Cape Peninsula University of Technology

Crop yields, secondary metabolites and anti-fungal activities of extracts of *Helichrysum cymosum* and *Helichrysum odoratissimum* cultivated in aquaponics

By

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Declaration

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

Signed Date

Abstract

The growing demand for herbal remedies has resulted in the significant over-harvesting of wild plants, exacerbating the problem of species extinction. This study evaluates two cultivation approaches that could be explored to cultivate medicinal plants. Production crops in aquaponics, which is a system that combines the growing of fish (aquaculture) and soilless cultivation of plants (hydroponics) under controlled conditions, is an alternative, innovative approach. The plants recycle the fish wastes from the fish tank by using up the nutrients in the wastewater for growth, and the clean water is then recirculated in a closed system. Hydroponics is another cultivation method widely used in the precision cultivation of vegetables. It minimizes water wastage and optimises the fertilization of plants. Thus far little evidence exists of the use of these technologies for cultivation of medicinal plants.

Helichrysum odorattisimum and *Helichrysum cymosum,* which belong to Asteraceae*,* are among the most sought-after medicinal plant species in South Africa. Herbal remedies are widely used and are becoming more popular. The harvesting of medicinal plant materials from wild populations is destroying their genetic diversity and their habitats. *Helichrysum* spp. are popular for their therapeutic values in traditional medicine. Extracts from numerous species of the same genus have been used to treat skin diseases, respiratory disorders, and circumcision procedures. This study was conducted to evaluate the antifungal activities, secondary metabolite content, and crop yields of *Helichrysum cymosum* and *Helichrysum odoratissimum* extracts cultivated in aquaponic and hydroponic systems to assess the prospects of using both systems in the sustainable cultivation of medicinal plants.

To evaluate the effectiveness of the aquaponic and hydroponic systems, plants were grown using three cultivation methods: aquaponic, hydroponic and field systems, and the data on antifungal and antioxidant activities, secondary metabolite content, and crop yield were compared. Six-week-old *H. odorattisimum and H. cymosum* seedlings were cultivated in an aquaponic (T1) and hydroponic systems (T2). Field-growing plants (T3), which were planted three months earlier were also screened for antifungal and antioxidant activities and secondary metabolite content. The bioactive chemicals and antifungal activities of the plants cultivated in these two systems were then compared to plants grown in the field (T3). In the aquaponic system, plants were cultivated on silica sand in a deep culture system and *Carassius auratus* (goldfish) produced the fish wastes. In the hydroponics, plants were cultivated in a substrate blend of Coco coir, vermiculite, and perlite as substrates at a ratio of 2:1:1, respectively and supplied with a hydroponic fertilizer (Nutrifeed®). Fifteen replicates of each species (*H. odoratissimum* and *H. cymosum*) seedlings were used in this study for both treatments.

The results for *H. odoratissimum* yield (plant height, fresh and dry weights) and the tissue nutrient contents did not change substantially ($p > 0.05$) between aquaponic and hydroponic

treatments. Gas chromatography–mass spectrometry (GC–MS) analysis showed that monoterpenes and sesquiterpenes were the most abundant compounds in *H. odoratissimum*; however, no statistical difference was observed among the field, hydroponic, and aquaponic plants (DF = 2; χ^2 = 2.67; p > 0.05). While there was no significant difference in polyphenol contents among the three treatments, remarkably, the flavonol contents in the leaves varied significantly (DF = 2; χ^2 = 6.23; p < 0.05) among the three treatments. A higher flavonol content occurred in leaves from the hydroponic system than in leaves from the aquaponic (p < 0.05) and field (p > 0.05) systems. The MIC results showed that the ethanolic extract of *H. odoratissimum* was fungistatic against *F. oxysporum*; however, this effect was more prominent in the ethanol extracts of plants grown in the aquaponic system, with a mean MIC value of 0.37 ± 0.00 mg/mL.

Helichrysum cymosum plants did not vary significantly (DF=1,28; χ^2 =1.63; p = 0.21) in plant heights which ranged from (22.83 ± 1.59 cm – 26.46 ± 0.94 cm) and dry weight among the treatments; however, T2 produced slightly better results. The fresh weight of *H. cymosum* differed significantly (p < 0.05) between the treatments. The results showed that there was no significant difference between aquaponics and hydroponics treatments on crop yields (plant height, fresh and dry weight). Interestingly, there were significant differences (DF = 2; χ^2 = 19.76; p = 0.00) in total polyphenol contents between the three cultivation techniques; however, the field-collected plants yielded higher polyphenol contents (452.10 ± 53.37 mg GAE/g) than hydroponics (433.49 ± 11.95 mg GAE/g) followed by aquaponics (136.46 ± 42.09 mg GAE/g). The flavonol contents differed significantly between the three cultivation techniques (DF = 2; χ^2 = 6.31; p = 0.03), with higher flavonol contents in the field-collected plants (250.62 ± 58.12 mg QE/g), followed by hydroponics (164.05 ± 14.89 mg QE/g) and aquaponics (71.60 ± 14.45 mg QE/g). The volatile chemical contents did not differ significantly according to the gas chromatography-mass spectrometry (GC-MS) analysis (DF = 2; χ^2 = 3.53; p = 0.17; p > 0.05) among the three treatments. However, plants grown in aquaponics had a higher number of compounds (106) than those grown in hydroponics (104) or field plants (103). The antifungal bioassay showed that the ethanol extracts of *H. cymosum* harvested from the field also had a higher fungistatic activity against *F. oxysporum*. The highest antioxidant capacity was obtained in plants cultivated in hydroponics followed by field-collected plants although there was no statistical difference among the treatments.

In conclusion, aquaponics and hydroponics performed better or similar to field cultivation and are viable alternative methods for cultivating *H. odoratissimum* plants. The results of the present study suggest that commercial cultivation of *H. odoratissimum* and *H. cymosum* using hydroponic and aquaponic systems may be feasible.

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Dedication

This work is devoted to Novirginia Zantanta, Katriva Zantanta, and Nosizwe Zantanta, my grandmother, father, and mother and to my entire family. Thank you for your love, support, and prayers during my academic journey.

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List of acronyms

˚C Degree Celsius

ANOVA Analysis of Variance

B Boron

Ca Calcium

Cu Copper

Fe Iron

FRAP Ferric reducing antioxidant power

DPPH 2,2'-diphenylpicrylhydrazyl

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

HCl Hydrogen chloride

K Potassium

LECA clay Light Expanded Clay Aggregate

GC-MS Gas Chromatography – Mass Spectrometry

MIC Minimum Inhibitory Concentration

INT *p*-iodonitrotetrazolium

Mg Magnesium

Mn Manganese

N Nitrogen

Na Sodium

S Sulphur

TA Total Activity

P Phosphorus

Zn Zinc

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Chapter 1 : Introduction

1.1 General introduction

Increasingly, medicinal plants are playing a significant role in the global healthcare systems. The current demand for aromatic and medicinal plants is high, and it is expected to maintain a steady increase. They are important commodities in international trade in recent years (Stafford *et al*., 2005). Currently, large volumes of medicinal plants leave South Africa for Europe. The medicinal plants are used to manufacture phytopharmaceutical, nutraceutical and cosmeceutical products (Vasisht *et al*., 2016). *Helichrysum* species such as *H. odorattisimum* and *H. cymosym* are indigenous to South Africa and are among the most sought-after medicinal plants in the country. For many years, *Helichrysum spp*. has been used as a source of medicines to cure a variety of ailments in Europe and African countries (Lourens *et al*., 2004). These plants are used to treat pain, coughs, and colds, as well as fever, headache, menstrual pains, wound dressing, and infection prevention (Giovanelli *et al*., 2018). These fragrant plants have also been used to treat respiratory and wound infections (Kutluk *et al*., 2018). Essential oils from *Helichrysum species* have antibacterial properties and could be used to treat tropical disorders like malaria (Van Vuuren *et al*., 2006).

The world's population has grown rapidly in the last century, and it is predicted that by 2050, the world's population will increase from 9.7 to 10 billion people (Barea, 2015; Chen *et al*., 2020). The rapid population growth is putting a huge strain on natural resources, including plants, water, and land. These resources are often over-exploited, leading to scarcity and environmental degradation (Hanjra and Qureshi, 2010; Borah, 2017; Goddek *et al*., 2020; Surendran *et al*., 2021). Unfortunately, the conventional agricultural practices are insufficient to meet near-and long-term demands (Chen *et al*., 2020) — they are unsustainable. Conventional (soil-based) cultivation is often associated with high inputs such as the heavy use of inorganic fertilizers, synthetic pesticides, and water, which are potentially damaging to the environment (Santos *et al*., 2012).

Various challenges in conventional (soil-based) cultivation have been encountered, for example, the excessive and continuous use of fertilizers, especially synthetic fertilizers, such as nitrogen-based fertilizers. Over time, excessive fertilization diminishes the soil ingredients required for crop production, resulting in soil infertility, decreased productivity, water management, and deterioration of the environment (Goddek *et al*., 2015; Chen *et al*., 2020). According to Konig *et al*. (2016) and Folorunso (2021), plant production has many negative impacts on the environment including groundwater pollution, destruction of soil biodiversity and soil erosion.

The above-mentioned challenges have motivated the search for alternative strategies that are environmentally friendly and cost effective. Soilless cultivation methods such as aquaponics and hydroponics can avoid the challenges faced by soil-based cultivation. These two systems have the potential to operate successfully under limited water resources and mitigate pollution (Palm *et al*., 2018; Mok *et al*., 2020). Furthermore, plant growth and chemical constituents can be manipulated in these systems by manipulating nutrient supply to plants (Seawright, 1998).

Using aquaponics to cultivate South African medicinal plants is an interesting innovative approach to sustainable cultivation of medicinal plants. This approach could limit the exploitation of some endangered medicinal plants from the wild, enhance fish production, and provide opportunity for commercial production of medicinal plants. Aquaponics has many other advantages, including high production, faster growth rate, crop maturity, yields, and consistency (Yousif and Köhler, 1990). Plants grown in aquaponics are less prone to diseases that attack plants such as soil-borne pests — it is one of the major advantages of using aquaponics in plant cultivation (Bakiu and Julian, 2014). Because of its ecologically friendly nature, aquaponics is regarded as one of the most resource-efficient production systems. In an aquaponic system, wastewater is recycled and reused as a substitute for dumping to adjacent water bodies (Nuwansi *et al*., 2021).

Hydroponics is also a soilless cultivation of plants. The plants are grown in a pure nutrient solution (water culture) or substrate culture, which is used to substitute typical agricultural soil (Pardossi *et al*., 2006). It is a well-established method for commercial cultivation of many food crops, including leafy vegetables, tomatoes, cucumbers, peppers, strawberries, and many others (Butler and Oebker, 1962). This approach is particularly useful in areas where environmental stress (cold, heat, desert, etc.) is a significant issue (Polycarpou *et al*., 2005). And for a variety of medicinal plant species, the viability and advantages of this growth system for the synthesis of secondary metabolites are potentially high (Papadopoulos *et al*., 2000; Hyden, 2006; Maggini *et al*., 2014). Over the last few years, many studies have shown that hydroponics is a feasible method for the cultivation of medicinal plants. Medicinal plants are increasingly being cultivated on a commercial scale and hydroponic systems can be manipulated to achieve high crop yield and excellent crop quality. Because it has significant advantages over conventional field cultivation, hydroponics may meet both legal and industrial criteria for medicinal plants. For example, hydroponics allows for more efficient use of water and fertilizers; plants can be grown all year; quantity and quality of production are highly predictable because they are not affected by geographic location or pedoclimatic conditions; plant contamination is absent or minimal; and plant material is easy to process and extract (Mulabagal and Tsay, 2004; Pardossi *et al*., 2006; Raviv and Lieth, 2019). Another significant advantage of hydroponics is the ability to purposely subject plants to stress stimuli that are

known to cause an increase in secondary metabolite concentrations (Brechner *et al*., 2007; Rahimi *et al*., 2012).

