it becomes important to introduce coastal edible species as new food crops which can tolerate water stress and saline conditions with appropriate irrigation scheduling. *Mesembryanthemum crystallinum* is an edible coastal halophyte that switches from the C3 photosynthetic mechanism to crassulacean acid metabolism (CAM) when coping with environmental stress induced by drought and salinity (Agarie *et al.,* 2009 & Choi *et al*., 2017). The plant can complete its life cycle on soils containing high salt levels equivalent to those contained in seawater (Agarie *et al*., 2009).

Even though *M. crystallinum* has been reported to have the above-mentioned properties, there is a dearth of information on the interactive effect of different salt concentrations and irrigation intervals on the vegetative growth of this plant (Stavridou *et al.,* 2019; Lu *et al*., 2021). These findings are crucial in understanding the physiological response of *M. crystallinum* to salt and water stress and in developing a suitable cultivation protocol for the species to promote its endorsement as an edible vegetable capable of maximizing food availability while saving water. The recommended growth protocol can be used by commercial farmers to grow this species and fill the existing knowledge gap on its adaptability to saline soils. This will help to mitigate the effects of water scarcity occasioned by high salinity that most crops cannot tolerate, hence, contributing to food security.

3.3 Methods and materials

3.2.1 Study area

The experiment was carried out in the research greenhouse of the Horticultural Science Department of the Cape Peninsula University of Technology (CPUT) in Cape Town, South Africa. The greenhouse temperature was set to range between 21 and 26 °C during the day and between 12 and 18 °C at night, with a relative humidity of 60%. The daily photosynthetic photon flux density (PPFD) was 420 μmol/m2/s on average, with a maximum of 1020 μmol/m2/s.

3.2.2 Plant Preparation, Irrigation, and Treatments

Seeds of *M. crystallinum* were obtained from a commercial garden centre, Renu-Karoo Nursery & Veld Restoration at Prince Albert, Western Cape, South Africa. The seeds were sown in two small seed trays containing a mixture of silica sand, coco coir, and vermiculite (1:1:1) as described by Loconsole *et al.* (2019). A layer of course bark was laid in the seed tray before the medium was added to prevent leaching. Approximately 2 kg of the medium was added to each tray. Thereafter, the seeds were evenly broadcasted on the trays, followed by the application of a thin layer of vermiculite on top of the seeds. The trays were then watered with Captab (4 g/L) manufactured by Universal Crop Protection (Pty) Ltd., Kempton Park, South Africa, to prevent the development of fungal diseases and were placed on a heating bed under mist irrigation sprayers for germination. After the first set of true leaves emerged, one hundred and ninety-two (192) uniformly germinated seedlings were washed using tap water to remove soil and other debris. They were then potted up in 12.5 cm black plastic pots containing river sand and, thereafter, were hardened off for a week in the greenhouse before moving to the experimental site (Figures 3.1a-d).

During this period, seedlings were irrigated daily with a nutritional solution formed by adding NUTRIFEED™ (manufactured by Starke Ayres Pty. Ltd., Gauteng, South Africa) to municipal water at 10 g per 5 L. The nutrient solution contained the following ingredients: N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg), and Zn (240 mg/kg). After acclimatization, plants were watered with distilled water for 5 days to wash off any salt residue and, thereafter, were organized into four irrigation treatments ((1) 100 mL once a day; (2) 100 mL once every 2 days; (3) 100 mL once every 4 days; (4) 100 mL once every 8 days) with four salt concentrations (0, 200, 400, and 800 ppm) applied in each treatment. Salt concentrations were set up by adding increasing concentrations of NaCl to the nutrient solution. A handheld EC meter (Martini EC 59, manufactured by Milwaukee®, Milwaukee, MI, USA) was used to measure the salt concentration in the solution. A completely randomized design was used to accommodate the two-way factorial experiment. Forty-eight (48) replicates per factorial combination were used, totalling to 192 plants for the entire experiment.

Figure 3.1a: Germinating *Mesembryanthemum crystallinum* seeds in flat trays (Source: Mndi).

Figure 3.1b: *Mesembryanthemum crystallinum* seedlings transplanted into plug trays (Source: Mndi).

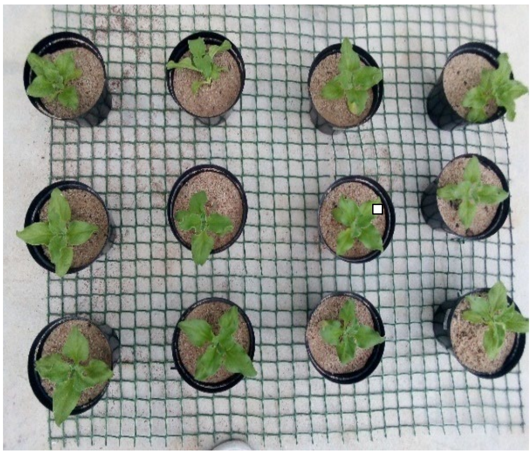

Figure 3.1d: *Mesembryanthemum crystallinum* transplanted plugs to 15 cm pots (Source: Okuhle Mndi).

Figure 3.1c: *Mesembryanthemum crystallinum* seedling growth one week after transplanting to plug trays (Source: Okuhle Mndi).

3.2.3 Data collection and statistical analysis

To determine vegetative growth, the stem length and quantity of leaves were employed as variables. Every two weeks, the stem length was measured using a 30 cm ruler from the substrate level to the tip of the tallest shoot, and leaf counting was performed manually. Shoots and roots were divided at the post-harvest stage, and the fresh weights of various samples were determined using a typical laboratory scale (RADWAG® Model PS 750.R2). The plant material was subsequently dried to a consistent weight in an oven at 55 °C using a LABTECHTM model LDO 150F (Daihan Labtech India Pty. Ltd., 3269 Ranjit Nagar, New Delhi, India). The difference between the fresh and dry weights was compared with the amount of water held within plants' tissues.

All the data collected was statistically analysed using two-way analysis of variance (ANOVA) using Minitab software. The significant difference between the mean values of treatments was compared at $p \leq 0.05$ using Tukey least significant difference. The calculations to obtain statistical data were accomplished through the Minitab software and then tabulated. The data of results are presented by graphs and tabulations.

3.4 Results

3.4.1 Number of leaves

Significant difference ($p \leq 0.05$) in the mean leaf number was first observed on week 4 where mean values of 10.33, 10.42, and 10.42 were recorded in treatments with salt concentrations of 200 ppm, 400 ppm, and 800 ppm irrigated every 4th day, respectively, however all treatments were comparable to one another. On week 6, the highest significance of leaf number was observed on treatment "800 ppm 4th day irrigation" with a mean value of 12.50 while the control treatment had a mean value of 10.17. On week 8, the highest significance ($p \leq 0.05$) of mean was still highest on treatment "800 ppm 4th day irrigation" with mean value of 14.75 when compared to the control treatment with a mean value of 12 (Table 3.1).

3.4.2 Stem length

Table 3.2 presents that stem length had the highest significant ($p \leq 0.05$) effect on 800 ppm 4th-day irrigation on week 2 when compared to all other treatments, including the control "0 ppm daily irrigation" with the mean value of 5.33. On week four, significance at $p \leq 0.05$ was observed to be the highest only on "0 ppm 2nd day irrigation". On weeks six and eight, the highest significance of $p \leq 0.05$ was observed more alike with week four level of significance. The experimental results show that the interaction of different salinity and irrigation intervals had a significant ($p \leq 0.05$) effect on the stem length growth of the species, where treatment with "0 ppm 2nd day irrigation" had the highest mean value from week 4 till week 8 of the experiment. Plants treated with eighth-day irrigation intervals and everyday irrigation indicated slower stem growth. It was also observed that as the salt concentration increased, the stem length of the crop increased as the plants continued growing (Table 3.2).

Table 3.1: Effects of different salt concentrations and irrigation intervals on the number of leaves of *Mesembryanthemum crystallinum*.

Salt concentration	Irrigation intervals	Week 4	Week 6	Week 8
0 ppm	Daily	9.42 ± 0.26 ab	10.17 ± 0.30 c	12.00 ± 0.35 bcd
	2nd Day	9.25 ± 0.28 ab	8.33 ± 0.41 d	12,42 ± 0.43 bc
	4th Day	10.00 ± 0.01 ab	11.00 ± 0.302 bc	11.67 ± 0.48 bcd
	8th Day	9.75 ± 0.18 ab	10.17 ± 0.167 c	10.67 ± 0.28 cd
200 ppm	Daily	9.00 ± 0.28 b	9.91 ± 0.31 c	10.00 ± 0.01 d
	2nd Day	9.58 ± 0.31 ab	10.17 ± 0.17 c	10.33 ± 0.23 cd
	4th Day	10.33 ± 0.23 a	10.50 ± 0.26 bc	10.67 ± 0.28 cd
	8th Day	9.92 ± 0.29 ab	10.17 ± 0.17 c	10.50 ± 0.26 cd
400 ppm	Daily	9.50 ± 0.23 ab	10.25 ± 0.18 c	11.33 ± 0.62 bcd
	2nd Day	9.33 ± 0.28 ab	10.00 ± 0.25 c	10.83 ± 0.52 cd
	4th Day	10.42 ± 0.23 a	11.75 ± 0.39 ab	13.17 ± 0.76 ab
	8th Day	9.83 ± 0.17 ab	9.83 ± 0.17 c	10.00 ±0.01 d
800 ppm	Daily	9.58 ± 0.89 ab	9.58 ± 0.38 cd	11.50 ± 0.44 bcd
	2nd Day	9.67 ± 0.33 ab	10.50 ± 0.26 bc	10.67 ± 0.57 cd
	4th Day	10.42 ± 0.29 a	12.50 ± 0.50 a	14.75 ± 0.79 a
	8th Day	9.67 ± 0.26 ab	10.58 ±0.26 bc	11.50 ± 0.50 bcd
Two-way ANOVA Tukey Statistics				

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

Table 3.2: Effects of different salt concentrations and irrigation intervals on the stem length of *Mesembryanthemum crystallinum.*

Salt concentration	Irrigation intervals	Week 2	Week 4	Week 6	Week 8
0 ppm	Daily	5.33 ± 0.33 cd	6.83 ± 0.51 bc	8.42 ±0.68 b	10.05 ±0.97 b
	2nd Day	6.67 ± 0.36 a-d	9.25 ± 0.49 a	12.08 ± 0.70 a	15.75 ± 0.93 a
	4th Day	5.75 ± 0.31 bcd	7.58 ± 0.36 abc	10.00± 0.56 ab	12.58 ± 0.78 ab
	8th Day	5.17 ± 0.27 d	6.75 ± 0.41 c	8.67 ± 0.67 b	10.75 ± 0.94 b
200 ppm	Daily	6.33 ± 0.26 a-d	7.75 ± 0.39 abc	9.75± 0.47 ab	11.83 ±0.98 ab
	2nd Day	6.75 ± 0.31 abc	8.33 ± 0.33 abc	10.67 ± 0.58 ab	13.50 ± 0.75 ab
	4th Day	6.67 ± 0.31 a-d	8.42 ± 0.42 abc	10.58 ± 0.69 ab	13.25 ±1.05 ab
	8th Day	5.42 ± 0.26 cd	7.42 ± 0.50 abc	9.92 ± 0.76 ab	12.33 ±0.98 ab
400 ppm	Daily	6.08 ± 0.26 a-d	7.41 ± 0.26 abc	9.08 ± 0.38 ab	10.92 ± 0.61 b
	2nd Day	6.500 ± 0.29 a-d	7.92 ± 0.38 abc	9.42 ± 0.56 ab	11.75± 0.96 ab
	4th Day	6.67 ± 0.47 a-d	7.83 ± 0.59 abc	9.58 ± 0.75 ab	12.08 ± 1.33 ab
	8th Day	5.92 ± 0.26 a-d	7.58 ± 0.38 abc	9.25± 0.64 ab	11.33 ± 0.91 ab
800 ppm	Daily	6.58 ± 0.29 a-d	8.25± 0.35 abc	10.25 ±0.55 ab	12.33 ± 0.76 ab
	2nd Day	7.08 ± 0.29 ab	8.500 ±0.38 abc	10.33 ± 0.54 ab	12.92 ± 0.80 ab
	4th Day	7.42 ±0.38 a	8.92 ± 0.43 ab	11.00± 0.76 ab	13.00 ±1.03 ab
	8th Day	5.75 ±0.33 bcd	7.25 ± 0.51 abc	9.00 ± 0.78 ab	10.50 ± 1.06 b

Two-way ANOVA Tukey Statistics

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

3.4.3 Fresh weight

The highest significant fresh weight (76.35 g) was obtained under 800 ppm salinity irrigated every 4th day while the least fresh weight (17.07 g) was observed in treatment 0 ppm with 8th day irrigation treatment (Figure 3.2) compared to all other treatments including the control treatment of "0 ppm daily irrigation" with mean value of 35.22 g. No significance was observed when comparing control with "0 ppm 4th day irrigation". However, all other treatments had significant effect when compared to the control treatment (Figure 3.2).

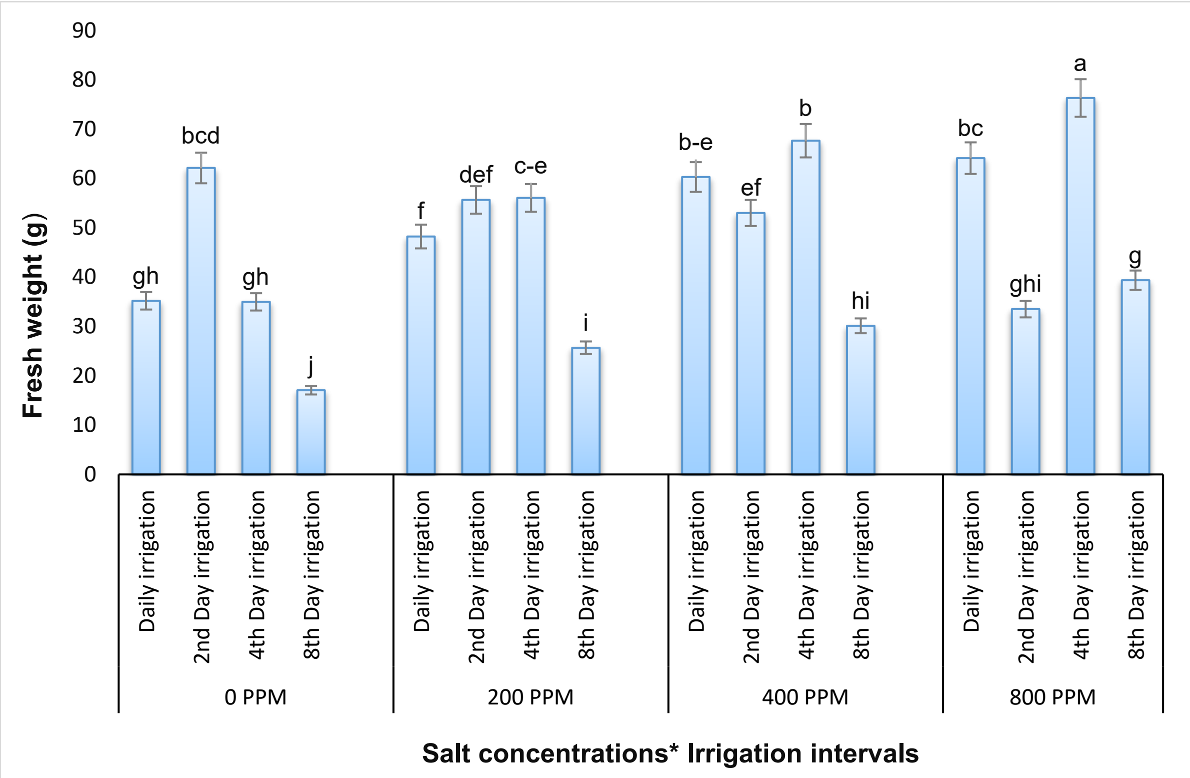

Figure 3.2: Effects of different salt concentrations and irrigation intervals on mean fresh weight of *Mesembryanthemum crystallinum.*

3.4.4 Dry weight

Almost all treatments had a statistically significant effect ($p \leq 0.05$) on dry weight except 0 ppm salinity under 8th-day irrigation" and 800 ppm at 2nd-day irrigation" when compared to the control. The highest mean dry weight was observed at 800 ppm salinity under 4th day irrigation" with mean value of 5.67 g compared to control with mean value of 2.11 g. Different salt concentrations and irrigation intervals had statistical significance ($p \leq 0.05$) in almost all treatments except for "0 ppm" at 4th-day irrigation, "200 ppm" at 8th-day irrigation, "800 ppm" under 2nd day irrigation and "0 ppm" at 8th-day irrigation with mean values of 2.45, 2.42, 2.14, and 1.96 g respectively.