Previous research has shown that aquaponics and hydroponics can be used to successfully grow a variety of vegetables; however, these systems are still underutilised for the cultivation of medicinal plants (Graber and Junge, 2009; Pantanella *et al*., 2010). While there are a few evidence on the successful cultivation of medicinal plants in hydroponics, few studies have investigated the suitability of cultivating medicinal plants in aquaponics. As a result, the current study's hypothesis is to demonstrate that plants grown in aquaponic and hydroponic systems can produce the same, if not better-quality plants than field grown plants.

Helichrysum odoratissimum and *Helichrysum cymosum* are among the most extensively utilized medicinal species from the genus *Helichrysum,* which belongs to the *Asteraceae* family. Therefore, this study was conducted to evaluate the antifungal activities, secondary metabolite content, and crop yield of *H. cymosum* and *H. odoratissimum* extracts cultivated in aquaponic and hydroponic systems to assess the prospects of using aquaponics and hydroponics as sustainable cultivation approaches to medicinal plant cultivation. In addition, the findings of this research could be used to bridge the knowledge gap in aquaponics and hydroponics cultivation and medicinal plant cultivation in South Africa in order to address water scarcity, environmental sustainability, and the short supply of plant-based medical materials. This research will contribute toward the development of a new protocol for large- and small-scale farmers to cultivate medicinal plants.

The study’s conceptual underpinning, scientific rationalizations, aim, hypothesis, main objective and specific objectives are described in Chapter one. Literature review is covered in chapter two. Chapter three evaluates the suitability of aquaponics in cultivating two *Helichrysum* species (*H. odorattisimum* and *H. cymosum*), by assessing growth parameters such as plant height, fresh and dry weights of the two species, nutrient uptake, and bioactive compounds, compared to hydroponics and field cultivation.

1.2 Structure of the thesis

This study is divided into five chapters which are briefly discussed.

Chapter one: Introduction, background to the research problem, structure of the thesis, statement of the research problem, hypothesis, aims and specific objectives.

Chapter one: Covers, the study's conceptual underpinning, scientific rationalizations, aim of the study, hypothesis, main and specific objectives of the study are described in this chapter, and brief information on the justification of the work.

Chapter two: The literature review is covered in chapter two.

Chapter three: The objective of chapter three was to compare crop yields, secondary metabolite contents and anti-fungal activities of extracts of *H*. *odoratisimum* cultivated in aquaponic, hydroponic and field systems. The rationale for the study, the materials and methods used, the findings, and the conclusions are presented.

Chapter four: Chapter four aimed to compare the crop yields, secondary metabolite contents, and anti-fungal properties of extracts of *H. cymosum* grown in the indicated field, aquaponic, and hydroponic systems. The study's rationale, the methodologies and materials employed, the outcomes, and the conclusions are presented.

Chapter five: This chapter covers the general discussion that integrates the previous chapters, as well as the study's outcomes. Recommendations for further research are provided.

1.3 Background to the research problem

Medicinal plants have long been used as a source of primary medicine to treat a variety of human and animal ailments globally. The practice continues to grow as evidenced with local and international demands for herbal and phytopharmaceutical materials (Sheldon *et al*., 1997; Bekalo *et al*., 2009). Previous research has revealed that medicinal plants are widely utilized as traditional medicines to meet the fundamental health care needs of an estimated 80% of the world's population (Oyebode *et al*., 2016). Traditional herbal medicines made from more than 1020 indigenous plants are used by 27 million people in South Africa (Reddy, 2007).

As the world's population is anticipated to reach 9 billion people by 2050, it is expected that the world's arable land would be insufficient to meet the increased food, water, and medicine needs in South Africa and globally (Okemwa, 2015). As a result, the necessity to conserve these resources now is crucial. Many studies have shown that conventional cultivation has a negative impact on the environment and good quality land needed for crop production has diminished due to toxicity caused using pesticides and insecticides, which negatively impact human health and the ecosystem (Murugan *et al*., 2013). Land shortages, weed infestations, plant diseases, pests, and soil infertility are all well-known issues associated with soil cultivation of plants (Palm *et al*., 2018; Goddek *et al*., 2020). These difficulties have driven the quest for ecologically acceptable alternative growth methods, such as hydroponics which is a method of growing plants without the use of soil by employing nutrient-rich water solutions as well as aquaponics. Aquaponics is a potentially beneficial farming strategy that, through nutrient and waste recycling, can helps alleviate the above-described issues associated with traditional plant growing while improving long-term environmental goals (Goddek *et al*., 2020).

In the horticultural and agricultural fields, growing plants in a controlled environment has gained favour. Many studies are being carried out in this area, contributing to the growing popularity and interest in the cultivation of plants under controlled environmental conditions (Giurgiu *et*

al., 2014). Because the grower has complete control over the process, hydroponic and aquaponic cultivation of medicinal plants can result in higher quantities of bioactive chemicals. The findings from previous research revealed that hydroponics could boost plant growth and yield of targeted plant parts even in places where these plants do not naturally grow (Xego *et al*., 2017).

1.4 Statement of the research problem

Using medicinal plants for their medical benefits is a common practice worldwide. The South African population is reliant on plant remedies for medical care, and these plants are more accessible and affordable (Reddy, 2007). This could lead to over-exploitation of plant resources, hastening the extinction of some plant species and placing a significant strain on plant biodiversity in general (Matanzima *et al*., 2014).

Considering the high demand for traditional medicine in South Africa and the over-exploitation of wild medicinal plants, effective and sustainable methods of raising medicinal plants such as hydroponics and aquaponics are needed to safeguard these resources. As a result, the goal of this study is to assess the antifungal activities, secondary metabolite content, and crop yield of two *Helichrysum* species (*Helichrysum cymosum* and *Helichrysum odoratissimum*) extracts cultivated in an aquaponic and hydroponic systems to determine the viability of aquaponics as a sustainable method of medicinal plant cultivation.

1.5 Hypothesis

Aquaponics is likely to have a good impact on the growth of *H. odoratissimum and H. cymosum*. It is hypothesized that the secondary metabolites contents of plants grown in aquaponics, hydroponics, and in the field will be comparable. Antifungal properties of *H. cymosum* and *H. odoratissimum* grown in aquaponic, hydroponics, and in the field systems against *F. oxysporum* are expected not to differ.

1.6 Aim of the study

The primary objective of this study is to assess the antifungal activities, secondary metabolite content, and crop yield of two *Helichrysum* species (*H. odoratissimum and H. cymosum*) extracts cultivated in an aquaponic and hydroponic systems to determine the viability of aquaponics and hydroponics as sustainable methods of medicinal plant cultivation.

1.7 Specific objectives

To achieve the aim of this study, the following objectives were studied:

a) To compare the growth parameters (heights, fresh, and dry weights) of *H. cymosum* and *H. odoratissimum* grown in aquaponics and hydroponics.
b) To measure the nutrient contents of *H. cymosum* and *H. odoratissimum* grown in aquaponics, hydroponics, and field grown plants to see if there will be differences among cultivation methods.
c) To compare the secondary metabolite (polyphenols and antioxidants) contents of *H. cymosum* and *H. odoratissimum* cultivated in aquaponics, hydroponics and in the field plants.
d) To compare the antifungal activities of *H. cymosum* and *H. odoratissimum* grown in the aquaponic system and in hydroponics against *Fusarium oxysporum*.

Chapter 2 :Literature review

2.1 Cultivation of medicinal plants

Herbal medications are widely used, and recent reports suggest that their use is on the rise (WHO, 2004). However, the sourcing of medicinal materials from the wild is destroying genetic diversity and plant habitats (Behrens and Vines, 2004). According to the World Health Organization, more than 80% of the world's populations in developing countries rely on herbal medicine for basic healthcare (Behrens and Vines, 2004). Herbal remedies are also becoming more popular in developed countries, with 25% of the UK population using them on a regular basis (Ekor, 2014). Around two-thirds of the 50 000 diverse medicinal plant species in use come from the wild (Edwards, 2004). In Southern Africa, traditional medicine is an essential aspect of the healthcare system. Most herbal medicines are made from plants that have been harvested in the wild. Throughout South Africa, over 700 plant species are actively traded for medical purposes, and extensive collection of wild medicinal materials is causing decrease of stocks of many sought-after species, posing a severe danger to biodiversity of some regions in South Africa such as Eastern Cape (Dold and Cocks, 2002). As a result, a substantial number of medicinal plant species are now endangered, reaching a tipping point where sustainable harvesting is almost impossible.

Although enhanced regulation and the implementation of sustainable wild harvesting methods can provide appropriate protection for some species, increasing domestic production of medicinal herbs is a more viable long-term alternative. Cultivation also opens up the prospect of applying biotechnology to overcome problems with herbal medicine production (Edwards, 2004).

2.2 Asteraceae

This genus (*Helichrysum*) is part of the Asteraceae family, which includes roughly 500–600 species worldwide, with 245–250 found in Africa, including Madagascar, Australia, and Eurasia (Matanzima *et al*., 2014; Kutluk *et al*., 2018; Leonardi *et al*., 2018). Flavonoids, sesquiterpenes, polyacetylenes, terpenes, phenolics, chalcones, phthalides, sterols, pyrones, coumarins, diterpenes and other secondary metabolites found in plant extracts are abundant in these plants (Van Vuuren *et al*., 2006; Giuliani *et al*., 2016). The medicinal qualities of these plant extracts obtained from *Helichrysum* species have been found to have prospective pharmacological applications as antioxidant, antibacterial, and anti-inflammatory agents (Giuliani *et al*., 2016; Leonardi *et al*., 2018). These secondary metabolites have global acceptance and potential for application as anti-inflammatory, antibacterial, antioxidant, and anti-diabetic medicines in the medical profession (Jadalla, 2020).

Scientific reports suggest that members of the genus *Helichrysum* have been used in folk medicine for over 2000 years to treat many ailments (Albayrak *et al*., 2010). These plants have been used in treating bladder disorder, infections and respiratory illnesses. Furthermore, *Helichrysum* species have been documented to be utilized for a variety of cultural purposes, including protection against calamities and supernatural causes for cultural ceremonies, and circumcision (Wiersum *et al*., 2006).

Inhaling 'Imphepho' smoke causes a trance-like condition, implying effects on the central nervous system (Serabele *et al*., 2021). Despite the fact that smoke inhalation is one of the most common traditional methods of 'Imphepho' administration, there have been reports on the phytochemistry of the smoke; its volatile scented compounds may play a key role in anti-infective treatments. *Helichrysum* oils have significant antimicrobial properties (Van Vuuren *et al*., 2006). Additionally, there has been a trend in recent years to use natural resources, such as plants, as ingredients in cosmetic goods (Twilley *et al*., 2021). Essential oils secreted in the epidermal cells on the petals, ovaries, and bracts, as well as on the leaves, are responsible for the aromatic features of many *Helichrysum* species (Perrini *et al*., 2009).