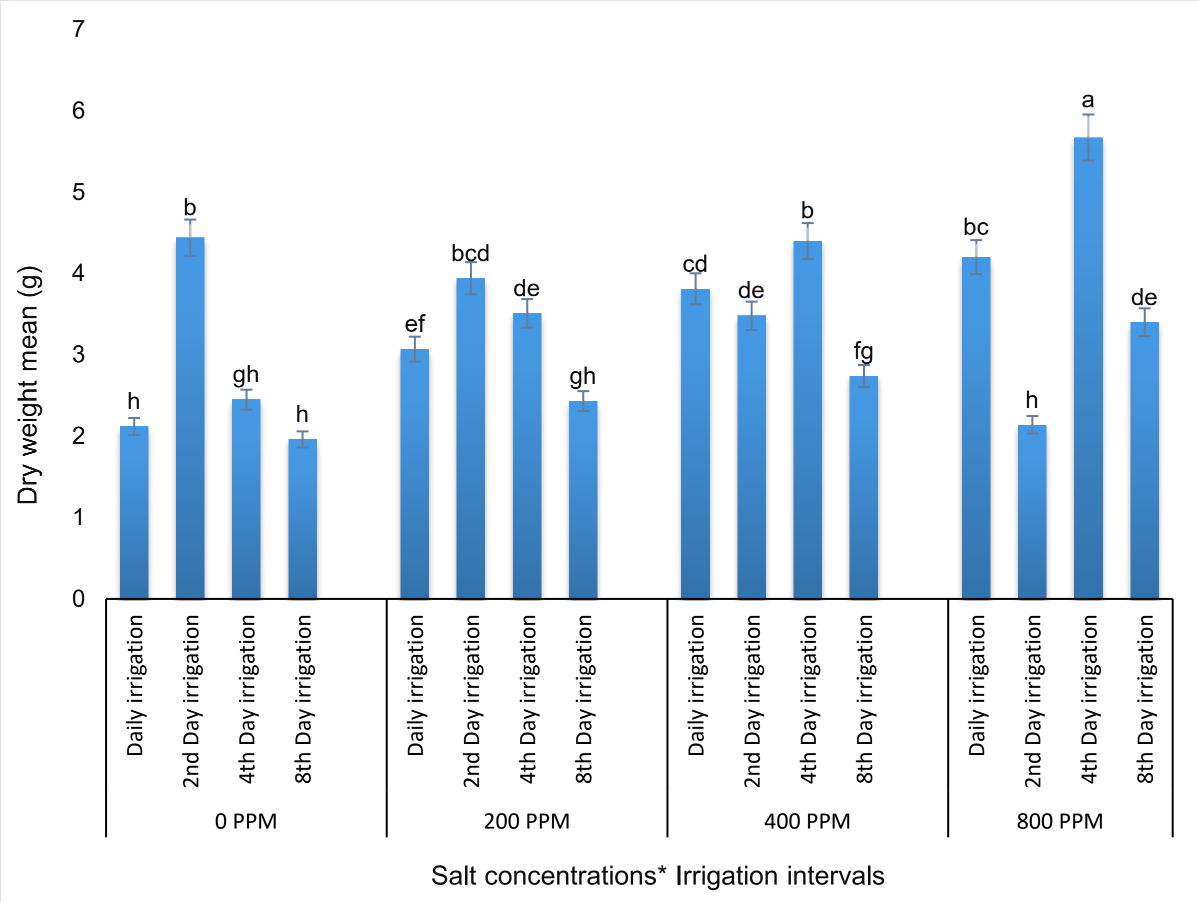

Figure 3.3: Effects of different salt concentrations and irrigation intervals mean dry weight of *Mesembryanthemum crystallinum.*

3.5 Discussion

M. crystallinum grown under different salinity and irrigation intervals treatments showed no significant differences in morphological parameters including number of leaves, stem height and fresh and dry weight of leaves. For some plants it has been reported that salt stress leads to drastic increase in H_2O_2 accumulation, abscisic acid biosynthesis, and reduced K^+ contents in the shoot (Saeidi-Sar *et al.,* 2013). Each of the aforementioned factors results in stomata closure, which reduces CO2 assimilation andimposes yield penalties (Sofy *et al*., 2021; Hedrich & Shabala, 2018). However, this is quite different in the case of halophytes as they are naturally salt-tolerant plant species which flourish under saline environments that would have impacted yield penalties in glycophytic crops (Hedrich & Shabala, 2018; Nikalje *et al*., 2019). This superior physiological response of halophytes to saline areas is supported by the findings of this study as higher salinity yielded more leaf numbers than the lower salt concentrations. The highest number of leaves was observed at maximum salinity of "800 ppm" with every fourth-day irrigation. Still, irrigation intervals also show that excessive water stress and incessant irrigation reduces plant growth as the highest yield of leaf numbers was observed on moderate irrigation.

Salinity may cause hyperosmotic stress, resulting in the production of ABA, which enhances stomatal closure while producing ABA, which enhances stomatal closure while reducing transpiration (Hedrich & Shabala, 2018). It has also been observed that all crop species respond to salinity stress by a rapidly increasing xylem sap ABA content (Niu *et al*., 2019). However, it has been revealed that not all halophytes respond alike to such exposure (Piovan *et al.,* 2019). *M. crystallinum* leaves have been reported to respond to NaCl exposure with CAM (crassulacean acid metabolism) mechanism induction and only marginal ABA accumulation (Hedrich & Shabala, 2018). Ice plant (*M. crystallinum*) is thus categorized to be in "obligatory halophytes" group, which require saline environments for optimal growth (Loconsole *et al*., 2019). The CAM mechanism is reported to be accelerated by salt stress (Madhavi *et al.,* 2022) and this adaptive mechanism improves water use efficiency to the plant *M. crystallinum*.

The results show that biomass accumulation is favoured by salinity, as crops treated with salt had larger mean values than no salt treatment species. These findings were similar with 'Raccoon' and 'Gazelle' biomasses, where 'Raccoon' outproduced 'Gazelle' at the two highest salinity levels, indicating that 'Raccoon' may outperform 'Gazelle' at higher NaCl concentrations (Ferreira *et al.,* 2020). Biomass accumulation in *C. vulgaris* was also not drastically reduced along the tested salinity gradient, affirming its tolerance for a wide range of salinity levels (Teh *et al*., 2021).

3.6 Conclusion

Different irrigation intervals and salinity concentrations displayed significant effect on the vegetative growth of *M. crystallinum*. The highest vegetative yield was observed under highest saline concentrations of 800 ppm in combination with moderate irrigation of every fourth day where the soil was moister than in damp and dry soils. Study with more salinity concentrations greater than 800 ppm needs to be conducted to evaluate the Ice plant's maximum salinity tolerance.

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Chapter four: Variation in chlorophyll contents of *Mesembryanthemum crystallinum* L. in response to different irrigation intervals and salinity levels

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4.1 Abstract

Chlorophyll is the most important class of green pigment found in the chloroplasts of plants and increase of this pigment results in better absorption of the light required for photosynthesis. Water scarcity immediately affects plants at the physiological, morphological, and molecular levels. In the present study, the influence of different salinity and irrigation intervals on the chlorophyll contents of *Mesembryanthemum crystallinum* were evaluated. The treatments consisted of varying salt concentrations of 0 ppm (control), 200 ppm, 400 ppm, and 800 ppm in conjunction with different irrigation intervals of daily (control), every second day, every fourth day, and every eighth day while the control treatment was 0 ppm salinity under daily irrigation. The results revealed the highest chlorophyll significance $p \leq 0.05$ on treatment “0 ppm fourth day irrigation” throughout the experimental period whereas the lowest chlorophyll contents mean values 0.98 and 1.03, were recorded under "400 ppm daily irrigation" and "800 ppm 8th day irrigation" respectively. The results indicate that moderate irrigation and no sodium chloride yield the highest chlorophyll content in *M. crystallinum*. Lowest chlorophyll content was observed on treatments “400 ppm under daily irrigation” and “800 ppm at eighth-day irrigation” and this was related with reduced CO2 inflow, which was attributed to stomatal closure and a decrease in mesophyll conductivity under water stress paired with high salinity. Chlorophyll content of *M. crystallinum* leaves increased over time as the plant ages, and these findings were associated with the seasonal light changes.

Keywords: chloroplast; chlorophyll; environmental stress; photosynthesis; salinity; water regimes

4.2 Introduction

Chlorophyll is the most important class of green pigment that is found in the chloroplasts of plants (Wang, *et. al*., 2020; Fu, *et al.*, 2021). This pigment plays a crucial role in plant growth and development as it provides gre en colour to the leaves, which is necessary for producing glucose and oxygen from atmospheric carbon dioxide and water absorbed from the soil during photosynthesis. (Kathpalia & Bhatla, 2018; Faitrouni, 2022). Chlorophyll pigment has been proven to exhibit antimutagenic and anti-carcinogenic effects, and this widely takes part in the nutrition of humans (Bologa, 2021; Yamgar & Dhamak, 2022). It has been noticed that increase in chlorophyll contents in vegetable crops leads to improved biological functions and nutritional importance of the vegetables. Furthermore, s results in toincreased chlorophyll pigment in plants, resulting in better absorption of the light required for photosynthesis (Simkin *et al*., 2022). The amount of chlorophyll in leaf tissue is influenced by nutrient availability and environmental stresses such as drought, salinity, temperature, excessive soil water (Zilaie *et al*., 2022).

Water is a vital element for the growth and development of plants (Sargentis *et al*., 2021). It enhances the transport and circulation of all essential minerals in the plant, including elements absorbed from the soil and organic substances produced in the leaves and circulating in the xylem and phloem saps (Melicherová *et al*., 2020). Water scarcity directly impacts plants at the physiological, morphological, and molecular levels (Seleiman *et al.,* 2021; Zia *et al*., 2021). Previous research has demonstrated that water stress affects chlorophyll levels, stomata conductance, and photosynthetic rate but not leaf nitrogen content, leading to the conclusion that the induced stress losses of chlorophyll are not influenced by nitrogen deficiency (Muhammad *et al*., 2022). Water stress causes stomata to close, resulting in a slower rate of photosynthesis due to reduced carbon dioxide availability (Singh *et al*., 2021). At a certain point, water stress causes leaves wilting and resultsin reduced metabolic activity performed by leaves. This is followed by reduced plant growth and a greater probability of early senescence (Ammar *et al*., 2022; Azmat *et al.,* 2022). When plants are subjected to water stress, their stomata response, reactive oxygen species (ROS) scavenging, metabolic changes, and photosynthesis are all altered. (Balfagón *et al*., 2020). These collective responses cause a change in plant growth rate as an adaptive mechanism for survival (Zia *et al*., 2021).

Chlorophyll content is an initial factor to determine if a plant is negatively impacted by or promoted by environmental stress (Vandana *et al*., 2020). It is, therefore crucial to determine the effect each salt and water stress would have on the chlorophyll contents of *M. crystallinum* to understand the species' tolerance to varying salt concentrations and water regimes. This study's assessment of *M. crystallinum* chlorophyll content provides critical understanding of the influence of different irrigation intervals and salinity in developing an appropriate growth protocol for this crop to contribute towards overcoming food insecurity since compromised chlorophyll contents may result in lowered yields, reduced nutrient contents, hence compromising food security (Yasir *et al*., 2021).

4.3 Materials and methods

4.3.1 Study area

The experiment was carried out in the research greenhouse of the Horticultural Science Department of the Cape Peninsula University of Technology (CPUT) in Cape Town, South Africa. The greenhouse temperature was set to range between 21 and 26 °C during the day and between 12 and 18 °C at night, with a relative humidity of 60%. On average, the daily photosynthetic photon flux density (PPFD) was 420 µmol/m2/s, with a maximum of 1020 µmol/m2/s.

4.3.2 Plant Preparation, Irrigation, and Treatments

Seeds of *M. crystallinum* were obtained from a commercial garden centre, Renu-Karoo Nursery & Veld Restoration at Prince Albert, Western Cape, South Africa. The seeds were sown in two small seed trays containing a mixture of silica sand, coco coir, and vermiculite (1:1:1), as Loconsole et al. (2019) described. A layer of course bark was laid in the seed tray before the medium was added to prevent leaching. Approximately 2 kg of the medium was added to each tray. Thereafter, the seeds were evenly broadcasted on the trays, followed by the application of a thin layer of vermiculite on top of the seeds. The trays were then watered with Captab (4 g/L) manufactured by Universal Crop Protection (Pty) Ltd., Kempton Park, South Africa, to prevent the development of fungal diseases. They were placed on a heating bed under mist irrigation sprayers for germination. After the first set of true leaves emerged, one hundred and ninety-two (192) uniformly germinated seedlings were washed using tap water to remove soil and other debris. They were then potted up in 12.5 cm black plastic pots containing river sand and, thereafter, were hardened off for a week in the greenhouse before they were moved to the experimental site. During this period, seedlings were irrigated daily with a nutritive solution formed by adding NUTRIFEED™ (manufactured by Starke Ayres Pty. Ltd., Gauteng, South Africa) to municipal water at 10 g per 5 L. The nutrient solution contained the following ingredients: N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg), and Zn (240 mg/kg). After acclimatization, plants were watered with distilled water for 5 days to wash off any salt residue and, thereafter, were organized into four irrigation treatments ((1) 100 mL once a day; (2) 100 mL once every 2 days; (3) 100 mL once every 4 days; (4) 100 mL once every 8 days) with four salt concentrations (0, 200, 400, and 800 ppm) applied in each treatment. Salt concentrations were set up by adding increasing concentrations of NaCl to the nutrient solution. A handheld EC meter (Martini EC 59, manufactured by Milwaukee®, Milwaukee, MI, USA) was used to measure the salt concentration in the solution. A completely randomized design was used to accommodate the two-way factorial experiment. Forty-eight (48) replicates per factorial combination were used, totalling to 192 plants for the entire experiment (Figures 4.1a-d).

Figure 4.1a: Germinating *Mesembryanthemum crystallinum* seeds in flat trays. (Source: Okuhle Mndi)

Figure 4.2b: Transplanted *Mesembryanthemum crystallinum* seedlings to plug trays. (Source: Okuhle Mndi)

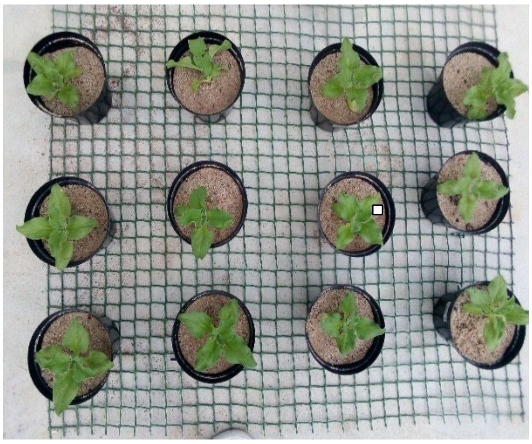

Figure 4.4d: Re-transplanted *Mesembryanthemum crystallinum* plants to 15cm pots. (Source: Okuhle Mndi)

Figure 4.3c: *Mesembryanthemum crystallinum* seedling growth one week after transplanting to plug trays (Source: Okuhle Mndi).

4.3.3 Water stress and salinity treatments

The experiment was set up in a randomized block factorial design with variable irrigation intervals and salinity levels explicitly created for this experiment. There were 4 different irrigation intervals (100 mL every day, 100 mL every two days, 100 mL after every 4 days, and 100 mL after every 8 days) in conjunction with 4 different salt concentrations of 0 ppm, 200 ppm, 400 ppm, or 800 ppm. Sodium Chloride purchased from Merck Chemicals (Pty) Ltd was used to manipulate the salinity of treatments.

4.3.4 Data collection and statistical analysis

The data for chlorophyll was taken from week two and after every two weeks till week 8 directly from actively growing fully developed leaves of *M. crystallinum*. The leaf SPAD readings (Chl SPAD) were obtained from two fully formed leaves of each plant using a chlorophyll meter (SPAD-502, Konica Minolta, Japan). The readings/figures were averaged out by the SPAD-502 m to produce a final number. The data was collected between 11 am and 16 pm, and the chlorophyll meter was first calibrated to adjust the chlorophyll meter to the greenhouse light intensity. All the data collected from the vegetative growth parameters of this study were statistically analysed using 2-way analysis of variance (ANOVA) using Minitab software. Tukey's least significant difference was used to compare the significant differences between treatment means at $p \leq 0.05$. The calculations carried out to obtain statistical data were accomplished through the computer program (Minitab) and were tabulated. The data results are represented by graphs and tabulations.

4.4 Results

4.4.1 Chlorophyll content of *M. crystallinum*

The mean values of chlorophyll content recorded over an 8-week experimental period, are presented in Table 4.1. When compared to all other treatments, including a control treatment, treatment "0 ppm" salinity on 4th-day irrigation had the highest on chlorophyll content throughout the experimental period with mean chlorophyll values of 1.13, 1.14, 1.06, and 1.24 mg/m^2 respectively from week 2 to week 8, but was comparable to all other treatments except for week eight. The results indicate that *M. crystallinum* grown with treatment 0 ppm 4th-day irrigation yields high chlorophyll contents compared to other treatment. In contrast, treatment 400 ppm daily and 800 ppm 8th day irrigation yielded lower chlorophyll contents.