2.3 *Helichrysum odoratissimum*

Helichrysum odoratissimum (L.) Sweet, also known as *Helichrysum bochstetteri* (Shc. -Bip. ex A. Rich.) Hook. f. and *Helichrysum bochstetteri var scabrum*, belongs to the Asteraceae family. It is an aromatic species that is widely distributed in intertropical region and Southern Africa (Puyvelde *et al*., 1989). *H. odoratissimum* is one of the most harvested and traded plants in South Africa (Serabele *et al*., 2021). Empirical studies have validated its use in traditional medicines; for examples, extracts of the plants have potent antifungal activities; hence it is not surprising that this species is used to treat abdominal pains, heartburn, fever, catarrh, headache, menstrual problems, urinary tract infections, and wounds in traditional medicines (Van Puyvelde *et al*., 1989; Serabele *et al*., 2021). The same plant is used to cure female sterility, menstrual discomfort, and eczema in other countries like Rwanda (Legoale *et al*., 2013; Serabele *et al*., 2021).

2.3.1 Morphological characteristics of *H. odoratissimum*

Helichrysum odoratissimum is a spreading perennial shrub with linear-oblong leaves that are greyish-white and wooly on both sides (Serabele *et al*., 2021) as presented in Figure (2.1). The flowers are pale golden yellow, with very little flower heads borne in clusters at the terminals of the branches, woody at the base, erect and up to 50 cm high, and blooming all year (Swelankomo, 2004; Matrose *et al*., 2021) (Figure 2.2) .

Figure 2.1: A photograph of *H. odoratissimum* showing linear oblong leaves)

Figure 2.2: Pale golden -yellow Inflorescence of *H.odoratissimum* and morphology (Sourced from: http%3A%2F%2Fwww.zimbabweflora.co.zw%2Fspeciesdata%2Fimages%2F15%2F159770-2.jpg&action=click)

2.3.2 Geographical distribution of *H. odoratissimum*

Helichrysum odoratissimum is widely distributed in South Africa, ranging from the Southpansberg in Limpopo to the Midlands and highlands of KwaZulu-Natal. Free State, Lesotho, the Cape Drakensberg, mountains and coastal areas of the Eastern Cape, around the Cape fold mountains of Cedarberg, Giftberg in Vanrhynsdorp and the Cape Peninsula in the Western Cape Province (Serabele *et al*., 2021) (Figure 2.3). *Helichrysum odoratissimum* grows in huge clusters on grassy or rocky slopes, and it quickly takes over empty places along roadsides and trails.

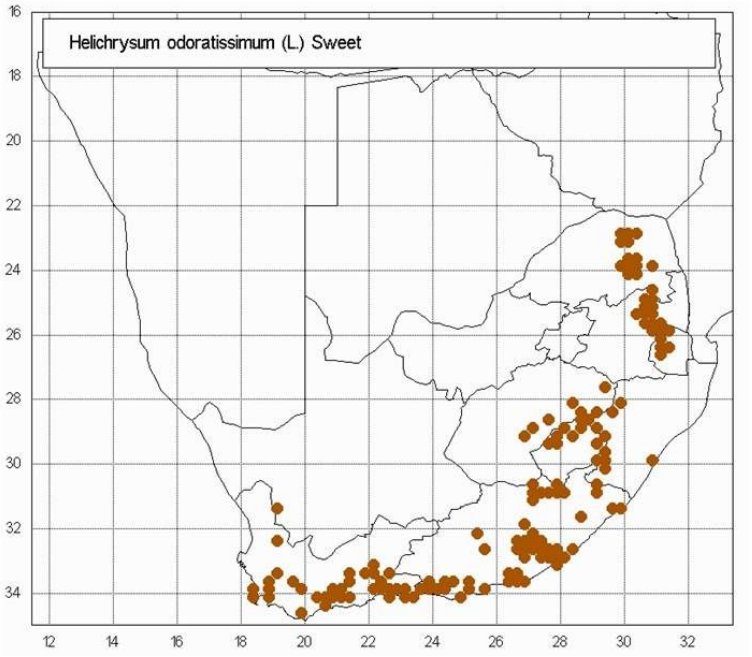

Figure 2.3: Map showing distribution of *H.odoratissimum* in South Africa (Adapted from: https://keys.lucidcentral.org/keys/v3/*helichrysum*/key/*Helichrysum*/Media/Html/images/*Helichrysum_odoratissimum*/*HELIODOR*.jpg)

2.3.3 Growth requirements and propagation of *H. odoratissimum*

Helichrysum odoratissimum is easy to grow in light, well-drained, composted soil, ideally poor sandy soil or rocky grasslands, invading overgrazed and wayside places (Mathekga, 1998). Stem cuttings and seeds are used for propagation. This fragrant plant can be used as a ground cover in groups of three to five or as a mass planting in a sunny location. *Helichrysum odoratissimum* is a fantastic choice for newly constructed gardens since it adds a splash of colour, especially while it's in bloom. Overwatering should be avoided at all costs, as *Helichrysum* species are susceptible to fungal infections, especially in the winter (Swelankomo, 2004).

2.3.4 Phytochemicals found in *H. odoratissimum*

Some of the possible chemical components were indentified in the plant through phytochemical investigation of *H. odoratissimum* extracts include sesquiterpenoids, sesquiterpenes, and alkaloids (Matrose *et al*., 2021). Compounds, such as α-humulene, curcumene, and caryophyllene were characterized from the volatile using gas chromatography–mass spectrometry analysis (De Canha *et al*., 2020). Monoterpenoides, particularly α-pinene, dominated the essential content of *H. odoratissimum* from several African countries (Asekun *et al*., 2007).

2.4 *Helichrysum cymosum*

The aromatic herb *H. cymosum* L. D.DON *subsp*. *cymosum* is abundantly distributed in Southern tropical Africa (Bhat and Jacobs, 1995; Bougatsos *et al*., 2004). *H. cymosum* is one of the most well-known *Helichrysum* species, and it is used to treat pain, coughs, and colds, as well as fever, headache, menstrual pains, wound dressing, and infection prevention (Giovanelli *et al*., 2018). This fragrant plant has long been used to treat respiratory and wound infections (Kutluk *et al*., 2018). Essential oils from *H. cymosum* have antibacterial characteristics and could be used to treat tropical disorders like malaria (Van Vuuren *et al*., 2006).

2.4.1 Morphological characteristics of *H. cymosum*

Helichrysum cymosum is a well-branched ground cover with thin greyish-white wooly branches densely covered with tiny linear-elongated to oval elongated leaves covered with thin silvery grey to paper-like hairs that can grow up to 1 m high (Matanzima *et al*., 2014), Figure (2.4). The dazzling canary-yellow flowers appear in flat-topped flower heads with a cluster of 6-20 flowers in late summer (Zenze, 2012), Figure (2.5).

Figure 2.4: A photograph of *H. cymosum* showing oval elongated leaves covered with thin silvery grey to paper-like hairs (Adapted from: https://www.hortweek.com/*helichrysum-cymosum*-silver-moon/ornamentals/article/1691692)

Figure 2.5: A photography showing the dazzling canary-yellow flowers of *H.cymosum* (Adapted from: http://ketenewplymouth.peoplesnetworknz.info/friends_of_te_henui/images/show/14365-*helichrysum-cymosum*-yellow-tipped-straw-flower-impepho)

2.4.2 Geographic distribution

Helichrysum cymosum grows in bunches in damp locations such as hollows between dunes, between bushes, and along forest edges. As shown in Figure (2.6), *H.cymosum* occurs in the Western Cape, Eastern Cape, and KwaZulu-Natal (Zenze, 2012).

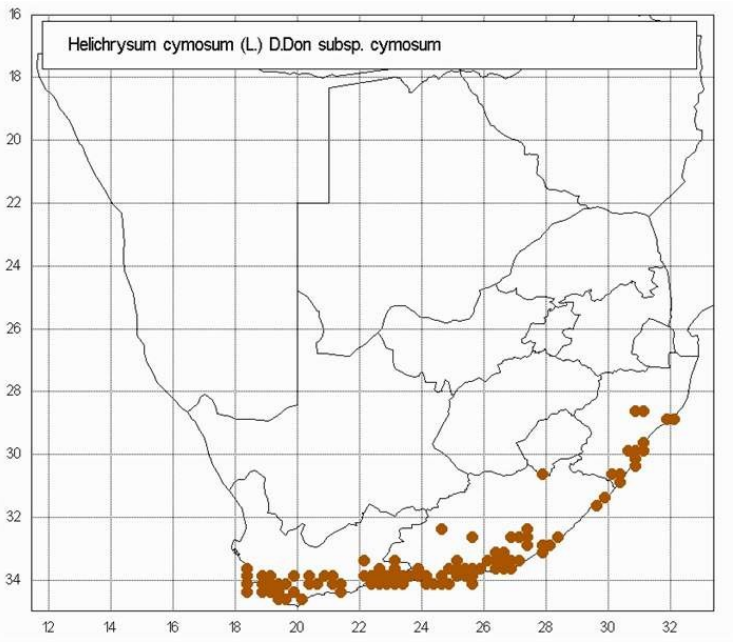

Figure 2.6: A map showing distribution of *H.cymosum* in South Africa (Adapted from: https://keys.lucidcentral.org/keys/v3/*helichrysum*/key/*Helichrysum*/Media/Html/*Helichrysum_cymosum_subsp._cymosum*.htm)

2.4.3 Growth requirements and propagation

Helichrysum cymosum is a hardy ground cover for dry areas that is easy to propagate from seeds sown in autumn or spring, cuttings rooted in sand-based medium with the addition of compost and fertilizer for nutrients, and cuttings rooted in sand-based medium with the addition

of compost and fertilizer for nutrients. Containers, mixed borders, window boxes, herb gardens, and fragrance gardens are all good places to put this plant. *H. cymosum* thrives in well-drained soils like sandy soils, making it an excellent choice for coastal gardens. It thrives in both sunny and shaded environments (Zenze, 2012).

2.4.4 Phytochemicals found in *H. cymosum*

A GC-MS analysis of the chemical composition of *H. cymosum* extracts revealed a total of 65 components. The primary compound identified were trans-caryophyllene, caryophyllene oxide, pinene, p-cymene, spathulenol, and bourbonene (Bougatsos *et al*., 2004). Several compounds, including helihumulone, helichromanochalcone,5-hydroxy-8-methoxy7-prenyloxyflavanone;1-Benzopyran-4-one,2,3-dihydro-5-hydroxy-8-methoxy-7-[(3-methyl-2-butenyl) oxo]; pinocembrin chalcone, were recovered from the alcoholic extracts of the leaves and roots of *H. cymosum*,-3-Phenyl-1-[2,4,6-trihydroxy-3-(3-methyl-2-buten-1-yl)phenyl]2,3-Dihydro-6,8-dihydroxy-5-methoxy,2-phenyl-4H-1-BENZOPYRAN-4-one;2,3-Dihydro-5.8-dihydroxy-6-methoxy-2-phenyl-4H-1-benzopyran-4-one;2,3-Dihydro-5.8-dihydroxy-6-methoxy-2-phenyl-4H-1-benzopyran-4-one (Bougatsos *et al*., 2004).

Table 2.1 : Review of bioactivities of *Helicrysum* spp (*Asteraceae*) in South Africa: the plant species, including part of the plant utilized, technique of preparation, traditional use, and biological activity of the plant used

Plant Species	Plant part used	Mode of Preparation	Traditional Use	Biological Activity	References
Helichrysum appendiculatum	Leaf	Eaten raw	Respiratory infections should be treated.	Antibacterial activity was determined, as well as antifungal activity linked to infections.	(Lourens, Viljoen and van Heerden, 2008; Heyman , 2009)
Helichrysum argyrosphaerum	–	–	Consumed by animals but toxic if a large amount is consumed	Antibacterial activity was determined, as well as antifungal activity, which was linked to infections.	(Lourens, Viljoen and van Heerden, 2008)
Helichrysum aureonitens	Leaves and stem	Burnt as incense	Used to invoke the ancestors' goodwill and to elicit dreams.	Antibacterial activity, Antifungal associated with infections, and antiviral activity were determined	(Van Puyvelde *et al.*, 1989; Moran, 1996; Abdel-Rahim *et al.*, 2019; Najar *et al.*, 2020; Etsassala, Hussein and Nchu, 2021; Pasch *et al.*, 2021; Serabele *et al.*, 2021)
Helichrysum caespititium	Plant	Smoke inhaled after being crushed and burnt.	Headaches and chest colds are treated with this herb.	Antibacterial activity, Antifungal associated with infections, Anti-inflammatory and Antimycobacterial activity determined	(Lourens, Viljoen and van Heerden, 2008),
Helichrysum cymosum	Leaf	Decoction/tea	Used to invoke the goodwill of the ancestors, treat	Antibacterial activity, Antifungal associated with infections, and antimalarial	(Bhat and Jacobs, 1995; Van Staden, 2005; Kutluk *et al.*,

			respiratory, wound infections and colds and coughs. Fresh leaves are boiled and the vapor bath for treating headaches. Used as nauseant	activity determined	2018; Cock and Vuuren, 2020)
Helichrysum decorum	Root Plant	Extract Burned and smoked inhaled	Used to stimulate trances	Antibacterial activity, Antifungal activity associated with infections determined	(Lourens, Viljoen and van Heerden, 2008)
Helichrysum foetidum	Plant	The extract is drunk/smoke inhaled.	Used to induce trances.	Antibacterial activity determined	(Lourens, Viljoen and van Heerden, 2008)
	Leaf	Extract	Used to treat flue.		
	Leaf	Wound dressing			
	Root	Extract	Used to treat eye problems		
Helichrysum gymnocomum	Stems and leaves	Burned as incense	Used to invoke the goodwill of ancestors	Antibacterial activity and antifungal activity determined	(Lourens, Viljoen and van Heerden, 2008)
Helichrysum herbaceum	Stems and leaves	Burned as incense	Used to invoke the goodwill of the ancestors.	Antibacterial activity and antifungal activity determined	(Lourens, Viljoen and van Heerden, 2008)

			Commercially sold			
	Stem and leaves					
Helichrysum kraussuii	Leaf	Decoction	Used to wash keloid scars, wound dressing for infections	Antibacterial activity and antifungal activity determined		(Bremner and Meyer, 2000 ;Lourens, Viljoen and van Heerden, 2008)
Helichrysum longifolium	Leaf	–	Used to treat circumcision wounds. Leaves are heated over very hot ash before being used to bandage for treatment of wounds after circumcision	Antibacterial activity and antifungal activity determined		(Lourens, Viljoen and van Heerden, 2008)
Helichchrysum miconiifolium	Leaf	Tea	Used medicinally as tea and Xhosa people grind and boil the leaves and use them to relieve pain after circumcision.	Antibacterial activity and antifungal activity determined		(Lourens, Viljoen and van Heerden, 2008)
			The root powder is used for intestinal parasites and ticks on poultry.			
	Root	Powder				
Helichrysum mundtii	Plant	Decoction	Used to treat chest complaints	–		(Lourens, Viljoen and van Heerden, 2008)
Helichrysum natalitium	Leaves and stems.	Burnt as incense.	Used to invoke the goodwill of the ancestors.	–		(Lourens, Viljoen and van Heerden, 2008)
	Leaves and stems		Commercially sold			
		–				

Helichrysum nudifolium	Leaf	Burnt as incense	To invoke the goodwill of the ancestors	Antibacterial, antifungal, and anti-inflammatory activity determined	(Lourens, Viljoen and van Heerden, 2008)
Helichrysum nudifolium var. *pilosellum*			Used for treating people who wish some deed hidden and who are afraid of being found out	Antibacterial activity and antifungal activity determined	(Lourens, Viljoen and van Heerden, 2008)
Helichrysum pedunculatum	Leaf	Extract	Used as an ointment to induce healing after circumcision to prevent inflammation externally.	Antibacterial activity and anti-inflammatory activity determined	(Bhat and Jacobs, 1995)
Helichrysum odoratissimum	Leaf	Leaf pulp	Used for wound dressing and burns.	Antibacterial activity, antifungal, and antimicrobial activity determined	(Lourens, Viljoen and van Heerden, 2008; Maroyi, 2019; Twilley *et al.*, 2021)
		Extract	Used as one of the ingredients to formulate skincare product (sunscreen)		
	Plant		Sotho people use it to fumigate huts.		
			The pleasant smell of ointment is mixed with fats and used by chief wives.		
		Ointment	Inhaled and used to treat insomnia and as a protective cleanser.		

	Plant, leaf, and stem	Smoke			
Helichrysum panduratum	leaf	Decoction	Used to treat feverish attacks in children	Analgesic activity and anti-inflammatory determined	(Lourens, Viljoen and van Heerden, 2008)
Helichrysum pedunculatum	Leaf and root	antiseptic	Used after circumcision to prevent inflammation externally	Antibacterial activity determined	(Lourens, Viljoen and van Heerden, 2008)

2.5 Other phytochemicals found in *Helichrysum spp.*

2.5.1 Secondary metabolites

Plant secondary metabolites are compounds produced by plants and their endophytes and include a wide range of active chemicals. They are regularly accumulated in lower amounts and are generated by specific cell types at different phases of development (Jain, 2019). Plants are living chemical factories that produce a wide range of secondary metabolites, which are the foundation for many commercial pharmaceutical medications and herbal therapies derived from medicinal plants. The various chemical compounds in medicinal plants have biological activity that can benefit human health through pharmaceutical and food businesses, but they also have significant value in the perfume, agrochemical, and cosmetic industries (Li *et al.*, 2020). Secondary metabolites production begins with basic pathways like glycolysis or shikimic acid biosynthesis and then diversifies based on cell type, developmental stage, and environmental factors (Patra *et al*., 2013). Based on their biochemical pathways, nitrogen-containing compounds (cyanogenic glycosides, alkaloids, and glucosinolates), phenolic compounds (flavonoids and phenylpropanoids), and terpenes are the three major families of secondary metabolites in plants (isoprenoids). As stated by Li *et al.* (2020) these substances are widely distributed in diverse plant cells, tissues, and organs. Furthermore, many environmental factors can either stimulate or impede plant growth and development.

2.5.2 Antioxidants

Plants have recently become more popular as sources of natural antioxidants for the scavenging of free radicals (Njenga and Viljoen, 2006). An antioxidant is described as "any substance capable of preventing or considerably delaying the oxidation of oxidizable materials in small amounts, generally at concentrations much lower than the oxidizable materials to be protected" (Ghosh and Deb, 2014). The fact that oxygen can be converted by metabolic activity into more reactive forms such as superoxide, hydrogen peroxide, singlet oxygen, and hydroxyl radicals, collectively known as active oxygen, makes antioxidants crucial to human physical well-being. Various metabolic mechanisms in live cells produce these chemicals.

According to De Beer *et al*. (2003), antioxidants other than vitamin C, E, and carotinoids have been readily found in many natural products. Antioxidants have been shown to protect against oxidative stress damage. Defense mechanisms that scavenge and stabilize free radicals protect all organisms against free radical attack, but when the pace of free radical generation surpasses the rate of oxidative stress, a harmful process that can damage cell components

such as lipids, proteins, and DNA, occurs (Jain *et al*., 2019).

Many antioxidant-based medication formulations are employed in the prevention and treatment of diseases whose processes involve the process of oxidative stress. Because synthetic antioxidants are restricted due to their carcinogenicity, researchers have been looking for naturally occurring antioxidants to substitute synthetic antioxidants in foods, cosmetics, and medicinal products.

The principal antioxidants constituents of natural products are phenolic compounds that comprised of phenolic acid and flavonoides (Kähkönen *et al*., 1999). Phenols have a significant function in plant photo-oxidation resistance and disease resistance (Kasote , 2013). Phenolic antioxidants are potent free radical terminators (Shahidi *et al*., 1992), they donate hydrogen to free radicals and hence, break the reaction of lipid oxidation at the initiation step. Many pathogenic problems are linked to free radicals, including inflammation, metabolic disorders, cell aging, atherosclerosis, and carcinogenesis. These characteristics have led to considerable plant screening for antioxidants and to see if medicinal plants may contain antioxidants that could potentially modify oxidative stress-related illnesses.

2.6 Cultivation approaches

2.6.1 Soil cultivation of plants

Traditional agriculture plays an important part in meeting the food demands of a growing human population, but it has also resulted in a greater reliance on synthetic fertilizers and pesticides (Santos *et al*., 2012). Chemical fertilizers are industrially modified compounds with known amounts of nitrogen, phosphorous, and potassium, and their use pollutes the air and ground water by eutrophicating bodies of water (Youssef *et al*., 2014).

The soil, as well as its features, play a vital role in agricultural production. On the one hand, it creates the conditions for the development and growth of cultivated plants; thus, one of the agricultural production processes' goals is to maintain it in the best possible condition; on the other hand, it is constantly subjected to unfavourable changes that result from natural processes as well as field operations carried out with the use of agricultural equipment, machines, and tractors. The world's area of soil degradation as a result of vehicle wheels compaction is estimated to be about 64 million ha (Bulinski and Sergiel, 2013). For plants, soil is usually the most readily available growth medium. It offers anchoring, nutrients, air, and water, among other things (Hasegawa, 2003). Soils, on the other hand, can sometimes hinder plant growth. It can harbour disease-causing organisms and nematodes (Ajilogba and Babalola, 2013). Also, inappropriate soil reactions, unfavourable soil compaction, poor

drainage, erosion degradation, can hinder plant growth (Bhargav, 2020). Furthermore, conventional crop growing in soil is challenging because it requires a lot of space, a large amount of labour, and plenty of water (Hussain *et al*., 2014).