Table 4.1: Effects of different salt concentrations and irrigation intervals on the chlorophyll contents of *Mesembryanthemum crystallinum.*

Salt concentration	Irrigation intervals	Week two	Week four	Week six	Week eight
0 PPM	Daily irrigation	0.97± 0.01 c	1.01± 0.01 bcd	1.03± 0.01 abc	1.14± 0.01 cd
	2nd Day irrigation	0.97± 0.04 c	1.01± 0.01 bcd	1.05± 0.01 ab	1.16± 0.01 bc
	4th Day irrigation	1.13± 0.01 a	1.14± 0.02 a	1.06± 0.01 a	1.24± 0.01 a
	8th Day irrigation	1.03± 0.01 abc	1.03± 0.02 a-d	1.05± 0.01 ab	1.22± 0.01 ab
200 PPM	Daily irrigation	0.97± 0.01 c	1.02± 0.04 a-d	0.99± 0.01 abc	1.05± 0.02 e
	2nd Day irrigation	1.00± 0.01 c	1.00± 0.01 cd	1.01± 0.00 abc	1.09± 0.01 de
	4th Day irrigation	1.12± 0.01 ab	1.12± 0.01 ab	1.01± 0.02 abc	1.08± 0.01 de
	8th Day irrigation	1.04± 1.01 abc	1.04± 0.01 a-d	1.03± 0.01 abc	1.19± 0.01 abc
400 PPM	Daily irrigation	0.98± 0.01 c	0.98± 0.01 d	0.99± 0.01 abc	1.08± 0.01 de
	2nd Day irrigation	1.05± 0.01 abc	1.05± 0.01 a-d	1.00± 0.01 abc	1.05± 0.01 e
	4th Day irrigation	1.00± 0.01 bc	1.10± 0.07 abc	1.01± 0.01 abc	1.05± 0.01 e
	8th Day irrigation	1.03±0.01 abc	1.03± 0.01 a-d	1.04± 0.01 ab	1.14± 0.01 cd
800 PPM	Daily irrigation	1.05± 0.07 abc	0.98± 0.01 d	0.98± 0.01 bc	1.09± 0.01 de
	2nd Day irrigation	1.07± 0.01 abc	1.08± 0.01 a-d	1.02± 0.01 abc	1.09± 0.01 de
	4th Day irrigation	1.01± 0.01 bc	1.03± 0.01 a-d	1.02± 0.01 abc	1.06± 0.01 de
	8th Day irrigation	1.01± 0.01 bc	1.03± 0.01 a-d	0.96± 0.05 c	1.01± 0.01 e
Two-way ANOVA Tukey Statistics					

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

4.5 Discussion

Plants grown under stress use more energy to compensate for the loss and jeopardize certain important elements that are essential for plant metabolism (Réthoré *et al*., 2019; Bonacina *et al*., 2022). To survive harsh environmental conditions, plants defend against this stress through multiple changes at physiological and biochemical levels (Moustafa-Farag *et al*., 2020). Water conservation constraints limit CO2 diffusion (Kaur *et al*., 2021). Under water stress, low CO2 inflow is often attributed to stomatal closure and a decrease in mesophyll conductivity (Kaur *et al*., 2021). Thus, leaf morphology influences the utilization of N and CO2, and a higher investment in photosynthetic tissue is related to increased leaf nitrogen concentration, chlorophyll concentration, and photosynthetic efficiency per leaf area (Hou *et al*., 2019).

Photosynthesis is influenced by major micromorphological factors such as stomatal density and size, mesophyll parenchymal thickness, and the quantity of intercellular space inside mesophyll tissue, and has substantial connections with them. (MacMillan *et al.,* 2021). Under high salinity, stomatal conductance decreases, effectively limiting CO2 influx; thus, leaf photosynthetic capacity is limited by the electron transport capacity of thylakoid proteins, Rubisco activity, and mesophyll resistance (Ermakova *et al*., 2019). In this study, the lowest chlorophyll content was observed in treatments subjected to excessive environmental stress, including excessive irrigation under saline conditions and water stress combined with excessive salinity, particularly under treatments 400 ppm daily irrigation and 800ppm eighth day irrigation. This may indicate that, while this species survived the environmental stress, there is a significant loss of metabolic resources that it accounts for, which affects its growth rate (Francisco *et al*., 2019).

The highest chlorophyll content was recorded under 0 ppm at 4th day irrigation. *Mesembryanthemum crystallinum* has been documented as an edible crop that yields best under moist soils other than wet or dry soil (Loconsole *et al*., 2019). Improved mesophyll thickness in non-saline soil provided the leaf with greater intercellular gaps and thus increased mesophyll conductivity (Carillo *et al.,* 2019). High photosynthetic capacity might theoretically boost water use efficiency since more carbon is assimilated per unit water transpired (Condon, 2020). Because these cells are largely photosynthetic, the amounts of leaf nitrogen and chlorophyll grow with the amount of palisade tissue, and chloroplasts contain up to 75% of the leaf organic nitrogen. (Ustin & Jacquemoud, 2020). Even though nutrients may be available to plant, water remains crucial for transporting minerals through all plant parts and reduction in water availability minimizes efficiency of sap movement through all plant parts which results to death of a plant (Bhatla & Lal, 2018). Therefore, it is crucial to adopt crops that can retain water during drought.

The weekly results also show that as the plants grew, all treatments' chlorophyll contents increased. Most studies show that plant chlorophyll levels are higher in younger and adult leaves (Yang *et al*., 2019). Chlorophyll concentrations were consistently greater under 0 ppm daily irrigation, even though chlorophyll contents were not constant. These swings in chlorophyll content were caused by variations in light intensity as seasonal conditions varied and plants matured, causing plants to increase chlorophyll content in low light days to absorb enough light, and vice versa. (He *et al*., 2019). This could imply that the chlorophyll content of *M. crystallinum* increased due to factors other than salt and water stress, as the experiment began in May, when the intensity of sunlight was consistently decreasing as the season progressed into winter, which is associated with low light intensity (Kaiser *et al*., 2019). It's also been reported that chlorophyll levels are directly related to crop growth and yield (Song *et al*., 2020). Few studies have shown that leaf nitrogen content is positively correlated with chlorophyll content (Li *et al*., 2018). Other studies, however, have shown that water and salt stress lower chlorophyll levels, stomata conductance, and photosynthetic rate, but not leaf nitrogen content (Sanchez *et al*., 1983; Hazrati *et al*, 2016; Ngxabi *et al*., 2021) leading to the conclusion that the induced stress losses of chlorophyll are not solely influenced by nitrogen deficiency although as the leaves age, nutrient resorption begins (Li *et al*., 2019). Findings from various experiments may have revealed that plants responses to stress differ, only chlorophyll content was studied in this study. It is therefore critical to monitor chlorophyll content to effectively identify and understand the effect that each environmental stress has on *M. crystallinum* growth and to harvest the leaves at the appropriate time before they begin nutrient resorption.

4.6 Conclusions

Moderate irrigation coupled with no sodium chloride “0 ppm 4th day irrigation” yielded high chlorophyll contents throughout the experimental period, while excessive irrigation under high saline conditions, particularly under treatments “400 ppm daily irrigation” and “800 ppm eighth-day irrigation” resulted in the lowest chlorophyll yield. It was further observed that chlorophyll content of *M. crystallinum* leaves increased as the experiment progressed, even though some studies show that plant chlorophyll levels are higher in younger leaves than in adult leaves. These findings may be associated with other environmental factors such as the seasonal light changes since the experimental study commenced from May, a month which is at the beginning of the winter period. Light availability influences Chlorophyll content more than plant age, as plants increase chlorophyll content in low light conditions to absorb sufficient light, and vice versa.

4.7 Recommendation

Low chlorophyll content is one of the most critical problems limiting crop output and nutritional composition. More research is needed to improve the understanding of the relationship between the nitrogen composition of *M. crystallinum* leaves and chlorophyll content under varying salinity and irrigation intervals.

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Chapter five: Phytochemical contents and antioxidant capacity of *Mesembryanthemum crystallinum* L. in response to different irrigation intervals and salinity levels

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5.1 Abstract

This present study evaluated the phytochemical and antioxidant capacity of *Mesembryanthemum crystallinum* under different salinity and irrigation intervals to establish an appropriate growth protocol for maximum productivity of natural phytochemicals and antioxidants for the increasing demand for these compounds. The treatments consisted of different salt concentrations of 0 ppm (control), 200 ppm, 400 ppm, and 800 ppm achieved by adding sodium chloride to reverse osmosis (RO) water with 0 ppm in conjunction with different irrigation intervals of daily (control), every second day, every fourth day, and every eighth day, resulting to a total of sixteen treatments. The results indicated that treatment 0ppm 8th day irrigation yielded the highest polyphenols (20.06 mg GAE/L) even though there was no significant difference observed $p \leq 0.05$ when compared to control treatment (18.19 mg GAE/L). The highest significance for flavonols (8.95 mg QE/L) was recorded at 0ppm on the 8th day of irrigation. Only 200ppm daily irrigation treatment had the least significant impact on flavonols. FRAP capacity was highest on treatments 0ppm 8^{th}-day irrigation (100,20 umol AAE/L) compared to control treatment (95,58 umol AAE/L) and all other treatments, however, no significant difference was observed between these two treatments. For HDDP, the greatest significance mean value (131,08 umol TE/L) was found with treatment 0 ppm 8^{th}-day irrigation, when compared to the control mean value of (99,61 umol TE/L). *M. crystallinum* exhibited true characteristics of halophytes as polyphenols, DPPH (umol TE/L), and TEAC (umol TE/L) capacity were increased with the decline in salinity and longer irrigation intervals, while increasing salinity had least effects on the production of these compounds. Under low salinity coupled with longer irrigation intervals stress, the concentration of these compounds to *M. crystallinum* increases making it a potential crop for consideration to contribute to natural production of these compounds.

Key words: Antioxidants, flavonols, halophytes, irrigation intervals, salinity, polyphenols, phytochemical, reverse osmosis, water stress.

5.2 Introduction

Almost all environmental and biotic challenges induce a broad molecular stress response known as oxidative stress, which can damage cell components and lead them to malfunction (Gómez *et al*., 2019). This is induced by over-production and accumulation of molecules containing activated oxygen called reactive oxygen species (Gómez *et al*., 2019). Reactive oxygen species (ROS) include hydroxyl radicals, singlet oxygen, and hydrogen peroxide in the plant cells that activates signalling pathways, leading to some disruptive changes in physiological, biochemical, and molecular mechanisms in cellular metabolism (Xie *et al*., 2019; Debnath *et al*., 2021). Excessive ROS, however, cause oxidative stress, a state of imbalance between the production of ROS and the neutralization of free radicals by antioxidants, resulting in damage to cellular components, including lipids, nucleic acids, metabolites, and proteins, which finally leads to cell death in plants (Xie *et al.,* 2019; Adwas *et al.,* 2019). Thus, maintaining a physiological level of ROS is crucial for aerobic organisms, which relies on the combined operation of enzymatic and nonenzymatic antioxidants (Xie *et al*., 2019; Tretter *et al*., 2021).

To improve the tolerance of plants towards the harsh environment, it is vital to reinforce the comprehension of oxidative stress and antioxidant systems and conditions that promote their biosynthesis (Xie *et al*., 2019). Salinity and water stress are twin factors influencing the physiology and biochemistry in many herbs (Bistgani *et al*., 2019). It is thought that high salinity and water stress can delay plant growth, inhibit enzymatic reaction, reduce photosynthetic capacity, and negatively impact agricultural production (Gengmao *et al.,* 2015; Medina *et al.,* 2015). To overcome the stress factors, plants build up some osmotically active compounds to neutralize or suppress the osmotic stress induced by excessive sodium and chloride ionssodium and chloride ions in the soil substrate (Lee *et al.,* 2013). Excess Na^+ and Cl^- change a growth medium's osmolarity and manipulate the transmission of metabolic signal cascades, resulting in increased production of secondary metabolites in plants (Medina *et al.,* 2015).

Polyphenols are plants' most abundant secondary metabolites with excellent antioxidant ability to capture oxidative free radicals (Xu *et al*., 2020). Depending on their chemical structure, they are sub-classified into flavonoids, phenolic acids, and tannins (Cui *et al.,* 2020). Flavonoids possess several medicinal benefits, including anticancer, antioxidant, anti-inflammatory, and antiviral properties (Ullah *et al*., 2020). They also have neuroprotective and cardio-protective effects (Jabeen *et al*., 2021). The protective roles of antioxidants against free radicals, which potentially result to cancer, heart disease, stroke and other chronic diseases in humans, are enormous (Njoya, 2021). Antioxidant ingredients can stabilize or deactivate free radicals, avoiding and diminishing cellular injury and maintaining health (Omidifar *et al*., 2021; Michalak *et al*., 2022).

Historically, physicians used leaf juice of *M. crystallinum* to soothe inflammation of the mucous membranes of the respiratory or urinary system (Mohlakoana & Moteetee, 2021) while in Europe, the fresh juice has been used to treat water retention painful urination and to soothe lung inflammation (Hirpara *et al.,* 2005). The presence of epidermal bladder cells on stems, flower buds and leaves of *M. crystallinum* with which the plant excretes excess salt via non-glandular trichomes (Agarie *et al*., 2007; Barkla *et al.,* 2016) is a critical adaptive strategy that enables the plant to survive harsh saline conditions. Since *M. crystallinum* has evolved this strategy, it is unclear how the introduced stress factors, namely, salinity and water regimes, will affect the yield of phytochemicals and antioxidant activity of the plant. It is therefore important to understand the impact of different irrigation intervals and salinity on antioxidant capacity of *M. crystallinum* to implement the most productive protocol to maximize naturally produced antioxidants from the plant. This study investigated, for the first time, the combined effects of salinity and irrigation intervals on polyphenols, flavonoids, and antioxidant contents of *M. crystallinum* using the DPPH, FRAP and TEAC assays to validate claims on its ethnobotanical usage and advance its pharmacological potential.

5.3 Materials and methods

5.3.1 Study area

The experiment was carried out in the research greenhouse of the Horticultural Science Department of the Cape Peninsula University of Technology (CPUT) in Cape Town, South Africa. The greenhouse temperature was set to range between 21 and 26 °C during the day and between 12 and 18 °C at night, with a relative humidity of 60%. On average, the daily photosynthetic photon flux density (PPFD) was 420 μmol/m2/s, with a maximum of 1020 μmol/m2/s.

5.3.2 Plant Preparation, Irrigation, and Treatments

Seeds of *Mesembryanthemum crystallinum* were obtained from a commercial garden centre, Renu-Karoo Nursery & Veld Restoration, at Prince Albert, Western Cape, South Africa. The seeds were sown in two small seed trays containing a mixture of silica sand, cococoir, and vermiculite (1:1:1), as Loconsole et al. (2019) described. A layer of course bark was laid in the seed tray before the medium was added to prevent leaching. Approximately 2 kg of the medium was added to each tray. Thereafter, the seeds were evenly broadcasted on the trays, followed by the application of a thin layer of vermiculite on top of the seeds. The trays were then watered with Captab (4 g/L) manufactured by Universal Crop Protection (Pty) Ltd., Kempton Park, South Africa, to prevent the development of fungal diseases and were placed on a heating bed under mist irrigation sprayers for germination (Figures 5.1 – 5.3).

Figure 5.1a: Germinating *Mesembryanthemum crystallinum* seeds in flat trays. (Source: Okuhle Mndi)

Figure 5.1b: Transplanted *Mesembryanthemum crystallinum* transplanted plugs to 15 cm pots (Source: Okuhle Mndi). seedlings to plug trays. (Source: Okuhle Mndi)

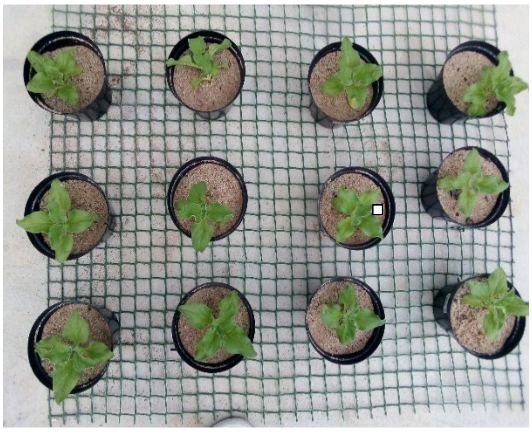

Figure 5.1d: Re-transplanted plants to 15cm pots. (Source: Okuhle Mndi)

Figure 5.1c: One week after transplanting *Mesembryanthemum crystallinum* seedlings to plug trays. (Source: Okuhle Mndi)

After the first set of true leaves emerged, one hundred and ninety-two (192) uniformly germinated seedlings were washed using tap water to remove soil and other debris. They were then potted up in 12.5 cm black plastic pots containing river sand and, thereafter, were hardened off for a week in the greenhouse before they were moved to the experimental site (See Figure 5.1a-d).

During this period, seedlings were irrigated daily with a nutritive solution formed by adding NUTRIFEED™ (manufactured by STARKE AYRES Pty. Ltd., Gauteng, South Africa) to municipal water at 10 g per 5 L. The nutrient solution contained the following ingredients: N

(65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg), and Zn (240 mg/kg).

After acclimatization, plants were watered with distilled water for five days to wash off any salt residue and, thereafter, were organized into four irrigation treatments ((1) 100 mL once a day; (2) 100 mL once every 2 days; (3) 100 mL once every 4 days; (4) 100 mL once every 8 days) with four salt concentrations (0, 200, 400, and 800 ppm) applied in each treatment. Salt concentrations were set up by adding increasing concentrations of NaCl to the nutrient solution. A handheld EC meter (Martini EC 59, manufactured by Milwaukee®, Milwaukee, MI, USA) was used to measure the salt concentration in the solution. A completely randomized design was used to accommodate the two-way factorial experiment. Forty-eight (48) replicates per factorial combination were used, totalling to 192 plants for the entire experiment.