2.6.2 Hydropnics cultivation of plants

Hydroponics is a method of growing plants without soil by immersing their roots in nutritional solution (Hussain *et al*., 2014). In South Africa, hydroponic farming is quickly becoming a popular method for growing food, plants, and flowers all year. Soilless agriculture may be successfully started and considered as an alternate option for growing nutritious food plants, crops, or vegetables in the current situation (Butler and Oebker, 2006). Previous research has shown that cultivating medicinal plants under regulated environmental conditions in an aeroponic or hydroponic system improves quality, bioactivity, and biomass output on a commercial scale (Jousse *et al*., 2010). Growing plants in soil-free culture has numerous advantages over soil-based cultivation. The hydroponic technique is a clean and reasonably simple technology with manageable risk of soil-borne diseases or pest infectations, decreasing or eliminating the usage of pesticides and their toxicity (Polycarpou *et al*., 2005). Furthermore, plants require less growing time than crops cultivated in the field, and plant growth is faster since there is no mechanical restriction to the roots and all nutrients are freely available to plants. When compared to soil cultivation, hydroponics requires far less water. In soil farming, most of the water we provide to the plants is leached deep into the soil and unavailable to the plants' roots, whereas in hydroponics, plant roots are either submerged in water or a film of nutrients mixed in water always surrounds the root zone, keeping it hydrated and nourished. Water is recovered, purified, replenished, and recycled in this process, so there is no waste (Choi *et al*., 2011). Growers of medicinal plants are also drawn to hydroponic systems for cultivation because growing plants in soilless cultures in controlled environments could results in higher concentrations of bioactive compounds in plants than traditional soil cultivation through manipulation of the growing conditions by the grower (Giurgiu, 2014). Growing plants without soil medium allows for more precise control of environmental conditions, increased productivity, and improved crop quality. For examples temperature, pH, electrical conductivity, and oxygen concentration of nutritional solutions can be easily manipulated (Walters and Lopez, 2022).

2.6.3 Aquaponic cultivation of plants

Aquaponics is a technique that combines farming and agricultural production by using aquaculture waste in conjunction with hydroponically growing plants as a natural fertilizer supply to sustain the plant life cycle (Goddek *et al*., 2015; Abdel-Rahim *et al*., 2019). Aquaculture waste nutrients are utilized to feed hydroponically-grown plants in an aquaponic system. Hydroponics, on the other hand, is a method of plant cultivation in which roots are immersed in nutritional solution and mechanical support is provided by an inert medium. Aquaculture is the farming of fish in tanks, ponds, or lakes (Palm *et al*., 2018). Combining these two cultivation methods have been shown to be effective at producing plants while also raising fish. Aquaponics conserves water and cutback on the use of iorganic fertilizers because the soilless-cultivated plants use up the nutrients contained in the fish wastes and re-supply the fish tank with recycled water (Folorunso, 2021). Alternatively, plants can help purify water by filtering dirt from the water and returning it to the fish in a mutually beneficial interaction (Bakiu *et al*., 2021).

An estimated 50% of land for cultivation will be unusable for farming as a result of population growth, which is expected to reach 9 billion people in the next few years. As a result, aquaponics provides the opportunity to cultivate crops in places where traditional cultivation is not possible thus offers a sustainable ecosystem in which both plants and fish may thrive while consuming less water and space and producing no waste or pollution (Goddek *et al*., 2015; Bakiu *et al*., 2021). Plant roots are submerged in nutrient rich water from fish waste without being supplied with chemicals or fertilizers to aid in plant development, as opposed to soil-based cultivation, which has potential advantages such as improved sustainability, reduced resource consumption, and reduced environmental impact. For example, aquaponics systems use about 2% of the water that traditional systems use, and plants roots are submerged in nutrient rich water from fish waste without being supplied with chemicals or fertilizers to aid in plant development (Panigrahi *et al*., 2016; Gashgari *et al*., 2018). Aquaponics have been deemed environmentally friendly due to its low use of non-renewable resources and close proximity to zero waste generation (Konig *et al*., 2016).

According to Gashgari *et al*. (2018), plants grown in a soilless system are healthier and more consistent. Furthermore, plants produced in an aquaponic system can produce 20–25% higher yields than plants grown in a soil-based system, with yields up to five times higher (Gashgari *et al*., 2018). Another issue faced by soil cultivation is soil erosion and degradation caused by traditional farming methods that rely heavily on soil and, as a result, deplete the soil vital minerals. Consequently, incorporating aquaponics in medicinal plant production could improve medicinal plant cultivation (Dubey *et al*., 2017).

Aquaponics has the potential to have a lower negative environmental impact, reducing the

need for industrial fertilizers even further (Folorunso, 2021). While many investigations have illustrated that aquaponic systems have been used for food production, studies on the cultivation of medicinal plants in aquaponics are scare. Konig *et al*. (2016) found that aquaponics can be used for other purposes other than food production, including as an educational tool in schools, providing a better climate in public infrastructure, homes, and social institutions such as hospitals. In Italy, for example, aquaponics has been utilized to help patients recover from shock in a psychiatric hospital. Also, Panigrahi *et al*. (2016) have identified aquaponics as one of the systems that allows plants to be more resistant to infections or plant pathogens, such as *Fusarium oxysporum*, that damage crops.

2.6.3.1 Fish species used in aquaponics systems

2.6.3.2 Gold fish (*Carassius auratus)*

Carassius auratus (L) goldfish are ornamental fish that are native to Central Asia, China, and Japan. They have been bred in captivity for a long time (Watson *et al*., 2004). Goldfish has already been considered as a promising species for monoculture aquaponic recirculating systems (Shete *et al*., 2013; Shete *et al*., 2014). Small, vegetated lakes and reservoirs are ideal for goldfish. Despite being a cool-water fish, goldfish can survive in a wide range of water temperatures. Goldfish can grow to be over 23 inches (58 cm) long and weigh up to 6 pounds (2.7 kg), with a 30-year lifespan. Because it can survive in low-quality water, the goldfish is considered a "hardy" fish. Goldfish prefers temperatures between 65- and 75-degrees Fahrenheit (18 and 24 ℃), although they can survive at temperatures as low as 32 degrees Fahrenheit (0 ℃) and as high as 95 ℃ Fahrenheit (35 ℃), especially if the temperature change is gradual (seasonal). Temperatures outside of the optimal range may suppress the fish's immune systems, resulting in reduced eating and growth (Watson *et al.*, 2004). Goldfish are omnivores that can be fed a variety of natural and prepared foods. During the grow-out period, most farmers employ a normal 25 to 32 percent protein diet.

2.6.3.3 Nile tilapia (*Oreochromis niloticus*)

Oreochromis niloticus Nile tilapia is a silver fish with olive, grey, or black bars on its body. During the breeding season, it frequently blushes crimson (Picker, 2013*)*. *O*. *niloticus* Nile tilapia is indigenous to the Middle East, central Africa, and northern Africa (Boyd 2004). It is a type of tropical freshwater and estuary fish. It favors shallow, quiet waters at lakes' edges and large rivers with enough flora (Picker, 2013). According to Rakocy (2005) Nile tilapia may consume a variety of foods, including other fish and fish eggs as well as phytoplankton,

periphyton, aquatic plants, invertebrates, benthic fauna, detritus, and bacterial films. The lifespan of nile tilapia exceeds ten years (Ogutu-Ohwayo, 1990). *Oreochromis niloticus'*s ability to grow appears to be constrained by its access to food and the temperature of its environment (McDaid Kapetsky and Nath, 1997). The ideal temperature range for growth is 28–36 ℃, and as the temperature drops, growth slows (Teichert-Coddington *et al*., 2017). Their capacity to change their nutrition may also have an impact on how they grow (Bwanika *et al*., 2007). *O. niloticus* can mature sexually in aquaculture ponds at a young age of 5 to 6 months Rakocy, (2005). Salinity and temperature are the main variables restricting the distribution of the Nile tilapia, which is said to flourish in any aquatic habitat except than torrential river systems (Shipton *et al*., 2008). *Oreochromis niloticus'*s reported temperature range for survival is between 11 and 42 ℃. Since Nile tilapia can withstand concentrations as low as 3–4 mg/l, the amount of dissolved oxygen is not a key limiting factor for them (Boyd, 2004).

2.6.3.4 Nutrients's sources

The fish feed and the water fed to the system (including Mg, Ca, and S) are the two principal sources of nutrients in an aquaponic system (Delaide *et al*., 2017; Schmautz *et al*., 2016). Fishmeal-based and plant-based feeds are the two main types of fish feed. Fishmeal is the most common type of aquaculture feed, with fish meal and fish oil serving as the primary sources of lipids and proteins (Geay *et al*., 2011). Concerns about the feed's long-term viability have been raised for some time, and attention have been given to plant-based diets (Boyd, 2015; Davidson *et al*., 2013; Hua and Bureau, 2012; Tacon and Metian, 2008). Fishes secrete waste, bacteria turn the waste into nutrients, and plants extract the nutrients, improving the water quality for the aquatic animals, in an aquaponic system (Love *et al.*, 2014).

2.6.3.5 Macronutrients

In an aquaponic system where carbon is given to fish through feed and plants through CO_2 fixation, fish feed is the source of carbon and nitrogen (Schmautz *et al*., 2015; Delaide *et al*., 2017). Fish can enhance their biomass and metabolism by using 22% of the carbon in their food. The remaining carbon is either exhaled as CO_2 (52%), dissolved (0.7–3%), or solid (25%) forms (Timmons and Ebeling, 2013). The carbon from the feedstock that isn't consumed is left to degrade in the system. The kind of carbohydrates found in fish feed (e.g., starch or non-starch polysaccharides) can affect the digestibility of the feed and the biodegradability of the waste in an aquaculture or aquaponic system (Meriac *et al*., 2014). On the other hand, plants need nitrogen in large amounts. The fish food also contains phosphate and potassium, among

other elements. Phosphorus is a necessary component of plant growth that can be absorbed by plants in its purest form. Potassium, on the other hand, is not required for fish, resulting in a low potassium content in fish feed and even lower potassium levels available to plants (Seawright *et al*., 1998; Graber and Junge, 2009; Suhl *et al*., 2016).

Tap water is the primary source of magnesium, calcium, and sulphur, which makes it easier for plants to absorb because the minerals are already there (Delaide *et al*., 2017). Calcium is available in aquaponics, although in insufficient amounts (Seawright *et al*., 1998) and is supplemented with calcium hydroxide Ca $(OH)_2$ (Eck, Körnerand Jijakli, 2019).

2.6.3.6 Micronutrients

Iron (Fe), manganese (Mn), and zinc (Zn) are primarily obtained from fish feed, whereas boron (B) and copper (Cu) are primarily obtained from tap water (Delaide *et al*., 2017). Key micronutrients are frequently present in aquaponics, although at insufficient amounts (Delaide *et al*., 2017), requiring nutrition supplement from external sources is vital. Due to the non-availability of the ferric ion form, iron shortages are common in aquaponics (Seawright *et al*., 1998; Schmautz *et al*., 2015). This deficit can be treated by bacterial protein (organic nutrient substances) produced by *Bacillus* or *Pseudomonas*, or iron supplementation with chemical chelated iron to minimize iron accumulation in the aquaponic system (Goddek *et al*., 2020). However, medicinal plants may be more capable of thriving under nutrient stress compared to cultivated species (Amirifar *et al*., 2022).

2.6.3.7 Hydroponics fertilizers

An important reasons for choosing the right fertilizer in hydroponics is that, in natural soil conditions, a plant can influence nutrient availability to some extent by exuding root exudates or exploring some new soil regions by growing roots, but in hydroponics, all nutrients must be provided in adequate amounts artificially and may vary from crop to crop (Page and Feller, 2013). Numerous hydroponic fertilizers are available; however, the choice depends on the crop and the system being used. For producing leafy vegetables in hydroponics, most studies used nutrient formulas such Cooper's, Imai's, Massantini's, and Hoagland's solution (Karimaei *et al*., 2001; Shah and Shah, 2009). The "made-from-scratch" approach to creating nutrient-dense dishes is another name for it (Mattson and Peters, 2014). According to Mattson and Peters (2014), tiny hydroponic growers find it challenging to control nutrient concentrations while creating their own hydroponic recipes, which has led to interest in commercially produced water-soluble fertilizers such as Nutrifeed®. This technique, which is appropriate for

hydroponic production, is also known as the one- or two-bag strategy (Mattson and Peters, 2014; Shah and Shah, 2009). Contrary to soil, which has differing growing conditions, hydroponics exposes plant roots directly to the nutrient solution.

2.7 Growing substrates

For most plants, soil is the most readily available growing medium. It promotes plant growth with anchoring, nutrients, air, and water, among other things (Hussain *et al*., 2014). Soils, on the other hand, can sometimes be a severe stumbling block for plant growth. Disease-causing organisms and nematodes, improper soil reactivity, unfavourable soil compaction, poor drainage, erosion deterioration, and so on are some of them (Hussain *et al.*, 2014). Many organic and inorganic substrates have been utilized to replace soil because they are disease and pest-free inert materials that can contain adequate moisture content and can be reused year after year including: rockwool, vermiculite, perlite, peat, leca clay, and others, as the most advanced technology for growing plants in greenhouses (Jankauskien *et al*., 2015). The basis of various media varies as mentioned; some are of organic sources, while others are produced artificially, and their physical, chemical, and biological qualities differ. As a result, substrate selection is one of the most important elements impacting plant growth and development, as well as plant health (Xego, 2017). In this study, five different types of growth media: silica sand, vermiculite, perlite, and coconut fibre (coco peat) are used. However, the effects of substrates were not tested.

2.7.1 Silica sand

Silica refers to a collection of minerals composed of silicon and oxygen, the two most abundant elements in the earth's crust. Sand is made up of mineral and rock pieces in microscopic grains or particles (Jolly and Lonergan, 2002). Sand can be used as a growth medium for plants that demand a dry, loose soil condition. Excess water will runoff rather than absorb into the sand as it would in clay. Because sand is light and easy to expand in, root plants like carrots and potatoes produce more vegetables. It also does not hold a lot of moisture; however, sand is commonly mixed with other growth substrates such as vermiculite, perlite, and coconut fibre and they aid in the retention of moisture as well as aeration of the mix for effective root development. (Shettel and Balke,1983; Hussain *et al.*, 2014).

2.7.2 Vermiculite

Vermiculite, like perlite, is a volcanic rock that expands when heated to high temperatures.

Potassium and magnesium are both present in this substance. It can hold lot of water and helps with drainage and aeration of the soil, but it's not as durable as sand or perlite; it is a very light, porous material that has a high capillary action and can store 3 to 4 times its weight in water (Raviv *et al*.,2002; Hussain *et al*., 2014).

2.7.3 Perlite

Perlite is the most frequent form of media used in soilless culture containerized systems (Boodley and Sheldrake, 1982). Perlite granules are made of a silicone mineral that occurs in volcanoes and are incredibly light. This media is available in small to big bags from merchants for use in growing mediums to improve drainage and aeration. Water can be held in perlite three to four times its own weight. It has a pH range of 6.0 to 8.0 and no buffering properties. It has little cation exchange capability and no mineral nutrients, in contrast to vermiculite. With particle and bulk densities of 0.9 and 0.1 g, respectively, expanded perlite is extremely light. It comes in a variety of sizes and diameters, the most common being 0–2 and 1.5–3.0 mm (Pardossi *et al*., 2011; Carlile *et al*., 2019).

2.7.4 Coconut fibre (coco peat)

Ultrapeat, Cocopeat, and Coco-tek are some of the brand names. It combines the water-holding properties of vermiculite with the air-holding properties of perlite. It's an entirely natural medium comprised of shredded coconut husks. The coconut husk provides two objectives for the seed: (1) protection from the sun and salt while floating in the ocean; and (2) a hormone-rich, fungus-free medium to promote germination and roots once the seed reaches landfall. Coconut coir, which has been finely shredded, and steam sterilized, is an excellent rooting medium that also protects against root infections and fungi (Hussain *et al*., 2014). Unlike peat moss, which is quickly depleting due to overuse, coir is an entirely renewable resource, high K, Na, and Mg content, and low cost.

2.8 *Fusarium oxysporum*

Fusarium is the most important genus of fungal plant pathogens, responsible for many diseases in nearly every commercially important plant species. *Fusarium oxysporum* is a

pathogen with a wide host range that has produced some of the most severe and economically destructive plant pandemic. With tomatoes (*Lycopersicom esculentum*) as hosts, *F. oxysporum* has recently emerged as a model for soil-borne fungal infections (Xego, 2017). This pathogenic fungus can survive in soils for up to ten years. Temperatures of 28 ℃ in the soil and air are ideal for disease growth. If the soil temperature is perfect but the air temperature is not, the pathogen will move to the lower parts of the stem, but the plants will not show visible symptoms. (Bawa, 2016).

The disease is spread over short distances by water and infected farming equipment, but it is usually spread over long distances by contaminated transplants or soil brought with them (Agrios, 2005). The pathogen is widely spread from plant to plant via root-to-root interaction during the growth stage. *F. oxysporum* can also be transmitted by soil contamination and root infection. Chlamydospores, which are prevalent in soil particles on contaminated shoes and plant stakes, are another way for the disease to spread (Ozbay *et al*., 2004). The development of disease resistant plant cultivars and chemical soil fumigation are the key methods for controlling such diseases (Fravel *et al*., 2003; Xego, 2017). *Fusarium* species are important model organisms for biological and evolutionary studies, in addition to their economic relevance.

2.9 Challenges associated with conventional cultivation

Several reports have revealed that agricultural land is negatively impacted by large amounts of pollutants or toxins, polluted water, and tonnes of dry sewage sludge deposited on South African soils, heavy metal contamination of rivers and soils as a result of polluted harvest sites or poor farming techniques, resulting in medicinal plant product contamination (Street *et al*., 2008). Bindraban *et al*. (2012), also mentioned that traditional farming is being challenged by increased energy and cost fluctuations, as well as climate change and pollution. These have resulted in resource constraints such as reduced cultivation surfaces, limited freshwater supplies, soil deterioration, and reduced soil nutrient levels. Furthermore, open-field agriculture is challenging because it necessitates a significant amount of space, al large amout of labour, and too much water. Because of their unfavourable geographical or topographical circumstances, soil is less available for crop production in most urban and industrial locations, and fertile cultivable arable lands are scarce in some areas.

2.10 References

Abdel-Rahim, M.M., Awad, Y.M., Abdallah, Y.A. and Radwan, S.M., 2019. Effects of four medicinal plants on the bioeconomic analysis and water-use efficiency of Nile tilapia, *Oreochromis niloticus* fry nursed under a small-scale aquaponics system. *Aquaculture, Aquarium, Conservation & Legislation*, *12*(3), pp.851–866.

Agrios, G.N., 2005. Plant Pathology. Elservier Academic Press London.

Ajilogba, C.F. and Babalola, O.O., 2013. Integrated management strategies for tomato *Fusarium* wilt. *Biocontrol science*, *18*(3), pp.117–127.

Albayrak, S., Aksoy, A., Sagdic, O. and Hamzaoglu, E., 2010. Compositions, antioxidant, and antimicrobial activities of *Helichrysum* (*Asteraceae*) species collected from Turkey. *Food Chemistry*, *119*(1), pp.114–122.

Asekun, O.T., Grierson, D.S. and Afolayan, A.J., 2007. Characterization of essential oils from *Helichrysum odoratissimum* using different drying methods. *Journal of Applied Sciences*, *7*(7), pp. 1005–1008.

Bakiu, R. and Shehu, Julian., 2014. Aquaponic systems as excellent agricultural research instruments in Albania. *Albanian Journal of Agricultural Sciences*, pp. 385–389.

Bakiu, R., Taçi, J., Nallani, E., Kamberi, E., Hala, E., Sadiku, E. and Buda, E., 2021.The Analyses of the Medicinal Plants Growth in the Integrated Production System of Aquaponics (Experimental and Commercial), *Albanian Journal of Agricultural Sciences*, *340*, pp. 0–5.

Barea, J.M., 2015. Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. *Journal of Soil science and Plant Nutrition*, *15*(2), pp.261–282.

Bawa, I., 2016. Management strategies of *Fusarium* wilt disease of tomato incited by *Fusarium oxysporum f. sp. lycopersici* (Sacc.) A Review. *International Journal of Advanced Research*, *2*(5).

Behrens, J. and Vines, G., 2004. Herbal Harvests with a Future: Towards Sustainable Sources for Medicinal Plants, Report for Plantlife International Salisbury. *Wiltshire: The Wild Plant Conservation Charity*.

Beibel, J.P., 1960. Hydroponics;The Science of Growing Crops Without Soil. Florida Department of Agriculture. *Bull*, *180*.

Bekalo, T.H., Woodmatas, S.D. and Woldemariam, Z.A., 2009. An ethnobotanical study of medicinal plants used by local people in the lowlands of Konta Special Woreda, southern

nations, nationalities and peoples regional state, Ethiopia. *Journal of Ethnobiology and Ethnomedicine*, *5*(1), pp.1–15.

Bhargav, A., 2020. Hydroponic: A Step Ahead for Soil and Water Conservation. *Agri Mirror: Future India*, *1*(1), p.21.

Bhat, R.B. and Jacobs, T.V., 1995. Traditional herbal medicine in Transkei. *Journal of Ethnopharmacology*, *48*(1), pp.7–12.

Bindraban, P.S., van der Velde, M., Ye, L., Van den Berg, M., Materechera, S., Kiba, D.I., Tamene, L., Ragnarsdottir, K.V., Jongschaap, R., Hoogmoed, M. and Hoogmoed, W., 2012. Assessing the impact of soil degradation on food production. *Current Opinion in Environmental Sustainability*, *4*(5), pp.478–488.

Boodley, J.W. and Sheldrake Jr, R., 1982. Cornell Peat–Lite Mixes for Commercial Plant Growing. *Cornell Cooperative Extension Information Bulletin 43*, pp. 1–8.

Borah, B.C., Technical viability of incorpora al viability of incorpora al viability of incorporation of low cost tion of low cost aquaponics in integra s in integra s in integrated chicken-fish f ted chicken-fish f ted chicken-fish farming system. *An Intrenational quartarlyJournal of Environmental Sciences*, *10*, pp. 1–8.

Bougatsos, C., Ngassapa, O., Runyoro, D.K. and Chinou, I.B., 2004. Chemical composition and in vitro antimicrobial activity of the essential oils of two *Helichrysum* species from Tanzania. *Zeitschrift für Naturforschung C*, *59*(5 –6), pp.368–372.

Boyd, C.E., 2004. Farm-level issues in aquaculture certification: Tilapia. *Report commissioned by WWF-US in*, pp.1–29.

Boyd, C.E., 2015. Overview of aquaculture feeds: Global impacts of ingredient use. In *Feed and feeding practices in aquaculture, Woodhead Publishing*, pp. 3–25.