5.3.3 Collection and processing of plant materials

Leaves of *M. crystallinum* were harvested after the eighth week of transplanting. Shoots and roots were divided at the post-harvest stage, and the fresh weights of each plant were determined using a typical laboratory scale (RADWAG® Model PS 750.R2). The plant material was dried to a crispy, consistent weight in an oven at 55 ◦C using a LABTECHTM model LDO 150F (Daihan Labtech India Pty. Ltd., 3269 Ranjit Nagar, New Delhi, India).

5.3.4 Phytochemical and Antioxidant Assays

5.3.4.1 Sample Preparation

The dried material of *M. crystallinum* was ground into a fine powder using a Junkel and Kunkel model A 10 mill. The samples were extracted by mixing 100 mg of the dried powdered material with 25 mL of 80% (v/v) ethanol (Merck, South Africa) for 1 hour. They were centrifuged at 4000 rpm for 5 min, and the supernatants were used for all analyses.

5.3.4.2 Total Polyphenols

The total polyphenol content of the extracts was determined using the Folin–Ciocalteu method, as reported by Sogoni et al. (2021), with slight modifications. About 25 µL of the sample was mixed with 125 µL of Folin–Ciocalteu reagent (Merck, Johannesburg, South Africa) that was diluted 10 times with distilled water in 96-well microplates. A 7.5% sodium carbonate solution was prepared and added to a 96-well microplate with extracts. The plate was incubated for 2 h at room temperature, and the absorbance was then measured at 765 nm in a Multiskan spectrum plate reader (Thermo Electron Corporation, Waltham, MA, USA). The standard curve was prepared using 0, 20, 50, 100, 250, and 500 mg/L gallic acid (Sigma, South Africa) in 10% EtOH, from which the polyphenolic content was extrapolated, and the results were expressed as mg gallic acid equivalent per g dry weight (mg GAE/g DW).

5.3.4.3 Estimation of Flavonol Content

The flavonol content of the extracts was determined using standard quercetin at 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol (Sigma-Aldrich, Johannesburg, South Africa) (Lim *et al*., 2022). A volume of 12.5 µL of the crude sample extracts was mixed with 12.5 µL 0.1% HCl (Merck, South Africa) in 95% ethanol and 225 µL of 2% HCl. The extracts were then incubated for 30 min at room temperature. The absorbance was read at 360 nm at a temperature of 25 °C. The results were expressed as milligram quercetin equivalent per gram dry weight (mg QE/g DW).

5.3.4.4 DPPH Free Radical Scavenging Activity

The DPPH radical was generated from a solution of 0.135 mM DPPH prepared in a dark bottle, as stated by Ohikhena *et al*. (2017). A volume of 300 µL of DPPH solution was reacted with graded concentrations (0 and 500 µM) of Trolox standard (6-Hydrox-2,5,7,8-tetramethylchroman-2-20 carboxylic acid) solution and 25 µL of crude extract. The mixtures were incubated for 30 min, after which absorbance was taken at 517 nM. The results were expressed as µM/Trolox equivalent per g dry weight (µM TE/g DW).

5.3.4.5. Ferric Reducing/Antioxidant Power (FRAP) Assay

The FRAP assay was performed using the method of Benzie and Strain (1999), and Jimoh, *et al*. (2020). FRAP reagent was prepared by mixing 30 mL of Acetate buffer (0.3 M, pH 3.6) (Merck, South Africa) with 3 mL of 2,4,6-tripyridyl-s-triazine (10 mM in 0.1 M hydrochloric acid) (Sigma, South Africa).13, 1026 6 of 21 3 mL of iron (III) chloride hexahydrate (FeCl3·6H2O) (Sigma, South Africa), and 6 mL of distilled water. In a 96-well plate, 10 µL of the crude sample extract was mixed with 300 µL of the FRAP reagent and incubated for 30 min at room temperature. The absorbance was then measured at 593 nm in a Multiskan spectrum plate reader (Thermo Electron Corporation, USA). The sample's FRAP values were calculated using an L-ascorbic acid (Sigma-Aldrich, South Africa) standard curve with concentrations varying between 0 and 1000 µM. The results were expressed as µM ascorbic acid equivalents (AAE) per g dry weight (µM AAE/g DW).

5.3.4.6 Determination of ABTS Antioxidant Capacity

The antioxidant capacity of ABTS was measured using a method described by Sogoni *et al*., (2021) with minor modifications. The stock solutions included a 7 mM ABTS solution and a 140 mM potassium peroxide sulphate (K2S2O8) solution (Merck, South Africa). 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. After allowing crude sample extracts (25 µL) to react with 300 µL ABTS in the dark at room temperature for 5 minutes, the absorbance was measured in a microplate reader at 734 nm at 25 C. M/Trolox equivalent per gram dry weight (M TE/g DW) was calculated.

5.3.4.7 Determination of antioxidant activity by the TEAC method

The radical cation (ABTS+) 2,2′-Azinobis (3-ethyl-benzothiazoline-6-sulfonic acid) diammonium salt was synthesized by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate (K2S208). The maximum absorbance of ABTS+ radical cation is 734 nm. The ABTS+ radical cation was diluted in PBS until its absorbance at 734 nm was 0.700 ± 0.02. The reaction was completed in 6 minutes at 30 °C after 100 µL of sample or Trolox solution prepared at different concentrations was added to 2 mL ABTS+ radical. The drop in absorbance at 734 nm was used to quantify the antioxidants' interaction with the radical. The extract's TEAC value was determined using the Trolox standard curve and represented as mmol TE/L extract (Aykın-Dinçer *et al.*, 2021).

5.3.4.8 Data collection and statistical analysis

The laboratory results were in triplicates per treatment and were statistically analysed using two-way analysis of variance (ANOVA) on Minitab software. The significant differences between treatment mean at $p \leq 0.05$ was compared using Tukey least significant difference. The calculations to obtain statistical data were accomplished through the computer program (Minitab) and presented in graphs.

5.4 Results

5.4.1 Polyphenols

When comparing control to the other treatments, salinity and water stress significantly reduced polyphenol capacity. The polyphenol concentration decreased as drought and salinity stress increased (Figure 5.2). Treatment 0 ppm salinity with 8th day irrigation intervals yielded the highest polyphenols (20.06 mg GAE/L) even though there was no significant difference $p \leq 0.05$ when compared to the control treatment (18.19 mg GAE/L). In each salinity treatment, the highest polyphenols were found on the eighth day of irrigation. The results showed that increasing salinity lowers polyphenol concentrations in *M. crystallinum* species correspondingly with increasing irrigation intervals (Figure 5.2).

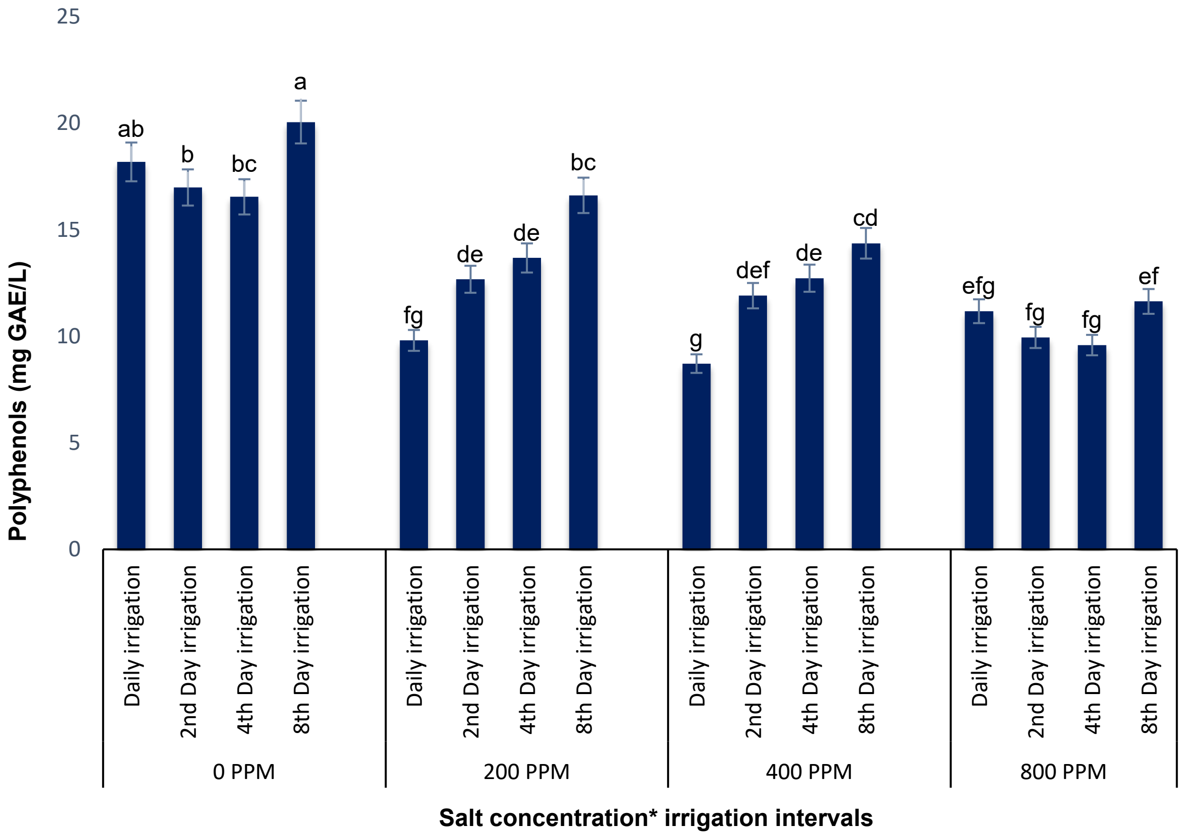

Figure 5.2: Effects of different salt concentrations and irrigation intervals on polyphenol contents of *Mesembryanthemum crystallinum.*

5.4.2 Flavonols

The highest flavonols (8.95 mg QE/L) were recorded at 0 ppm salt concentration under 8th day irrigation (Figure 5.3). Only treatment 200 ppm salinity with daily irrigation had the least significant impact on flavonols. When compared to the control treatment, all treatments in 200 ppm, 400 ppm, and 800 ppm salinity recorded a lower mean flavonols value (Figure 5.3). Under these salinity treatments, a maximum mean value of 5.24 mg QE/L was recorded at 400 ppm salinity with every 8th day irrigation, while a minimum mean value of 2.79 mg QE/L of flavonols was recorded at 400 ppm salinity with everyday irrigation (Figure 5.3).

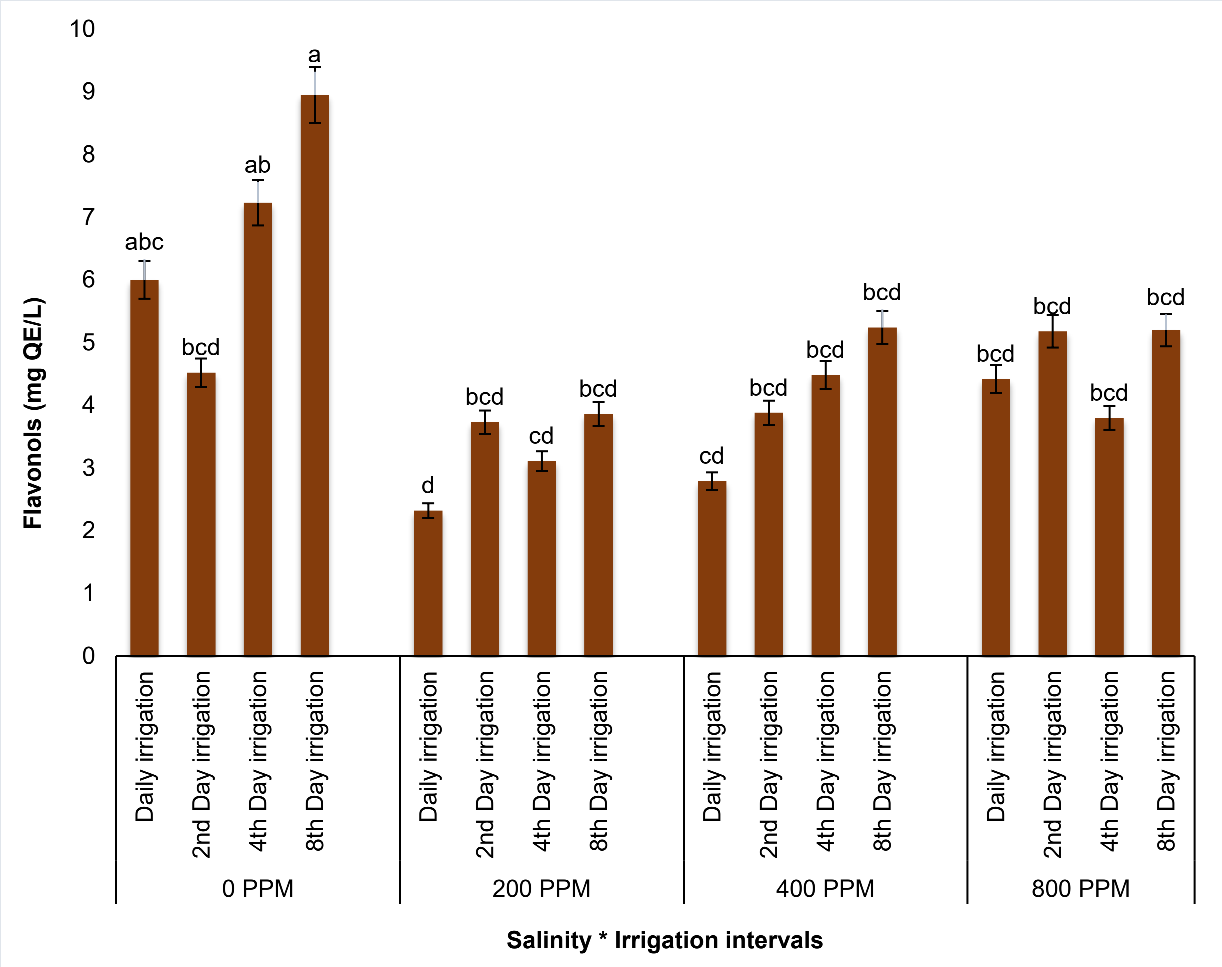

Figure 5.3: Effects of different salt concentrations and irrigation intervals on flavonols (mg QE/L) content of *Mesembryanthemum crystallinum.*

5.4.3 The FRAP antioxidant content (µmol AAE/L)

The FRAP antioxidant content was highest (100.20 µmol AAE/L) in samples of *M. crystallinum* treated with 0 ppm salinity and 8th-day irrigation interval compared to the control treatment 95.58 µmol AAE/L and all other treatments, despite no significant difference being observed between these two treatments (Figure 5.4). Compared to daily, 2nd, and 4th day irrigation, the 8th day irrigation yields the highest FRAP antioxidant under all salinity treatments. On every eighth day irrigation, the effect of the control and all salinity treatments was not statistically significant (α = 0.05) on the FRAP content of the tested plant samples (Figure 5.4).

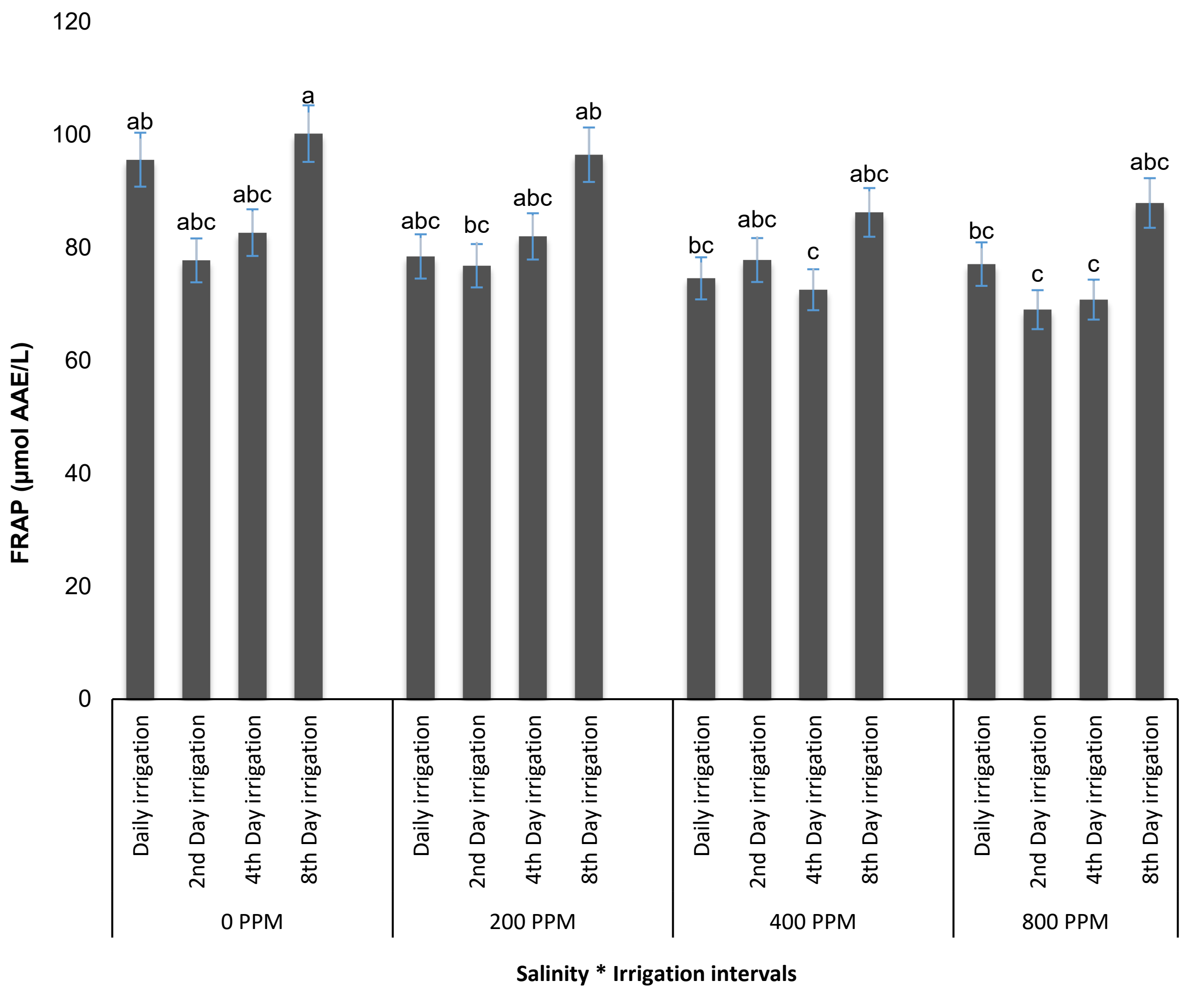

Figure 5.4: Effects of different salt concentrations and irrigation intervals on FRAP content of *Mesembryanthemum crystallinum.*

5.4.4 DPPH capacity (µmol TE/L)

The greatest significance mean value (131.08 µmol TE/L) was found with treatment 0 ppm under 8th day irrigation, when compared to the control mean value of 99.61 µmol TE/L (Figure 5.5). The treatments with the lowest salinity and the longest irrigation intervals had the highest DPPH antioxidant capacity. In general, increased salinity coupled with more frequent irrigation resulted in a decrease in DPPH antioxidant capacity. Under all treatments, frequent irrigation reduces the DPPH antioxidant capacity of *M. crystallinum*. Increased irrigation frequency results in frequent reductions in this crop's DPPH capacity. Compared to the control treatment, all daily irrigation treatments under 200 ppm, 400 ppm, and 800 ppm had significantly lower mean values (Figure 5.5).

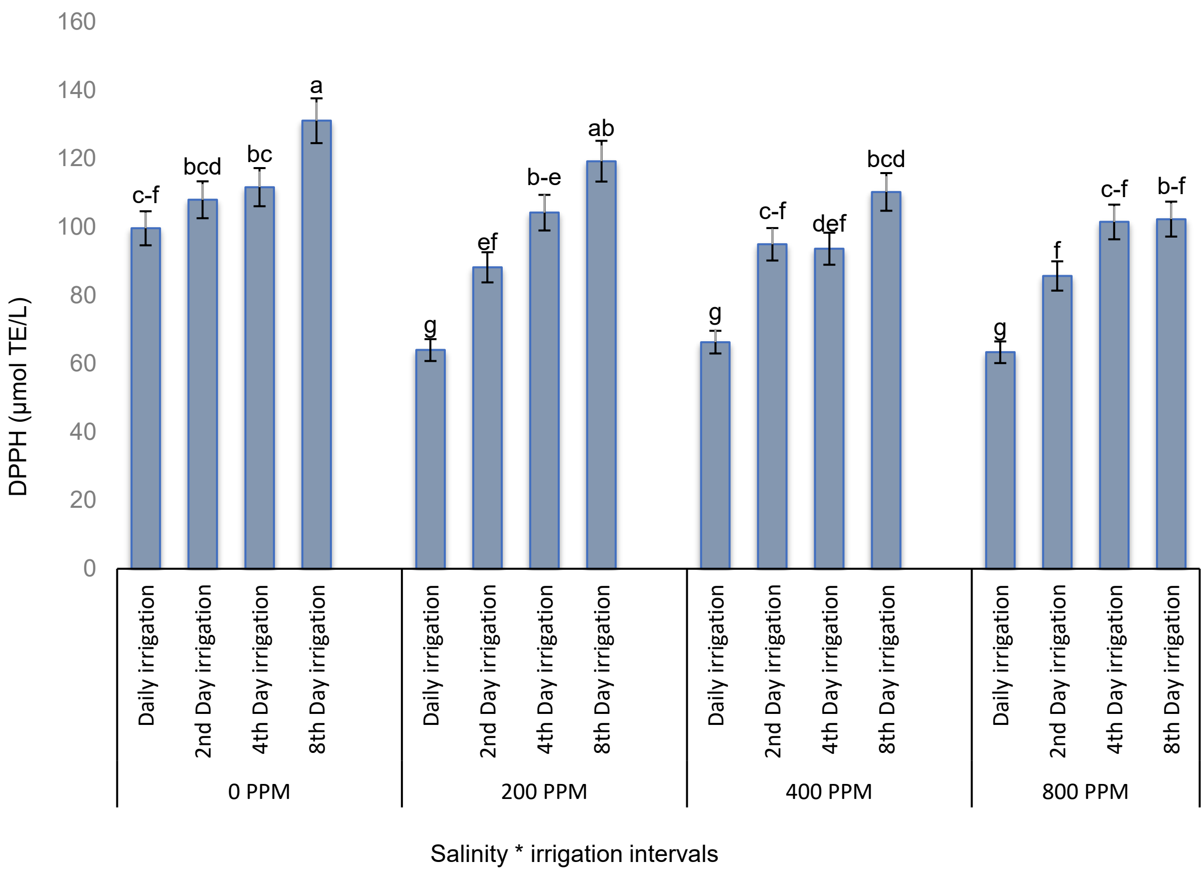

Figure 5.5: Effect of different salinity concentrations and irrigation intervals on DPPH (µmol TE/L) capacity mean values of *Mesembryanthemum crystallinum.*

5.4.5 TEAC contents (umol TE/L)

In terms of TEAC concentration, the control treatment (0 ppm salinity, daily irrigation) mean was significantly ($p \leq 0.05$) higher (141.14 µmol TE/L) than all other treatments (Figure 5.6). The lowest mean value (88.65 µmol TE/L) was recorded on treatments with 400 ppm daily irrigation. A high level of TEAC antioxidant (133,41 µmol TE/L) was also recorded in harvested plant samples grown under 200 ppm salinity and 8th day irrigation, whereas the TEAC values varied in tested samples of *M. crystallinum* harvested from other treatments (Figure 5.6).

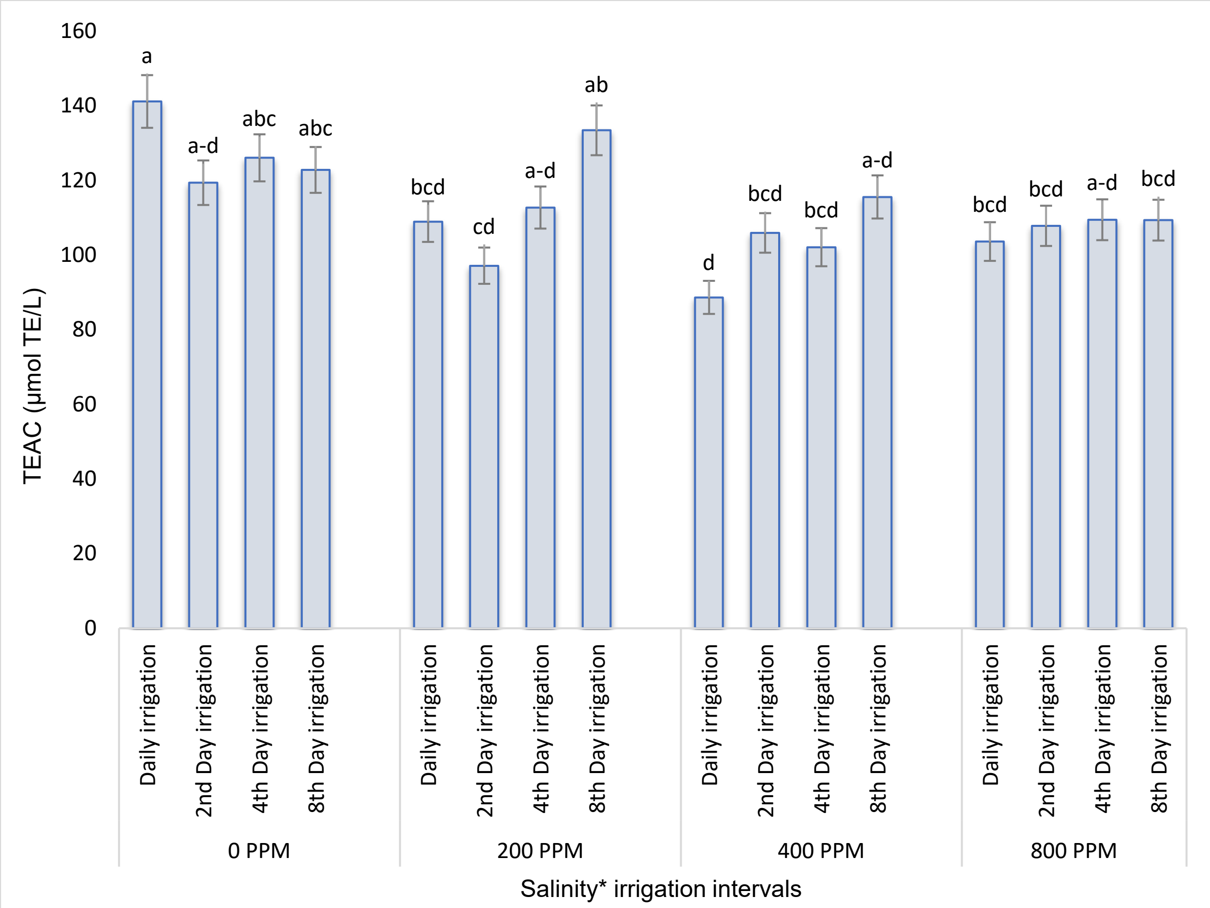

Figure 5.6: Effect of different salinity concentrations and irrigation intervals on TEAC (µmol TE/L) capacity of *M. crystallinum.*

5.5 Discussion

Not all plants respond to salinity similarly; some exhibit a considerable increase in free radical-scavenging activity, while others exhibit a significant decline in biological activity (Ngxabi *et al*., 2021). In this study, an increase in salinity and water stress completely reduced the polyphenol capacity on *M. crystallinum,* while low salinity with frequent irrigation resulted in high production of polyphenols. In *Thymus vulgaris* and *Thymus daenensis* for instance, it was observed that salinity stress improved the production of phenolic compounds (Bistgani *et al.*, 2019; Bahcesular *et al*., 2020). Generally, a rise in the level of secondary metabolite is a stress defence strategy that plants employ in the detoxification of free radicals to adapt to stress factors and sustain critical plant activities (Haida *et al.,* 2022). The decrease in polyphenol production is suggested to be due to elevated salt concentrations produced by reduced uptake of phosphorus and potassium, which are important ingredients in the creation of secondary metabolites like polyphenols (Ngxabi *et al.,* 2021). However, this may be different for *M. crystallinum*, as this species has taken further its salt and drought tolerance by evolving non-glandular epidermal bladder cells on stems, flower buds and leaves with which it excretes excess salt (Atzori *et al*., 2017; Agarie *et al.,* 2007; Barkla *et al.,* 2016).

High polyphenols production was observed on the eighth day of irrigation interval in all salinity treatments, which was inversely related to salt concentration. This corroborates earlier findings that drought (water stress) optimizes the concentration of polyphenols in plants (Barchet *et al.,* 2014; Popović *et al.*, 2016) although species, stress intensity or nature of stress may increase or decrease the phenolic levels in plants (Cheruiyot *et al.,* 2007; Griesser *et al.,* 2015; Cherit-Hacid *et al.,* 2015). Furthermore, plants living in saline environments have a higher endogenous concentration of high-nutrient molecules (Atzori *et al.,* 2017) which are essential components of complex defence signalling networks that control the dynamic and specific responses of plants to environmental stress (Balbi & Devoto *et al.,* 2008).

Even though flavonoids such as flavonols, anthocyanins, and proanthocyanidins have been shown to improve salt tolerance (Wang *et al*., 2021), when compared to the experimental control treatment, the maximum significance for flavonols (8.95mg QE/L) was reported on the combined treatment of 0 ppm, 8th day irrigation. This is consistent with previous findings in which high salt levels (200 mM or above) were found to be ineffective for flavonol accumulation (Ngxabi *et al*., 2021). This also agrees with Agudelo *et al*., (2021), who showed that 100 mM salinity was the most effective concentration on the antioxidant capacity of a halophyte, whereas the lowest mean value was observed in the treatment of 200 mM, which was the greatest salt concentration. All treatments in salinities of 200 ppm, 400 ppm, and 800 ppm had a lower mean flavonols values, ranging from 2.79 mg QE/L at 400 ppm daily irrigation to 5.24 mg QE/L at 400 ppm every 8th day irrigation. Salt favours the vegetative growth of halophytes but inhibits non-halophytes' growth at optimum amounts (Yuan *et al*., 2019). According to reports, an increase in total flavonoid accumulation increases salt tolerance in several crops (Wang *et al*., 2021).

Surprisingly, no significant difference was seen between these two treatments, the highest FRAP capacity (100.20 umol AAE/L) was recorded on treatment 0ppm 8th day irrigation when compared to the control treatment 95.58 umol AAE/L and all other treatments. The FRAP capability dropped as irrigation salinity increased, with extracts from freshwater irrigated plants exhibiting the highest activity (Rodrigues *et al*., 2020). Even though varied observations have been made on the activity of extracts, for example, *Sesuvium portulacastrum* L. was depending on the plant organ; for example, leaves and roots had enhanced activity with increasing salt levels, whereas stems had decreased antioxidant activity (Nikalje *et al*., 2018).

5.5 Conclusions and recommendations

M. crystallinum exhibited true characteristics of halophyte species as it produced these compounds normally under high saline state needing less excretion of these compounds to sustain normal growth. Polyphenols, DPPH (umol TE/L) capacity, and TEAC (umol TE/L) capacity was increased with decline in salinity and longer irrigation intervals. Further studies focusing on the salinity pathways in the plant and how it utilized salt to produce secondary metabolites is crucially recommended for effective understanding of potential pharmacological uses.

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Chapter six: The effects of different irrigation intervals and salt concentrations on full feed contents of *Mesembryanthemum crystallinum* L. leaves

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6.1 Abstract

Vegetable crops are the most affordable and accessible source of essential nutrients for nutritional diversity. Many countries are expected to focus more on food security projects that promote vegetables for human health. This is impelled by the increasing food crisis, malnutrition, and environmental stress such as salinity and drought which continuously limit production of adequate food quantities and quality. The effects of different salt concentrations and irrigation intervals on *Mesembryanthemum crystallinum* as a potential coastal edible crop was evaluated using proximate analysis (protein, fat, ash, ADF, NDF, and moisture), macro nutrients (nitrogen, phosphorous, potassium, calcium, and Magnesium), and micronutrients content (Zinc, copper, iron, boron, and manganese). The treatments consisted of different salt concentrations of 0 ppm, 200 ppm, 400 ppm, and 800 ppm in conjunction with different irrigation intervals of daily, every second day, every fourth day, and every eighth day, resulting to a total of sixteen treatments. The results show that an increase in salinity levels being inversely proportional to the acid detergent fibre (ADF), fat, protein, nitrogen (N), and Magnesium (Mg) of *M. crystallinum* leaves. At the same time, the increase in salinity was directly proportional to the increase to ash content, copper (Cu), zinc (Zn), and sodium (Na) increase. Adding to the evidence that *M. crystallinum* is a true halophyte with nutritional composition superior to Ethiopia kale (*Brassica carinata*), Swiss chard (*Beta vulgaris*), carrot (*Daucus carota*), tomato (*Lycopersicon esculentum*), and cabbage (*Brassica oleracea*).

Keywords: salinity, edible, macro nutrients, micronutrients, proximities, drought, malnutrition, food security.

6.2 Introduction

Plants require 17 elements to complete their life cycle, and a lack of any of these element's results in diminished metabolic and biological activities (Soetan *et al.*, 2010; Kathpalia & Bhatla, 2018). The shortage of these minerals could lead to plant mortality while reducing plant growth and development (Hajiboland, 2018; Gaikwad & Maitra, 2020). Apart from carbon, hydrogen, and oxygen which plants acquire from air and water, the remaining 14 elements are obtained from soil or through fertilizers, manures, and amendments (Amoah-Antwi *et al*., 2020). Plant nutrients are classified into two groups: macronutrients (nitrogen, potassium, phosphorous, magnesium, calcium, and sulphur) and micronutrients (manganese, molybdenum, iron, chloride, zinc, aluminium, boron, and copper), which are needed in smaller amounts but are necessary for plant development (Hansonis, 2022; Vejan *et al*., 2021). Each element has a distinct purpose in the plant, adding to the plant's overall development (Kathpalia & Bhatla, 2018).

However, plant's access to these vital elements is constantly limited by drought and salinity (Brito *et al*., 2019). Drought and salinity are two of the most damaging environmental conditions in agriculture, affecting plant development and metabolic activities such as water relations, photosynthetic assimilation, and nutrient uptake (Farooq *et al*., 2009; Brito *et al*., 2019). This reduction in growth and metabolic processes is caused by two factors: the relatively high osmotic potential of soil solution and ion toxicity, both of which result in a water deficit within each plant, and subsequent secondary stresses such as nutritional deficiency and oxidative stress (Soylemezoglu *et al*., 2009; Yue *et al*., 2012).