Brechner, M.L., Albright, L.D. and Weston, L.A., 2007, March. Impact of a variable light intensity at a constant light integral: effects on biomass and production of secondary metabolites by *Hypericum perforatum*. In *International Symposium on Medicinal and Nutraceutical Plants 756* (pp. 221–228).

Bremner, P.D. and Meyer, J.J.M., 2000. Prenyl-butyrylphloroglucinol and kaurenoic acid: two antibacterial compounds from *Helichrysum kraussii*. *South African Journal of Botany*, *66*(2), pp.115–117.

Bulinski, J. and Sergiel, L.E.S.Z.E.K., 2013. Soil considerations in cultivation of plants. Annals of Warsaw University of Life Sciences-SGGW. *Agriculture*, (61 Agricultural and Forest Engineering) ,*61*(61), pp. 5–15.

Butler, J.D. and Oebker, N.F., 1962. Hydroponics as a hobby: growing plants without soil. *Circular; 844*.

Bwanika, G.N., Makanga, B., Kizito, Y., Chapman, L.J. and Balirwa, J., 2004. Observations on the biology of Nile tilapia, *Oreochromis niloticus* L., in two Ugandan crater lakes. *African Journal of Ecology*, *42*, pp.93–101.

Carlile, W.R., Raviv, M. and Prasad, M., 2019. Organic soilless media components. *Soilless Culture*, pp.303–378.

Chen, P., Zhu, G., Kim, H.J., Brown, P.B. and Huang, J.Y., 2020. Comparative life cycle assessment of aquaponics and hydroponics in the Midwestern United States. *Journal of Cleaner Production*, *275*, p.122888.

Choi, B.S., Lee, S.S. and Ok, Y.S., 2011. Effects of waste nutrient solution on growth of chinese cabbage (*Brassica campestris* L.) in Korea. *Korean Journal of Environmental Agriculture*, *30*(2), pp.125–131.

Cock, I.E. and Van Vuuren, S.F., 2020. The traditional use of southern African medicinal plants for the treatment of bacterial respiratory diseases: A review of the ethnobotany and scientific evaluations. *Journal of Ethnopharmacology*, *263*, p.113204.

Davidson, J., Good, C., Barrows, F.T., Welsh, C., Kenney, P.B. and Summerfelt, S.T., 2013. Comparing the effects of feeding a grain-or a fish meal-based diet on water quality, waste production, and rainbow trout Oncorhynchus mykiss performance within low exchange water recirculating aquaculture systems. *Aquacultural engineering*, *52*, pp.45–57.

De Beer, D., Joubert, E., Gelderblom, W.C. and Manley, M., 2003. Antioxidant activity of South African red and white cultivar wines: free radical scavenging. *Journal of Agricultural and Food Chemistry*, *51*(4), pp.902–909.

De Canha, M.N., Komarnytsky, S., Langhansova, L. and Lall, N., 2020. Exploring the anti-acne potential of impepho [*Helichrysum odoratissimum* (L.) Sweet] to combat cutibacterium acnes virulence. *Frontiers in pharmacology*, *10*, p.1559.

Delaide, B., 2017. A study on the mineral elements available in aquaponics, their impact on lettuce productivity and the potential improvement of their availability (Doctoral dissertation, Université de Liège, Liège, Belgique).

Delaide, B., Goddek, S., Gott, J., Soyeurt, H. and Jijakli, H.M., 2016. Lettuce (*Lactuca sativa L. var. Sucrine*) growth performance in, complemented solution encourages the development of decoupled aquaponics. *Water*, *8*, p.467.

Dold, A.P. and Cocks, M.L., 2002. The trade in medicinal plants in the Eastern Cape Province, South Africa. *South African Journal of Science*, *98*(11), pp.589–597.

Dubey, A.K., Singh, D., Rajput, P.S., Kumar, Y., Verma, A.K. and Chandraker, S.K., 2017. Effect of NPK on plant growth, yield, and quality of Capsicum (*Capsicum annum* L.) cv Swarna Under Shade Net Condition. *International Journal of Current Microbiology and Applied Sciences*, *6*(3), pp.1085–1091.

Eck, M., Körner, O. and Jijakli, M.H., 2019. Nutrient cycling in aquaponics systems. In *Aquaponics Food Production Systems* ,pp. 231–246. Springer, Cham.

Edwards, R., 2004. No remedy in sight for herbal ransack. *New Scientist*, *181*(2429), pp.10–11.

Ekor, M., 2014. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. *Frontiers in pharmacology*, *4*, p.177.

Etsassala, N. G. E. R., Hussein, A. A. and Nchu, F., (2021.) 'Potential application of some *lamiaceae* species in the management of diabetes', *Plants*, *10*(2), pp. 1–22.

Folorunso, E.A., Roy, K., Gebauer, R., Bohatá, A. and Mraz, J., 2021. Integrated pest and disease management in aquaponics: A metadata-based review. *Reviews in Aquaculture*, *13*(2), pp.971–995.

Fravel, D., Olivain, C. and Alabouvette, C., 2003. *Fusarium oxysporum* and its biocontrol. *New phytologist*, *157*(3), pp.493–502.

Gashgari, R., Alharbi, K., Mughrbil, K., Jan, A. and Glolam, A., 2018, August. Comparison between growing plants in hydroponic system and soil-based system. In *Proceedings of the 4th World Congress on Mechanical, Chemical, and Material Engineering* ,pp. 1–7.

Geay, F., Ferraresso, S., Zambonino-Infante, J.L., Bargelloni, L., Quentel, C., Vandeputte, M., Kaushik, S., Cahu, C.L. and Mazurais, D., 2011. Effects of the total replacement of fish-based diet with plant-based diet on the hepatic transcriptome of two European sea bass (*Dicentrarchus labrax*) half-sibfamilies showing different growth rates with the plant-based diet. *BMC genomics*, *12*(1), pp.1–18.

Ghosh, R. and Deb, P., 2014. A study on antioxidant properties of different bioactive compounds. *Journal of Drug Delivery and Therapeutics*, *4*(2), pp.105–115.

Giovanelli, S., De Leo, M., Cervelli, C., Ruffoni, B., Ciccarelli, D. and Pistelli, L., 2018. Essential oil composition and volatile profile of seven *Helichrysum* species grown in Italy. *Chemistry & biodiversity*, *15*(5), p.e1700545.

Giuliani, C., Lazzaro, L., Calamassi, R., Calamai, L., Romoli, R., Fico, G., Foggi, B. and Lippi, M.M., 2016. A volatolomic approach for studying plant variability: the case of selected *Helichrysum* species (*Asteraceae*). *Phytochemistry*, *130*, pp.128–143.

Giurgiu, R.M., Morar, G.A., Dumitraș, A., Boancă, P., Duda, B.M. and Moldovan, C., 2014. Study regarding the suitability of cultivating medicinal plants in hydroponic systems in controlled environment. *Research Journal of Agricultural Science*, *46*(2).

Goddek, S., Delaide, B., Mankasingh, U., Ragnarsdottir, K.V., Jijakli, H. and Thorarinsdottir, R., 2015. Challenges of sustainable and commercial aquaponics. *Sustainability*, *7*(4), pp.4199–4224.

Goddek, S., Delaide, B., Mankasingh, U., Ragnarsdottir, K.V., Jijakli, H. and Thorarinsdottir, R., 2015. Challenges of sustainable and commercial aquaponics. *Sustainability*, *7*(4), pp.4199–4224.

Goddek, S., Joyce, A., Kotzen, B. and Burnell, G.M., 2020. Correction to: Aquaponics Food Production Systems. In *Aquaponics Food Production Systems* (pp. C1-C1). Springer, Cham.

Graber, A. and Junge, R., 2009. Aquaponic Systems: Nutrient recycling from fish wastewater by vegetable production. *Desalination*, *246*(1–3), pp.147–156.

Hanjra, M.A. and Qureshi, M.E., 2010. Global water crisis and future food security in an era of climate change. *Food policy*, *35*(5), pp.365–377.

Hasegawa, H., 2003. Crop ecology, management & quality. *Crop Sciences*, *43*, pp.921–926.

Hayden, A.L., 2006. Aeroponic and hydroponic systems for medicinal herb, rhizome, and root crops. *HortScience*, *41*(3), pp.536–538.

Heyman, H.M., 2009. *Metabolomic comparison of selected Helichrysum species to predict their antiviral properties* (Doctoral dissertation, University of Pretoria).

Hua, K. and Bureau, D.P., 2012. Exploring the possibility of quantifying the effects of plant protein ingredients in fish feeds using meta-analysis and nutritional model simulation-based approaches. *Aquaculture*, *356*, pp.284–301.

Hussain, A., Iqbal, K., Aziem, S., Mahato, P. and Negi, A.K., 2014. A review on the science of growing crops without soil (soilless culture)-a novel alternative for growing crops. *International Journal of Agriculture and Crop Sciences*, *7*(11), pp.833–842.

Jadalla, B.M.I.S., 2020. Phytochemical and biological studies of *Helichrysum cymosum*. (Master's. Dissertation, University of the Western Cape,South Africa).

Jain, C., Khatana, S. and Vijayvergia, R., 2019. Bioactivity of secondary metabolites of various plants: a review. *International Journal of Pharmaceutical Sciences and Research, 10*(2), pp.494–504.

Jankauskienė, J., Brazaitytė, A. and Viškelis, P., 2015. Effect of different growing substrates on physiological processes, productivity and quality of tomato in soilless culture. Soilless

culture: Use of substrates for the production of quality horticultural crops. *InTech, Rijeka, Croatia*, pp.99–124.

Jolly, R.J. and Lonergan, L., 2002. Mechanisms and controls on the formation of sand intrusions. *Journal of the Geological Society*, *159*(5), pp.605–617.

Jousse, C., Vu, T.D., Le Minh Tran, T., Al Balkhi, M.H., Molinié, R., Boitel-Conti, M., Pilard, S., Mathiron, D., Hehn, A., Bourgaud, F. and Gontier, E., 2010. Tropane alkaloid profiling of hydroponic *Datura innoxia* Mill. plants inoculated with Agrobacterium rhizogenes. Phytochemical Analysis: *An International Journal of Plant Chemical and Biochemical Techniques*, *21*(1), pp.118–127.

Kähkönen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S. and Heinonen, M., 1999. Antioxidant activity of plant extracts containing phenolic compounds. *Journal of Agricultural and Food Chemistry*, *47*(10), pp.3954–3962.

Karimaei, M.S., Massiha, S. and Mogaddam, M., 2001, September. Comparison of two nutrient solutions'effect on growth and nutrient levels of lettuce (*Lactuca sativa* l.) cultivars. In *International Symposium on Growing Media and Hydroponics 644*, pp. 69–76.

Kasote, D.M., 2013. Flaxseed phenolics as natural antioxidants. *International Food Research Journal*, *20*(1), p.27.

König, B., Junge, R., Bittsanszky, A., Villarroel, M. and Kőmíves, T., 2016. On the sustainability of aquaponics. *Ecocycles*, *2*(1), pp.26–32.

Kutluk, I., Aslan, M., Orhan, I.E., and Özçelik, B., 2018. Antibacterial, antifungal, and antiviral bioactivities of selected *Helichrysum* species. *South African Journal of Botany*, *119*, pp.252–257.