Limited nutrient availability and uptake by plants have a huge impact on food production and the economy, and this has a continual impact on global food security (Fita *et al*., 2015; Cheeseman, 2016; Wen *et al*., 2020; & Gomez-Zavaglia *et al*., 2020). Between 2015 and 2018, the Western Cape Province of South Africa was severely impacted by drought (Dube *et al*., 2020; Meza *et al*., 2021). Due to water shortages, green industries in the province suffered substantially, with 50% less onions and 80% less potatoes in the Ceres district (Williams, 2019). Reduced crop planting in impacted areas led to an estimated loss of income of approximately 2.5 m $ (R40 million) due to seasonal worker employment (Ziervogel, 2019; Zwane, 2019; Hunter & Cronin, 2020; Ringas *et al*., 2020). The drought also reduced the quantity and quality of fruit and vegetable products exported, resulting in an estimated loss of 16.1m $ (R259 million) (Williams, 2019).

Vegetables are an important part of everyday meals since they offer multiple benefits, including serving as a colourful side dish, improving the palatability of starchy staple foods, and delivering critical nutrients to the diet (Vandebroek & Voeks, 2018; Beck *et al*., 2019). Likewise, vegetables are the most affordable and accessible source of micronutrients, and it is expected that countries should pay more attention to food security projects that promote vegetables for good health (Hoffman *et al*., 2018; Singh *et al*., 2019). According to World Health Organisation (WHO, 2020) nutritionists, a daily ration of diverse vegetables is critical for a well-balanced diet, especially for children and pregnant women. However, in tropical Africa, nutritional inadequacy is reported to be the leading cause of health problems, potentially leading to high mortality rates and low economic productivity (Singh *et al*., 2019; Vianna *et al*., 2020).

The most common micronutrient deficiencies identified worldwide include zinc, iron, vitamin A, folate, iodine, vitamin B12, and other vitamin B (Misra *et al*., 2008; Bharadva *et al*., 2019). To achieve a balanced diet, WHO-FAO (2020) advises a daily global average intake of 400 g of fruit and vegetables per person. However, vegetables contain more micronutrients than fruits alone (Grubben *et al*., 2014). Traditional leafy vegetables' nutritional value is higher than other popularly known vegetables (Sarker & Oba, 2019). Although most of these traditional leafy vegetables have a potential for income generation, they still fail to compete with exotic vegetables at present due to lack of awareness and knowledge in terms of how they should be prepared or grown (Misra *et al*., 2008; Bokelmann *et al*., 2022).

Mesembryanthemum crystallinum has been documented as an edible plant species that was formerly served as a vegetable and used as a substitute for crops such as spinach (*Spinacia oleracea*) and cucumber (*Cucumis sativus*). The species strives through drought period with minimal water availability (Herppich *et al*., 2012). Consumption of traditional plant-based diets known to old civilizations is said to have many beneficial effects, including prevention of some age-related degenerative diseases such as arteriosclerosis, stroke, and cardiovascular crisis (Misra *et al*., 2008). Being a facultative halophyte, it is speculated that growing *M. crystallinum* under semi-hydroponic conditions with different salt concentrations and irrigation intervals could improve the nutritional values of the species. Hence, this study aimed to investigate the effect of salinity and irrigation regimes on the nutritional properties of *M. crystallinum* to promote its commercial production as a leafy option that could combat food shortages and malnutrition.

6.3 Materials and methods

6.3.1 Study area

The experiment was carried out in the research greenhouse of the Horticultural Science Department of the Cape Peninsula University of Technology (CPUT) in Cape Town, South Africa. The greenhouse temperature was set to range between 21 and 26 °C during the day and between 12 and 18 °C at night, with a relative humidity of 60%. On average, the daily photosynthetic photon flux density (PPFD) was 420 µmol/m2/s, with a maximum of 1020 µmol/m2/s.

6.3.2 Plant Preparation, Irrigation, and Treatments

Seeds of *M. crystallinum* were obtained from a commercial garden centre, Renu-Karoo Nursery & Veld Restoration, at Prince Albert, Western Cape, South Africa. The seeds were sown in two small seed trays containing a mixture of silica sand, cococoir, and vermiculite

(1:1:1), as described by Loconsole *et al.* (2019). A layer of course bark was laid in the seed tray before the medium was added to prevent leaching. Approximately 2 kg of the medium was added to each tray. Thereafter, the seeds were evenly broadcasted on the trays, followed by the application of a thin layer of vermiculite on top of the seeds. The trays were then watered with Captab (4 g/L) manufactured by Universal Crop Protection (Pty) Ltd., Kempton Park, South Africa, to prevent the development of fungal diseases and were placed on a heating bed under mist irrigation sprayers for germination. After the first set of true leaves emerged, one hundred and ninety-two (192) uniformly germinated seedlings were washed using tap water to remove soil and other debris. They were then potted up in 12.5 cm black plastic pots containing river sand and, thereafter, were hardened off for a week in the greenhouse before they were moved to the experimental site.

During this period, seedlings were irrigated daily with a nutritive solution formed by adding NUTRIFEED™ (manufactured by STARKE AYRES Pty. Ltd., Gauteng, South Africa) to municipal water at 10 g per 5 L. The nutrient solution contained the following ingredients: N (65 mg/kg), P (27 mg/kg), K (130 mg/kg), Ca (70 mg/kg), Cu (20 mg/kg), Fe (1500 mg/kg), Mo (10 mg/kg), Mg (22 mg/kg), Mn (240 mg/kg), S (75 mg/kg), B (240 mg/kg), and Zn (240 mg/kg). After acclimatization, plants were watered with distilled water for 5 days to wash off any salt residue and, thereafter, were organized into four irrigation treatments ((1) 100 mL once a day; (2) 100 mL once every 2 days; (3) 100 mL once every 4 days; (4) 100 mL once every 8 days) with four salt concentrations (0, 200, 400, and 800 ppm) applied in each treatment. Salt concentrations were set up by adding increasing concentrations of NaCl to the nutrient solution. A handheld EC meter (Martini EC 59, manufactured by Milwaukee®, Milwaukee, MI, USA) was used to measure the salt concentration in the solution. A completely randomized design was used to accommodate the two-way factorial experiment. Forty-eight (48) replicates per factorial combination were used, totalling 192 plants for the entire experiment.

6.3.3 Collection and processing of plant materials

Leaves of *M. crystallinum* were harvested after the eighth week of transplanting. Shoots and roots were divided at the post-harvest stage, and the fresh weights of each plant were determined using a typical laboratory scale (RADWAG® Model PS 750.R2). The plant material was dried to a crispy, consistent weight in an oven at 55 ◦C using a LABTECHTM model LDO 150F (Daihan Labtech India Pty. Ltd., 3269 Ranjit Nagar, New Delhi, India).

Figure 6.1a: Germinating *Mesembryanthemum crystallinum* seeds in flat trays. (Source: Mndi Okuhle)

Figure 6.1b: Transplanted *Mesembryanthemum crystallinum* transplanted plugs to 15 cm pots

Figure 6.1c: *Mesembryanthemum crystallinum* seedling growth one week after transplanting to plug trays (Source: Okuhle Mndi).

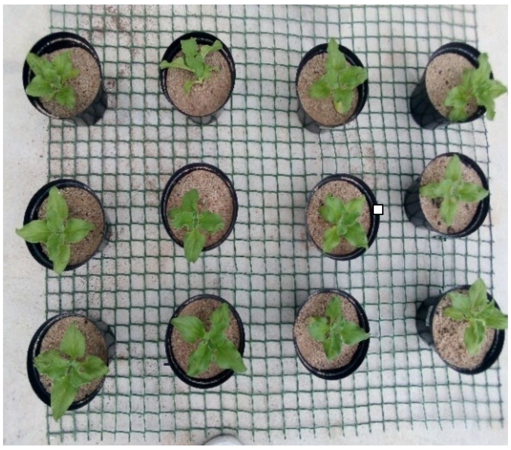

Figure 6.1d: Transplanted *Mesembryanthemum crystallinum* plants to 15 cm pots. (Source: Okuhle Mndi)

6.3.4 Nutritional Analysis

6.3.4.1 Sample Preparation

Dried leaves of each set of replicates were pulverized with an electric motor blender and transferred into airtight containers, which were kept in a refrigerator at 4 °C for nutritional analyses.

6.3.4.2 Mineral Analysis

The elemental analysis was performed using the Inductively Coupled Plasma–Optical Emission Spectrometer in the analytical laboratory of the Department of Agriculture and Rural Development, KwaZulu Natal Province, South Africa, as described by Bulawa *et al*. (2022), to determine the mineral composition of each set of replicates in the experiment.

6.3.4.3 Proximate Analysis

a. Moisture Content

The procedure given by Jimoh *et al*. (2020) with slight modifications was used to determine the moisture content. Empty porcelain vessels were dried in an oven at 105 ◦C for one hour, allowed to cool in a desiccator, and weighed W1. One gram of pulverised M. C crystallinum (W2) samples were placed in a vessel and oven-dried to a constant weight at 105 °C. The vessel and its contents were cooled in a desiccator before being reweighed (W3). The calculation below was used to determine the percentage of moisture content.

$$\%\ \text{Moisture content } = \frac{\text{W2} - \text{W3}}{W2 - W1} X100$$

b. Crude Fat Content

Following the recommendations and guidelines from the AOAC techniquedescribed by Nielsen (2017), the crude fat was determined. A pulverized sample of about 1 g was extracted in 100 mL of diethyl ether and shaken on an orbital shaker for 24 h. The mixture was then filtered, and the filtrate was collected in previously weighed clean beakers. The ether extract was then equilibrated with 100 mL of diethyl ether and shaken for another 24 h on an orbital shaker, and the filtrate was collected in a beaker (W1). The ether filtrate was concentrated to dryness in a steam bath and oven-dried at 55 ◦C before being reweighed in the beaker (W2). The proportion of crude fat was calculated using the formula below.

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

c. Ash Contents

To calculate the percentage ash content of plant samples, the AOAC technique described by Nielsen (2017) was utilized. After being marked with sample codes using a heat-resistant marker, porcelain crucibles were oven-dried at 105 ◦C for one hour. The crucibles were weighed after cooling in a desiccator (W1). Thereafter, 1 g of ground samples was added to porcelain crucibles that had already been weighed (W2). The crucibles with the contents were placed in a muffle furnace set to 250 ◦C for 1 h and then 550 ◦C for 5 h to completely ash the samples. After desiccator cooling, the samples were weighed (W3). The samples' ash content was calculated as:

$$\%\ \text{Ash content } = \frac{\text{W2}-\text{W3}}{\text{W2}-\text{W1}} \times 100$$

d. Crude Protein

Crude protein was determined by boiling 2 g of ground samples in a Kjeldahl flask with concentrated H2SO4 (20 mL) until a clear mixture was obtained, with a digestion tablet acting as a catalyst. The digested extracts were distilled after being filtered and dissolved in 250 mL. An aliquot containing 50 mL of 45% NaOH was distilled further in a 500 mL round-bottomed flask, and 150 mL of the distillate was transferred into a flask containing 100 mL of 0.1 M HCl. This was then titrated with methyl orange against 2.0 mol/L NaOH. A yellow colour change indicated the endpoint of titration, and the percentage nitrogen content was calculated as shown in the equation below.

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

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

e. Neutral Detergent Fibre (NDF)

The NDF composition of the samples was determined using the equation below, as described by Jimoh *et al*. (2020).

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

6.3.5 Statistical analysis

All the results of this study's proximate, macronutrients, and micronutrients tests were statistically analysed using two-way analysis of variance (ANOVA). Tukey's least significant difference was used to compare the significant differences between treatment means at p≤0.05. The computations used to obtain statistical data were completed using a computer program (Minitab) and tabulated. Tables are used to display the data outcomes.

6.4 Results

6.4.1 Proximate analysis

The results show that the acid detergent fibre (ADF) of *M. crystallinum* leaves decreases significantly as the salt concentration increases (Table 6.1). When compared to the control mean value (20.26%), the highest mean value (24.21%) was obtained from 0 ppm 8th day irrigation; however, no significant difference existed between these two treatments. The lowest significant (P≤0,05) mean value (14,54) for ADF was found under 800 ppm irrigation on the eighth day. The increase in salinity was directly related to the increase in ash content, with the greatest mean values recorded under 800 ppm treatments, with a maximum mean value

(52,51%) on the fourth-day irrigation treatment. Under 0 ppm 4th day irrigation, the lowest significant mean value (35,67%) was found.

Fat contents were substantially greater in the control treatment (2.03%) when compared to other treatments, with a trend indicating that increased salinity and longer irrigation intervals restrict fat formation, whilst 800 ppm 2nd day irrigation yielded the lowest significant fat content of 1.15% (Table 6.1). Non-detergent fiber was also found to be significantly high (29,63%) under low salinity but highest under treatment 0 ppm 4th day irrigation, despite no significant differences being identified when compared to the control treatment (27,48%). Furthermore, protein content (%) was substantially (15, 26%) high at 0 ppm 8th day irrigation compared to control treatment (12,37%) and other treatments, indicating a reduction in protein content as salinity concentration increases while an increase in protein contents as irrigation intervals got longer were noted.

6.4.2 Macronutrients

Nitrogen concentrations were substantially high (2440 mg/100g) with 0 ppm 8th day irrigation than in the control treatment (1978 mg/100g). Irrigation intervals and salinity both had an effect on the nitrogen content of this crop, as shown in table 6.2, with longer irrigation intervals promoting an increase in nitrogen content than short irrigation intervals, and an increase in salt concentration consistently resulting in a decrease in nitrogen content of the crop.

On phosphorous contents, almost all treatments had no significant differences when compared to the control treatment mean value (335 mg/100g), except for treatment "400 ppm 2nd and 8th day irrigation, 800 ppm 2nd and 8th-day irrigation," which had the lowest mean values; 130 mg/100g, 120 mg/100g, 130 mg/100g, and 115 mg/100g, respectively, which were significantly lower than the control treatment (335 mg/100g).

Potassium (K) concentrations were highest (9855 mg/100g, 8790 mg/100g, 8525 mg/100g, and 7880 mg/100g) with "0 ppm 8th day, 200 ppm 8th day, 0 ppm 2nd day, and 0 ppm 4th day irrigation," despite no significant p0.05 effect as compared to the control treatment (7090 mg/100g). There was no significant change when comparing the control treatment to all other least mean value treatments. Although there was no significant difference ($p \leq 0.05$) between the two treatments, the highest calcium (Ca) contents (2350 mg/100g) were recorded under 400 ppm 4th day irrigation when compared to the control treatment mean value (2115 mg/100g).

Magnesium content had greatest mean values of 1135 mg/100g, 980 mg/100g, 870 mg/100g, and 725 mg/100g which were only reported on the eighth day of irrigation in all salt concentrations ranging from 0ppm to 800 ppm. When compared to the control treatment (1150 mg/100g) mean value, the highest significance means value (1660 mg/100g) of K/Ca+Mg was recorded at "800 ppm" 8th day irrigation. Furthermore, as indicated in table 6.2, longer irrigation intervals with increasing salinity were directly related to an increase in K/Ca+Mg concentrations.

6.4.3 Micronutrients

Longer irrigation intervals increased manganese content more than salt concentrations, as highest mean values (15,70 mg/100g, 14,30 mg/100g, 15,20 mg/100g, and 15,35 mg/100g) with no significance from each other were obtained on the 8th day irrigation from all salt concentrations "0 ppm, 200 ppm, 400 ppm, 800 ppm" respectively. When compared to the control treatment mean value (43,35 mg/100g), the treatment "200 ppm 8th day irrigation" had the highest significant mean value (214 mg/100g). When compared to the control therapy, no additional therapies showed a meaningful effect.

Zinc concentrations were significantly higher (19,15 mg/100g) in treatment "400 ppm 8th day irrigation" (according to table 6.3) than in control treatment (11,7 mg/100 g) with a trend showing that the longest irrigation intervals (8^{th} day irrigation) yielded higher zinc contents while least zinc contents were recorded at daily 800 ppm. Longer watering intervals boosted *M. crystallinum* consumption of Zn contents in all salinity treatments. When compared to the other salinity and irrigation interval treatments, copper (Cu) was considerably higher (1,05 mg/100 g) in the control treatment, although no significant effect p0.05 was seen in these treatments when compared to each other. Sodium (Na) is directly proportional to salinity treatment, with the maximum mean value (14530 mg/100 g) achieved at 800 ppm and the lowest mean value (2235 mg/100g) obtained at "0 ppm”. Under salt concentration treatments of 200 ppm, 400 ppm, and 800 ppm, the control treatment (3135 mg/100g) was found to be lower than all other treatments. Furthermore, salt and water stress suppressed the nutritional content of some nutrients while improving the nutritional content of others

Table 6.1: The effects of different salinity levels and irrigation intervals on proximate quantities of *Mesembryanthemum crystallinum* leaves.

Salt concentration	Irrigation intervals	ADF %)	Ash (%)	fat (%)	moisture (%)	NDF (%)	Protein (%)
0 ppm	Daily	20,26± 0,99 ab	37,22± 2,01de	2,03± 0,02 a	9,36± 0,22 abc	27,48± 0,96 ab	12,37± 0,02 cde
	2nd day	23,42± 0,90 a	37,61± 1,66cde	1,63± 0,021 abc	7,98± 0,05 bc	29,63± 1,03 a	12,81± 0,80 bcd
	4th day	19,86± 0,94 ab	35,67± 1,55e	1,66± 0,06 abc	8,39± 0,20 bc	26,09± 1,099 a-d	13,31± 0,061 bc
	8th day	24,21± 0,38 a	40,29± 1,91b-e	1,86± 0,01 ab	7,7± 0,09 c	29,83± 1,22 a	15,26± 0,901 a
200 ppm	Daily	16,72± 1,35 bc	43,26± 0,02 b-e	1,50± 0,012 bcd	10,09± 0,63 ab	21,84± 0,09 de	11,15± 0,61 e-h
	2nd day	18,36± 0,99 bc	44,94± 0,25 a-d	1,38± 0,03cd	8,64± 0,111 abc	24,46± 0,22 b-e	11,44± 0,67 d-g
	4th day	17,61± 1,49 bc	42,38± 2,11 b-e	1,49± 0,61 bcd	9,42± 1,00 abc	23,94± 0,001 be	11,67± 0,084 def
	8th day	19,89± 1,65 ab	41,83± 2,03 b-e	1,7± 0,066 abc	8,85± 0,28 abc	26,57± 0,11 abc	14,34± 1,610 ab
400 ppm	Daily	17,03± 1,01 bc	45,41± 2,27 abc	1,17± 0,02 d	10,77± 0,081 a	22,15± 1,011 cde	10,30± 0,91 fgh
	2nd day	16,12± 1,09bc	46,35± 1,83 ab	1,21± 0,023 d	9,44± 0,22 abc	21,83± 0,88 d-e	10,62± 0,08 fgh
	4th day	16,47± 2,09 bc	46,81± 1,67 ab	1,42± 0,071 bcd	8,86± 0,023 abc	21,87± 0,08 d-e	10,48± 0,061 fgh
	8th day	16,89± 1,00 bc	42,77± 2,32 b-e	1,64± 0,25 abc	8,61± 0,09 abc	23,65± 0,130 b-e	12,66± 0,21 cde
800 ppm	Daily	15,98± 2,331 bc	48,11± 1,20 ab	1,22± 0,01 d	9,24± 0,22 abc	20,73± 0,32 e	9,78± 1,22 h
	2nd day	16,34± 1,22 bc	48,18± 2,991 ab	1,15± 0,031 d	9,31± 0,61 abc	21,95± 1,22 cde	10,07± 0,33gh
	4th day	17,8± 1,49 bc	52,51± 1,85 a	1,32± 0,02 cd	8,25± 0,08 bc	22,42± 2,11 cde	9,88± 0,51 gh
	8th day	14,54± 0,89 c	45,71± 1,367 ab	1,16± 0,111 d	8,62± 1,00 abc	20,48± 1,33 e	11,81± 0,15 c-f

Two-way ANOVA Tukey Statistics

Mean values ± Standard Error are shown in columns. The mean values followed by different letters are significantly different at P ≤0.05 (*) and ns = not significant as calculated by Tukey’s least significant difference.

Table 6.2: The effect of different salt concentration and irrigation intervals on macro nutrition quantities of *Mesembryanthemum crystallinum* leaves.

Salt concentration	Irrigation intervals	Nitrogen (mg/100g)	Phosphorus (mg/100g)	Potassium (mg/100g)	Calcium (mg/100g)	Magnesium (mg/100g)	K/Ca+Mg (mg/100g)
0 ppm	Daily	1978± 8,30 cde	335± 2,61 a	7090± 12,11 a-e	2115± 0,12 ab	640± 2,11 c-f	1150± 0,05 cd
	2nd day	2049± 9,61 bcd	330± 2,89 a	8525± 14,14 ab	2095± 0,11 abc	740± 2,76 bcd	1315± 1,10 a-d
	4th day	2129± 12,61 bc	310± 1,88 a	7880± 11,35 abc	1800± 0,27 a-d	745± 1,89 bcd	1330± 0,02 a-d
	8th day	2440± 11,77 a	230± 2,59ab	9855± 9,22 a	1620± 0,05 a-d	1135± 2,03 a	1450± 0,06 abc
200 ppm	Daily	1784± 7,22 e-h	230± 2,84 ab	3565± 8,25 efg	930± 2,22 d	370± 1,88 gh	1190± 0,66bcd
	2nd day	1831± 5,72 d-g	200± 5,71 ab	4910± 6,65b-g	1215± 1,88 bcd	450±1,37 e-h	1285± 0,98 bcd
	4th day	1868± 11,5 def	270± 3,22 ab	6330± 1,22 a-g	1300± 2,44 a-d	630± 0,88 c-g	1390± 1,89 a-d
	8th day	2294± 6,33 ab	165± 1,98 ab	8790± 3,72 a	1305± 2,88 a-d	980± 3,11 ab	1540± 2,65 ab
400 ppm	Daily	1648± 8,22 fgh	180± 2,88 ab	3310± 1,58 fg	905± 2,55 d	340± 1,75 h	1155± 1,87 cd
	2nd day	1698± 6,21 fgh	130± 2,11 b	4095± 1,93 d-g	1010±3,78 bcd	395± 1,22 fgh	1265± 2,65 bcd
	4th day	1676± 11,3 fgh	335± 1,88 a	6995± 2,08 a-f	2350± 4,11a	675± 1,99 cde	1060± 2,88d
	8th day	2025± 8,66 cde	120± 1,74 b	7500± 0,97 a-d	750± 2,58 d	870± 1,55 bc	1435± 3,11 abc
800 ppm	Daily	1567± 7,55 h	165± 1,88 ab	3170± 4,33 g	950± 3,88 d	340± 2,76 h	1080± 1,98 cd
	2nd day	1612± 12,25 gh	130± 2,30 b	3870± 8,14 d-g	860± 4,01 d	345± 2,31 h	1395± 1,66 a-d
	4th day	1581± 11,21gh	210± 2,91 ab	4540± 3,98c-g	1340± 2,94 a-d	520± 1,89 d-h	1055± 2,89 d
	8th day	1888± 9,22 cde	115± 1,82 b	7070± 2,38 a-d	985± 1,96 cd	725± 2,11 bcd	1660± 2,11 a
Two-way ANOVA Tukey Statistics							

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

Table 6.3: The effect of different salt concentration and irrigation intervals on micronutrients quantities of *Mesembryanthemum crystallinum* leaves.

Salt concentrations	Irrigation intervals	Mn (mg/100g)	Fe (mg/100g)	Zn (mg/100g)	Cu (mg/100g)	Na (mg/100g)
0 ppm	Daily	5,35± 0,02d	43,35± 1,05 b	11,7± 0,01bc	1,05± 0,001 a	3135± 1,10 de
	2nd day	9,65± 0,31a-d	107,55± 2,08 b	14,25± 0,02 abc	0,4± 0,011ab	2290± 1,98 e
	4th day	1,07± 0,06 a-d	44± 0,85 b	14,55± 0,011abc	0,1± 0,01b	5510± 1,11 cde
	8th day	15,70± 0,01 a	96,10± 0,140 b	16,45± 0,11 ab	0,1± 0,08 b	2235± 1,20 e
200 ppm	Daily	6,40± 0,091d	27,15± 0,18 b	9,45± 0,05 c	0,05± 0,01 b	10099± 0,11 a-d
	2nd day	9,35± 0,066a-d	37,15± 0,12 b	13,45± 0,52 abc	0,25± 0,011 ab	9190± 0,21 a-e
	4th day	9,25± 0,089a-d	32,40± 0,17 b	11,80± 0,02 bc	0± 0,001 b	11100± 0,22a-d
	8th day	14,30± 0,08 abc	214± 0,21 a	16,45± 0,03 ab	0,05± 0,01 b	6305± 0,18 b-e
400 ppm	Daily	6,30± 0,05 d	40,35± 0,14 b	9,65± 0,09 c	0,1± 0,02 b	11115± 0,52 a-d
	2nd day	11,05 ± 0,05 a-d	55,15± 0,09 b	11,95± 0,21 bc	0,1± 0,012b	10535± 0,91 a-d
	4th day	8,75± 0,02 bcd	67,65± 0,10 b	13,00±0,05 bc	0± 0,001 b	7120± 0,87 a-e
	8th day	15,20± 0,25 ab	57,75± 0,09 b	19,15± 0,15 a	0,1± 0,001 b	9875± 0,66 a-e
800 ppm	Daily	6,65± 0,02d	40,70± 0,11 b	8,60± 0,02 c	0,15± 0,001b	11605± 0, 47 abc
	2nd day	8,40± 0,12cd	37,50± 0,08 b	10,15± 0,22 c	0,15± 0,001b	11350± 0,57 abc
	4th day	9,60± 0,28 a-d	37,10± 0,11 b	9,20± 0,11c	0,05± 0,012 b	14530± 0,66 a
	8th day	15,35± 0,03 a	57,75± 0,13 b	17,55± 0,12 ab	0± 0,011 b	13980± 1,52 ab

Two-way ANOVA Tukey Statistics

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

6.5 Discussion

In the study, protein contents of *M. crystallinum* dried leaves were consistently greater on the eighth day of irrigation throughout the longest drought durations with or without salinity. Environmental stress causes changes in protein synthesis by disrupting plant protein metabolism and stimulate the creation of several new stress-specific proteins, which provide evolutionary value to plants for improved survival under severe environmental conditions (Sharma & Dubey, 2019).

Salinity elevations in all irrigation intervals, however showed a comparable decline trend in protein contents of *M. crystallinum* dried leaves. These findings were also corroborated by a study in which NaCl treatments resulted a decrease in *Vicia faba* soluble proteins grown under salinity stress, and these findings have been reported in other plant species (Alzahrani *et al*., 2019). Based on nitrogen nutrition, the decrease in protein content due to salinity stress is caused by a decrease in chlorophyll, 5-ALA, proline, and heme (Nawaz et al., 2020).

Nitrogen concentrations were recorded higher on eighth day irrigation with or without salinity than other comparable treatments (Table 6.2), while increase in salt concentration consistently resulted in a decline trend of crop nitrogen content. An early study of the relationships between water availability and N-fertilizer responses revealed that fertilizer N will not increase yield unless there is adequate water available to the plant and increasing soil-water availability will not increase production unless there is an adequate N supply (Hu & Schmidhalter 2005; & Wang *et al*., 2010)

Under water scarce conditions, absorption of N by the plants is essential for their growth because of its active role in structural and metabolic processes (Nawaz *et al*., 2020). Nitrogen maintains the metabolic function of plant at low tissue water potential, thereby playing an important role to alleviate drought stress in cereal crops (Nawaz et al., 2020). Salt stress reduces crop growth and production by impairing metabolic activities and physiological processes such as NO3 assimilation, uptake, and translocation, and increases sap osmolality in roots from 305 to 530 ms mol kg1 (Nawaz *et al.,* 2020). The nitrate reductase enzyme of the nitrate reduction pathway is severely harmed by salinity under salt-stress conditions (Rohilla & Yadav, 2019).

Fat contents indicated that increased salinity and longer irrigation intervals reduce fat synthesis. The findings are consistent with those of Keyvan *et al*., (2022), who discovered that the fat content of plants subjected to mild and severe drought treatments were significantly lower than that of plants subjected to well-watered conditions. Drought stress reduces fat content by affecting the lipid biosynthesis pathways and their enzymatic panel and would reduce the number of pods or pod length, reducing available carbon assimilation for fat synthesis in the plants (Keyvan *et al*., 2022). According to Custódio *et al*. (2021), such reduced fat saturation profiles are typically found in halophytes and are likely related to salt tolerance mechanisms such as decreased membrane permeability to sodium chloride (NaCl).

Almost all treatments had no significant differences in phosphorus (P) contents compared to the control treatment mean value (335 mg/100g), except for a few treatments that had significantly reduced phosphorus contents as a result of a combined treatment of salt and irrigation intervals starting at 400 ppm on the second day. P availability in saline soils can be reduced due to ionic-strength effects that reduce P activity and because P concentrations in the soil solution are tightly controlled by absorption processes and the low solubility of Ca-P minerals (Hu & Schmidhalter, 2005).

Potassium (K) concentrations increased with longer irrigation intervals and consistently decreased with increasing salinity levels. This may be due to potassium's role in stomatal regulation, osmoregulation, energy status, charge balance, protein synthesis, and homeostasis (Waraich *et al.,* 2011; Rangani *et al*., 2018). Plants' improved K nutrient status is responsible for increased photosynthesis and plant growth under drought stress conditions (Ahanger *et al*., 2016). Because drought and salinity cause water deficits in plants, K+ is equally important for maintaining turgor pressure under either stress (Hu & Schmidhalter 2005; Aslam *et al*., 2013). Furthermore, higher K+: Na+ ratios improve plant resistance to salinity; thus, under high Na ratios, K activity was suppressed (Jiang *et al*., 2016). However, because of the decreased mobility of K+ under salinity and drought, the availability of K+ to the plant decreases with decreasing soil water content (Hu & Schmidhalter, 2005; Heidari & Karami, 2014).

During the initial adjustment phase of plants coping with drought stress, accumulation of K+ may be more important than production of organic solutes, because osmotic adjustment via ion uptake like K+ is more energy efficient (Hu & Schmidhalter, 2005; Fanaei *et al*., 2009). High Na+ concentrations in the external solution reduce K+ and Ca2+ concentrations in many species' tissues (Hu & Schmidhalter, 2005). Plants' ion balance is altered by high salt concentration (Karimi *et al*., 2009). For example, competition between Na+ and K+ uptake changes the K+/Na+ ratio (Talaat *et al*., 2015). The protoplasm has a high K+/Na+ ratio under normal physiological conditions (Shabala *et al*., 2010). However, the similarity of K+ and Na+ radii makes it difficult for plants to distinguish between them, so the normally high K+/Na+ ratio is altered when Na+ enters through K+ pathways (Kronzucker *et al*., 2013).

Calcium (Ca) content consistently decreased as salinity increased. Similarly, calcium, potassium, and zinc ion contents decline with increasing salinity (Uddin *et al.,* 2012). Because sodium and chloride compete with nutrients like potassium, calcium, and nitrates, salinity can cause nutrient deficiencies or imbalances (Martnez-Ballesta *et al.,* 2010). Contrarily, nutrient absorption and acropetal transfer of some nutrients may be hampered by drought (Khatun *et al.,* 2021). Different calcium and sodium ratios, however, had no impact on rice's ability to absorb sodium (Wu & Wang, 2012). Calcium distribution in salt-sensitive plants' shoots dropped significantly under salt stress, suggesting that salt resistance and the ability of plants to retain calcium are connected (Hu & Schmidhalter, 2005).

The magnesium greatest mean values of 1135 mg/100g, 980 mg/100g, 870 mg/100g, and 725 mg/100g were only reported on the eighth day of irrigation in all salt concentrations, while sequential decline in Mg contents occurred as salinity increased. According to Liang (2018), drought reduces Mg and S uptake. Furthermore, the presence of competing cations such as Ca, Al, H, NH4, and Na causes its deficiency in plants (Liang 2018). Magnesium significantly impacts salt-stressed plants' water status (Liu et al., 2014). Increased leaf stomatal conductance in Mg-rich plants could be attributed to decreased leaf water potential and an increase in leaf turgor pressure (Nawaz *et al*., 2020).

Zinc concentrations in the treatment 400 ppm 8th day irrigation were significantly higher (19,15 mg/100g) than in the control treatment (11,7 mg/100g), with a trend indicating that longer watering intervals increased *M. crystallinum* Zn content in all salinities. Drought stress has been linked to changes in auxin's normal function in plants (Salehi-Lisar & Bakhshayeshan-Agdam, 2016). Zn supplementation during drought conditions has been shown to balance plant hormones and ensure plant survival (Nawaz *et al*., 2020; Umair 2020). Its presence under such harsh conditions acts as a co-enzyme for the synthesis of tryptophan, a precursor for the production of auxin. It thus promotes root development for improved plant water status (Nawaz *et al*., 2020). Furthermore, Zn nutrition protects plants from oxidative damage caused by ROS by increasing antioxidant activity and decreasing the activity of membrane-bound NADPH oxidase under drought-stress conditions (Waraich *et al*., 2011).

Micronutrient availability in saline and/or sodic soils is determined by the solubility of the micronutrients, the pH, and the electrical conductivity (EC) of the soil solution in dry soils (Dahlawi *et al*., 2018). Furthermore, salinity can affect micronutrient concentrations in plants differently depending on crop species and salinity level (Parihar *et al*., 2015). For example, salinity increased

Mn and Zn concentrations in barley shoots but decreased in corn (Rajabi *et al*., 2019). Salinity increased Fe concentration in low land rice shoots but decreased it in barley and corn (Hu & Schmidhalter, 2005). Salinity had little effect on the micronutrients Mn, Zn, Fe and B in growing and mature wheat leaves (Moradi & Jahanban, 2018).

Sodium (Na) increases in direct proportion to salinity, with the highest mean value (14530 mg/100g) obtained at 800 ppm and the lowest mean value (2235 mg/100g) obtained at "0 ppm." Similarly, under drought and saline conditions, the relative ratios of Na+/K+, Na+/Ca++, Na+/Mg++, Mg++/Ca++, and Mg++/K+ increased with increasing salinity treatment in Purslane (*Portulaca oleracea*) (Uddin *et al*., 2012). The leaves exposed to the highest salinity levels had the highest mineral residue content (Uddin *et al*., 2012). Salinity levels also influenced the mineral composition, as Na+, Mg++, and Cl- uptake and accumulation increased as salinity increased (Essa, 2002; Uddin *et al*., 2012).

Salt and water stress suppressed the concentration of some nutrients while increasing the concentration of others. However, no element or nutrition was found to be lower than the nutritional contents of Ethiopia kale (Brassica carinata), Swiss chard (Beta vulgaris), carrot (Daucus carota), tomato (Lycopersicon esculentum), and cabbage (Brassica oleracea) (Abdi *et al*., 2022), as this crop proved to contain more nutrients than these vegetable crops even under the highest salt and longest irrigation intervals.

6.6 Conclusions

Salinity and drought both influenced proximate, macronutrient, and micronutrient contents, indicating that the effects of these two stresses on plants may have the same or different effects on mineral or nutritional contents. For instance, increased salinity and drought reduced fat content, whereas zinc was more influenced by longer watering intervals at all salinity levels. Increasing salinity reduced Zn contents. This had more to do with mineral mobility, mineral availability to plants, mineral role, the influence of these environmental factors on *M. crystallinum* biological activities, and the nature of plant genetic characteristics. *M. crystallinum* contained comparable amounts of nutrients to other wild and coastal edible species, enabling it to be suitable for human consumption to contribute to food security. Further research should focus on supplementing plants under salt stress or drought with the elements that were increased by salinity or drought stress to determine whether such a strategy would effectively reduce or minimize the injury cost caused to plants by these environmental factors.

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Chapter seven: General discussions, conclusion, and recommendations

7.1 General discussions

The expansion of the global human population is anticipated to treble by 2050, which would demand higher food consumption and augment the pressure on food security within a global climate change of water scarcity (Dinar *et al*., 2019; Islam & Karim, 2019; Wang, 2019). The world agricultural economy has been under further pressure to increase sustainable food production by 70% for an additional 2.3 billion people by 2050 (Rahneshan *et al.,* 2018; Polash *et al.,* 2019; Gupta 2019; Searchinger *et al*., 2019). The impacts of global climate change have already caused a significant decline in food supplies (Leisner, 2020) with shortages of fresh vegetables in food markets (Srinieng & Thapa, 2018; Polash *et al.,* 2019; Abbas *et al.,* 2020). This decline has prompted further research on underutilized wild edible vegetables to supplement daily diets (Ngxabi *et al.,* 2021b; Munir *et al*., 2022). This study has shown that the cultivation and domestication of wild coastal edible species such as *Mesembryanthemum crystallinum* can be successful in contributing to food security as these plants are adaptable to harsh environmental conditions of salinity and water stresses while contributing to relevant compatible vegetative biomass, nutritional contents, sufficient secondary metabolites, and antioxidant capacity.

In chapter two, an overview of the natural habitat, distribution, and uses of *M. crystallinum*, world food security status, plant responses to water stress and salinity, economic and nutritional values of vegetables, effects of drought and soil salinization on food production, economy, and food security has been presented. Food insecurity and freshwater scarcity review revealed that coastal edible plants need to be considered to maximise food availability while minimising water usage (Ngxabi *et al.,* 2021b; Munir *et al*., 2022). The physiological properties of *M. crystallinum* indicate that this species has potential to be utilised as an edible coastal species which naturally adapts to grow in saline conditions along coastal regions (Munir *et al*., 2022). Even though the common ice plant has these potentials, information on how it responds towards combined treatments of salinity and irrigation intervals was missing and had to be investigated to determine the interaction between these two treatments for a more precise growth protocol of this plant (Stavridou *et al*., 2019; Lu *et al*., 2021).

In chapter three, the influence of various irrigation intervals and salinity concentrations on the vegetative parameters of *M. crystallinum*, namely the number of leaves, stem height, fresh weight, and dry weight, has been studied. The results showed that moderate irrigation with the highest salinity treatment, ‘‘4th-day irrigation 800 ppm salinity’’, consistently resulted in higher number of leaves when compared to other treatments, and this has also resulted in higher biomass formation of wet and dry weights, however, no significant difference observed. These results support the findings of Sogoni *et al*. (2021), where authors observed a significant increase in leaf/ branch number and total fresh and dry weight in *Tetragonia decumbens* subjected to salinity and drought. Furthermore, biomass accumulation of *C. vulgaris* was likewise not significantly reduced throughout the studied salinity gradient, confirming its tolerance for a wide range of salinities (Teh *et al*., 2021). Leaves of *M. crystallinum* have been reported to respond to NaCl exposure with CAM (crassulacean acid metabolism) mechanism induction and only marginal ABA accumulation (Hedrich & Shabala, 2018). The CAM mechanism is reported to be accelerated by salt stress, and this adaptive mechanism improves water use efficiency in *M. crystallinum* (Madhavi *et al.,* 2022). This behaviour can be explained by the availability of epidermal bladder cells in the leaves of many halophytes, including the common ice plant (Loconsole *et al*., 2019). *M. crystallinum* is categorized as "obligatory halophytes" group, which require saline environments for optimal growth (Loconsole *et al*., 2019). Furthermore, plants irrigated every two days without salinity had longer stem length. However, this was also comparable to plants subjected to salinity and drought, suggesting that the combined effects did not significantly affect stem length. These findings concur with results reported by Alam *et al*. (2022) on *Salsola imbricata* (Fetid Saltwort), where the combined effect of salt and water stress did not significantly affect shoot length. However, these results contradict the findings of Calone *et al.* (2023) on *Limonium angustebracteatum*, where individual salt stress's effect did not negatively affect leaf length but was remarkably affected by both factors. This suggests that the combined tolerance of drought and salinity could be species-specific among halophytes.

In chapter four, the effect of different irrigation intervals and salinity concentrations on the chlorophyll contents of *M. crystallinum* is evaluated. Moderate irrigation coupled with no sodium chloride “0 ppm 4th day irrigation” yielded high chlorophyll contents throughout the experimental period, while treatments “400 ppm daily irrigation” and “800 ppm eighth day irrigation” resulted in the lowest chlorophyll contents. However, treatments were comparable to each other except for the eighth-week data. Even though chlorophyll concentrations were not constant, chlorophyll contents were consistently greater under moderate irrigation with no salinity (0 ppm 4^{th} day irrigation). *M. crystallinum* has been documented as an edible crop that yields best under moist soils other than wet or dry soil (Loconsole, *et al*., 2019). These changes in chlorophyll concentration were related to light intensity variation due to seasonal conditions as plants age, causing plants to increase chlorophyll content on low-light days to absorb enough light, and vice versa (He *et al*., 2019).

Nevertheless, this reduction in chlorophyll content did not significantly negatively affect plant growth parameters. These findings were also observed by Atzori *et al.* (2017) in a field experiment, where the common ice plant was not negatively affected by the reduction in photosynthetic apparatus as the plant ages under increased seawater irrigation. These findings confirm that the species can tolerate salt and drought stress under cultivation.

In chapter five, the effect of different irrigation intervals and salinity tolerance on phytochemicals and antioxidants capacity of *M. crystallinum* was evaluated. The species exhibited true halophyte characteristics as they proved to produce these compounds less under a high saline state, needing less excretion of these compounds to sustain normal growth, while individual effects of water stress optimised the production of these compounds. Polyphenols, DPPH (μmol TE/L) capacity, and TEAC (μmol TE/L) capacity were increased with a decline in salinity and longer irrigation intervals, showing that less sodium chloride triggers stress signal which causes it to produce more of these secondary metabolites to sustain the stress. Loconsole *et al*. (2019) stated that *M. crystallinum* is categorized as an "obligatory halophyte" group, which requires saline environments for optimal growth. The positive effect of salinity in drought-stressed halophytes has also been reported in *Atriplex halimus* L. where the accumulation of antioxidants was reduced in samples subjected to the combined effect of salinity and drought (Alam *et al.,* 2022). Most halophyte dicots accumulate toxic ions in the aerial part, compensating with saline excretion mechanisms (glands or bladder), salt dilution (succulence), and low cytosolic ion concentrations because the H+/Na+ antiporter in the tonoplast and plasma membrane transports these ions toward the vacuole and/or out of the cell (Bueno & Cordovilla, 2020). The buildup of sodium chloride in the vacuole has the advantage of lowering the osmotic pressure in the leaf tissues, lowering the water potential to less than the soil water potential and allowing the plant to absorb water. (Bueno & Cordovilla, 2020; Sun *et al.,* 2020). Nevertheless, the leaves of ice plant subjected to both salinity and water stress possessed more polyphenols and flavonols than other promising edible halophytes in South Africa such as *Chenopodium album*, *Trachyandra divaricata, Trachyandra ciliata* (Ngxabi *et al*., 2021a; Bulawa *et al*., 2022; Chamkhi *et al.,* 2022; Tshayingwe *et al.,* 2022) and *Tetragonia decumbens* (Nkcukankcuka *et al*., 2022; Sogoni *et al*., 2021). This suggests that the leaves of this plant may be a good source of nutritional antioxidants.

In chapter six, the effect of different irrigation intervals and salinity tolerance on nutritional composition (proximate, micronutrients, and macronutrients) of *M. crystallinum* has been evaluated. The nutritional quality of comm This study eva This study evaluated the individual and combined impacts of salinity and drought stress on plant development, mineral content, proximate content, and phytochemical composition of *Mesembryanthemum crystallinum*. luated the individual and combined impacts of salinity and drought stress on plant development, mineral content, proximate content, and phytochemical composition of *Mesembryanthemum crystallinum*. ercial crops worldwide has been severely impacted by salinity and drought, with a greater impact in dry places (Ali *et al*., 2022). This has sparked increased global interest in the nutritional and nutraceutical benefits of halophytehalophytes' nutritional and nutraceutical benefits lophytes' nutritional and nutraceutical benefits in combating malnutrition and boosting food security in drought and salinity-affected nations (Alexopoulos *et al*., 2023). In this study, salinity and longer dry irrigation intervals influenced proximate, macronutrient, and micronutrient contents in *M. crystallinum*, indicating that the effects of these two stresses on plants may have the same or different effects on mineral or nutritional contents. Under these twin factors, all treatments had a high yield of minerals present in the leaves of *M. crystallinum*, which was noted to be comparable to other consumable halophytes such as *Sarcocornia perennis*, *Aster tripolium*, *Arthrocnemum macrostachyum*, and *Salcornia ramosissima*, which are already consumed mostly in restaurants and some supermarkets around the world (Ventura *et al.,* 2015; Barreira *et al*., 2017; Petretto *et al.,* 2019).

It was also observed that both increased salinity and drought contributed to the suppression of the fat content of the crop species. Dietary fats are an intrinsic component of an "unhealthy" diet due to their higher caloric density, as increased fat consumption has been blamed for obesity, cardiovascular disease, and related metabolic illness (Wali *et al*., 2020). This led federal guidelines for a low-fat diet for "high-risk" patients and as a preventive health intervention for everyone except infants (Seid & Rosenbaum, 2019). This makes *M. crystallinum* one of the great food sources with low-fat contents while exhibiting comparable mineral composition.

Zinc was more influenced by watering intervals at all salinity levels, as the longest dry irrigation interval yielded the highest zinc contents in *M. crystallinum*. Zinc is the second most important trace element in the body after iron, as it participates in many metabolic processes and serves three major biological functions as a structural, catalytic, and regulatory component (Chasapis *et al*., 2020; Maares & Haase, 2020). However, because Zn is not stored in the body, a daily intake of Zn is required to maintain essential levels and support all its functions (Chasapis *et al*., 2020). Zn supplementation during drought conditions has been shown to regulate plant hormones and ensure plant survival in plants (Nawaz et al., 2020; Umair 2020). Its presence under such extreme conditions functions as a co-enzyme for synthesising tryptophan, a precursor for creating auxin. It hence promotes root development for improved plant water status (Nawaz et al., 2020). This demonstrates that this crop can control its metabolism and absorb more zinc under drought stress, increasing Zn content that will benefit agricultural consumers.

Mesembryanthemum crystallinum contained higher nutrients, enabling it to be suitable for human consumption, combat hidden hunger and malnutrition, and foster food security. Ash has been used to measure nutritional value of food and is believed to be an indicator of the mineral contents that have been conserved in food items (Sousa *et al*., 2014). The ash content of the tested samples ranged from 35 to 52%, which is higher than 5% reported on other wild vegetables and corresponds to the composition found in processed foods (Liu *et al*., 2022). These results concur with Ntuli's (2019) research on two species of water spinach (*Ipomea plebeian* R.Br. and *Ipomea wightii* (Wall.) Choisy), where the ash level was reported to range from 20 to 38%.

7.3 Conclusion and recommendations

A combination of low sodium chloride concentrations and frequent irrigation intervals induced longer stem length growth. The highest sodium chloride concentrations on moderate irrigation induced a higher number of leaves and fresh weight, indicating that *M. crystallinum* can be successfully cultivated in saline environments with moderate water application for higher harvest yields. Chlorophyll contents were consistently higher under the interactive effect of moderate irrigation intervals with low salinity (0 ppm 4th day irrigation) even though chlorophyll contents were not stable because *M. crystallinum* has been documented as an edible crop that yields best under moist soils other than wet or dry soil. The fluctuations in chlorophyll contents were associated with light intensity variations due to seasonal changes, causing plants to increase chlorophyll content in low light days to absorb enough light, and vice versa.

M. crystallinum demonstrated true halophyte features in that it produced smaller contents of secondary metabolites under high saline conditions, requiring less synthesis of these compounds to maintain normal development. Polyphenols, DPPH (μmol TE/L) capacity, and TEAC (μmol TE/L) capacity rose with decreased salinity contents and longer irrigation intervals, indicating that lack of sodium chloride produces a stress signal, causing the plant to synthesise more of these secondary metabolites to restrain the stress. Longer irrigation intervals and salinity alter proximate, macronutrient, and micronutrient levels, implying that the effects of these two stresses on plants may have the same or distinct consequences on mineral or nutritional contents. Increased salinity and irrigation interval result in lower fat content, whereas longer irrigation intervals enhance zinc content at all salinity levels. *M. crystallinum* had comparably higher nutrient contents, making it appropriate for human consumption and a good candidate for contributing towards combating malnutrition and food insecurity. *Mesembryanthemum crystallinum* is ready to be considered for large-scale commercial production as a commercial crop.

Further research on other edible coastal plants will support the challenge of promoting versatile coastal edible species better to understand the physiological responses and adaptations to coastal environments. *Mesembryanthemum crystallinum's* adaptive mechanisms to salinity and drought stress can be compared to the physiological responses of its close relatives and other crop species that are potentially edible coastal crops. More research on responses to salt and drought is needed to establish the crop's physiological response to these environmental effects. The roots or the species need to be evaluated for potential pharmacological or nutritional values under these environments as the crop has been recently utilised for medicinal purposes.

7.4 References

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Appendix A:

agriculture

MDPI

Article

Interactive Effects of Salinity Stress and Irrigation Intervals on Plant Growth, Nutritional Value, and Phytochemical Content in *Mesembryanthemum crystallinum* L.

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Abstract: Halophytes such as ice plants are concurrently subjected to salt and drought stresses in their natural habitats, but our knowledge about the effects of combined stress on plants is limited. In this study, the individual and combined effects of salinity and irrigation intervals on the plant growth, mineral content, and proximate and phytochemical composition of *M. crystallinum* were evaluated. Treatments consisted of four irrigation treatments ((1) 100 mL once a day; (2) 100 mL once every 2 days; (3) 100 mL once every 4 days; (4) 100 mL once every 8 days) with four salt concentrations (0, 200, 400, and 800 ppm) applied in each treatment. Salt concentrations were set up by adding increasing concentrations of NaCl to the nutrient solution, while the control treatment was irrigated daily without NaCl. The results revealed a significant increase in the leaf number and fresh and dry weights of plants irrigated with 800 ppm salinity every four days. However, the highest chlorophyll content was consistently recorded in the control treatment (0 ppm, 4-day irrigation interval), although no significant variability in chlorophyll content was observed at week 6. The highest yields of N, Mg, and Cu were consistently recorded in plants without saline treatment, while P, K, Ca, Na, Zn, and Fe were consistently recorded in plants subjected to a combination of salinity and irrigation intervals. The combination of salinity and irrigation intervals was significant for Fe and Ca, whereas, for other elements, no significant differences occurred. The salt concentration did not influence the high yields of acid detergent fibre (ADF), crude fat, protein, or neutral detergent fibre (NDF), as they were recorded in high amounts in plants subjected to irrigation intervals only, whereas a combination of salinity and irrigation intervals resulted in the highest ash and moisture contents. Invariably, the 8-day irrigation interval without salinity optimized the yields of assayed polyphenols, flavonols, Ferric Reducing/Antioxidant Power (FRAP), and 2,2-diphenyl-1-picrylhydrazyl (DPPH), suggesting that salt stress does not influence the quantities of phytochemicals and antioxidants of *M. crystallinum*. These findings suggest that *M. crystallinum* can minimize the impact of salt stress on the accumulated minerals, phytochemicals, and proximate and antioxidant substances. Therefore, it is a suitable vegetable for regions affected by both salinity and water stress, as it can provide additional minerals, phytochemicals, antioxidants, and proximate nutrients when cultivated in saline soils.

Keywords: Aizoaceae; bio-saline agriculture; edible halophytes; functional foods; underexploited vegetables

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1. Introduction

As the world's population grows, so will global food production, putting additional pressure on already-scarce resources such as clean irrigation water and arable land [1,2]. In addition, increasing soil salinity and dry conditions caused by climate change are regarded as the most critical and adverse environmental factors for plants, leading to enormous