Legoale, P.B., Mashimbye, M.J. and van Ree, T., 2013. Anti-inflammatory and antioxidant flavonoids from *Helichrysum kraussii* and *H. odoratissimum* flowers. *Natural product communications*, *8*(10), pp. 1403–1404.

Leonardi, M., Giovanelli, S., Ambryszewska, K.E., Ruffoni, B., Cervelli, C., Pistelli, L., Flamini, G. and Pistelli, L., 2018. Essential oil composition of six *Helichrysum* species grown in Italy. *Biochemical Systematics and Ecology*, *79*, pp.15–20.

Li, Y., Kong, D., Fu, Y., Sussman, M.R. and Wu, H., 2020. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiology and Biochemistry*, *148*, pp.80–89.

Litescu, S.C., Eremia, S. and Radu, G.L., 2010. Methods for the determination of antioxidant capacity in food and raw materials. In *Bio-Farms for Nutraceuticals* (pp. 241–249). Springer, Boston, MA.

Lourens, A.C.U., Reddy, D., Başer, K.H.C., Viljoen, A.M. and Van Vuuren, S.F., 2004. In vitro biological activity and essential oil composition of four indigenous South African Helichrysum species. *Journal of Ethnopharmacology*, *95*(2–3), pp.253–258.

Lourens, A.C.U., Viljoen, A.M. and Van Heerden, F.R., 2008. South African *Helichrysum* species: A review of the traditional uses, biological activity and phytochemistry. *Journal of Ethnopharmacology*, *119*(3), pp.630–652.

Love, D.C., Fry, J.P., Genello, L., Hill, E.S., Frederick, J.A., Li, X. and Semmens, K., 2014. An international survey of aquaponics practitioners. *PloS one*, *9*(7), p.e102662.

Maggini, R., Kiferle, C. and Pardossi, A., 2014. Hydroponic production of medicinal plants. *Medicinal plants: Antioxidant properties, traditional uses and conservation strategies. Nova Science Publishers, Inc., Hauppauge, NY*, pp.91–116.

Maroyi, A., 2019. A synthesis and review of medicinal uses, phytochemistry and biological activities of *Helichrysum odoratissimum* L. *Sweet. Asian Journal of Pharmaceutical and Clinical Research*, *12*, pp.15–23.

Matanzima, Y., 2014. Quantitative and qualitative optimization of antimicrobial bioactive constituents of *Helichrysum cymosum* using hydroponics technology (Doctoral Master's dissertation, Cape Peninsula University of Technology).

Mathekga, A.D.M. and Meyer, J.J.M., 1998. Antibacterial activity of south african *Helichrysum* species. *South African Journal of Botany*, *64*(5), pp.293–295.

Matrose, N.A., Obikeze, K., Belay, Z.A. and Caleb, O.J., 2021. Impact of spatial variation and extraction solvents on bioactive compounds, secondary metabolites, and antifungal efficacy of South African Impepho [*Helichrysum odoratissimum* (L.) Sweet]. *Food Bioscience*, *42*, p.101139.

Mattson, N.S. and Peters, C.A.R.I., 2014. A recipe for hydroponic success. *Inside Grower*, *2014*, pp.16–19.

McDaid Kapetsky, J. and Nath, S.S., 1997. A strategic assessment of the potential for freshwater fish farming in Latin America. *COPESCAL Technical Paper (FAO)*, 10, p. 128.

Mok, W.K., Tan, Y.X. and Chen, W.N., 2020. Technology innovations for food security in Singapore: A case study of future food systems for an increasingly natural resource-scarce world. *Trends in food science & technology*, *102*, pp.155–168.

Moran, M., 1996. Health policy and management: The health-care agenda in a British political context-Paton, C.

Mulabagal, V. and Tsay, H.S., 2004. Plant cell cultures-an alternative and efficient source for

the production of biologically important secondary metabolites. *International Journal of Applied Science and Engineering*, *2*(1), pp.29–48.

Murugan, A.V., Swarnam, T.P. and Gnanasambandan, S., 2013. Status and effect of pesticide residues in soils under different land uses of Andaman Islands, India. *Environmental monitoring and assessment*, *185*(10), pp.8135–8145.

Najar, B., Nardi, V., Cervelli, C., Mecacci, G., Mancianti, F., Ebani, V.V., Nardoni, S. and Pistelli, L., 2020. Analysis of African *Chrysanthemum* essential oils and volatiles pca Hca, *Molecules*, 25(14), pp. 1–17.

Njenga, E.W. and Viljoen, A.M., 2006. In vitro 5-lipoxygenase inhibition and anti-oxidant activity of *Eriocephalus* L.(Asteraceae) species. *South African Journal of Botany*, *72*(4), pp.637– 641.

Nuwansi, K.K.T., Verma, A.K., Chandrakant, M.H., Prabhath, G.P.W.A. and Peter, R.M., 2021. Optimization of stocking density of koi carp (*Cyprinus carpio var*. koi) with gotukola (*Centella asiatica*) in an aquaponic system using phytoremediated aquaculture wastewater. *Aquaculture*, *532*, p.735993.

Nuwansi, K.K.T., Verma, A.K., Rathore, G., Prakash, C., Chandrakant, M.H. and Prabhath, G.P.W.A., 2019. Utilization of phytoremediated aquaculture wastewater for production of koi carp (*Cyprinus carpio var. koi*) and gotukola (*Centella asiatica*) in an aquaponics. *Aquaculture*, *507*, pp.361–369.

Ogutu-Ohwayo, R., 1990. The decline of the native fishes of lakes Victoria and Kyoga (East Africa) and the impact of introduced species, especially the Nile perch, Lates niloticus, and the Nile tilapia, *Oreochromis niloticus*. *Environmental biology of fishes*, *27*(2), pp.81–96.

Okemwa, E., 2015. Effectiveness of aquaponic and hydroponic gardening to traditional gardening. *International Journal of Scientific Research and Innovative Technology*, *2*(12), pp.21–52.

Oyebode, O., Kandala, N.B., Chilton, P.J. and Lilford, R.J., 2016. Use of traditional medicine in middle-income countries: a WHO-SAGE study. *Health policy and planning*, *31*(8), pp.984–991.

Ozbay, N. and Newman, S.E., 2004. *Fusarium* crown and root rot of tomato and control methods. *Plant Pathology Journal*, *3*(1), pp.9–18.

Page, V. and Feller, U., 2013. Selection and hydroponic growth of bread wheat cultivars for bioregenerative life support systems. *Advances in space research*, *52*(3), pp.536–546.

Palm, H.W., Knaus, U., Appelbaum, S., Goddek, S., Strauch, S.M., Vermeulen, T., Jijakli, M.H. and Kotzen, B., 2018. Towards commercial aquaponics: a review of systems, designs, scales, and nomenclature. *Aquaculture international*, *26*(3), pp.813–842.

Panigrahi, G., Panda, S. and Padhi, S.N., 2016. Aquaponics: An innovative approach of symbiotic farming. *International Journal of Bioassays*, *5*(09), pp.4808–4814.

Pantanella, E., Cardarelli, M., Colla, G., Rea, E. and Marcucci, A., 2010, August. Aquaponics vs. hydroponics: production and quality of lettuce crop. In *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 927* (pp. 887–893).

Papadopoulos, A.P., Luo, X., Leonhart, S., Gosselin, A., Pedneault, K., Angers, P., Gaudreau, L. and Dorais, M., 2000. May. Soilless greenhouse production of medicinal plants in North Eastern Canada. In *World Congress on Soilless Culture: Agriculture in the Coming Millennium 554* (pp. 297–304).

Pardossi, A., Carmassi, G., Diara, C., Incrocci, L., Maggini, R. and Massa, D., 2011. Fertigation and substrate management in closed soilless culture. *Pisa: University of Pisa*.

Pardossi, A., Malorgio, F., Incrocci, L. and Tognoni, F., 2006. Hydroponic technologies for greenhouse crops. In Crops: Quality, Growth and Biotechnology; Dris, R., Ed.; *WFL Publisher*, pp. 360–378.

Pasch, J., Ratajczak, B., Appelbaum, S., Palm, H.W. and Knaus, U., 2021. Growth of Basil (*Ocimum basilicum*) in DRF, Raft, and Grow Pipes with Effluents of African Catfish (*Clarias gariepinus*) in Decoupled Aquaponics. *AgriEngineering*, *3*(1), pp.92–109.

Patra, B., Schluttenhofer, C., Wu, Y., Pattanaik, S. and Yuan, L., 2013. Transcriptional regulation of secondary metabolite biosynthesis in plants. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, *1829*(11), pp.1236–1247.

Perrini, R., Morone-Fortunato, I., Lorusso, E. and Avato, P., 2009. Glands, essential oils and in vitro establishment of *Helichrysum italicum* (Roth) G. Don ssp. microphyllum (Willd.) Nyman. *Industrial crops and products*, *29*(2–3), pp.395–403.

Picker, M., 2013. *Alien and invasive animals: A South African perspective*. Penguin Random House South Africa, p.240.

Picker, M., 2013. *Alien and invasive animals: A South African perspective*. Penguin Random House South Africa.

Polycarpou, P., Neokleous, D., Chimonidou, D. and Papadopoulos, I., 2005. A closed system for soil less culture adapted to the Cyprus conditions. *F. El Gamal, AN Lamaddalen, C. Bogliotti, and R. Guelloubi. Non-conventional water use*, pp.237–241.

Primitivo, M.J., Neves, M., Pires, C.L., Cruz, P.F., Brito, C., Rodrigues, A.C., de Carvalho, C.C., Mortimer, M.M., Moreno, M.J., Brito, R.M. and Taylor, E.J., 2022. Edible flowers of *Helichrysum italicum*: Composition, nutritive value, and bioactivities. *Food Research*

International, *157*, p.111399.

Rahimi, M., Farhadi, R. and Balashahri, M.S., 2012. Effects of heavy metals on the medicinal plant. *International Journal of Agronomy and Plant Production*, *3*(4), pp.154–158.

Rakocy, J.E., 2005. Cultured aquatic species information programme. *Oreochromis niloticus*. *FAO Fisheries and Aquaculture Department, Rome. www. fao. org/fishery/culturedspecies/Oreochr omis_niloticus/*en (Cited: 26 February 2019).

Raviv, M., Lieth, H. and Bar-Tal, A. eds., 2019. *Soilless culture: Theory and practice: Theory and practice*. Elsevier.

Raviv, M., Wallach, R., Silber, A. and Bar-Tal, A., 2002. Substrates and their analysis. *Hydroponic production of vegetables and ornamentals*, pp.25–105.

Reddy, D., 2007. The Phytochemistry and Antimicrobial Activity of Selected Indigenous *Helichrysum* species. Ph.D. Dissertation, University of the Witwatersrand, Braamfontein, South Africa.

Santos, V.B., Araújo, A.S., Leite, L.F., Nunes, L.A. and Melo, W.J., 2012. Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems. *Geoderma*, *170*, pp.227–231.

Schmautz, Z., Graber, A., Mathis, A., Griessler Bulc, T. and Junge, R., 2015. Tomato production in aquaponic system: mass balance and nutrient recycling. *Aquaculture Europe*, *15*, p.15.

Schmautz, Z., Loeu, F., Liebisch, F., Graber, A., Mathis, A., Griessler Bulc, T. and Junge, R., 2016. Tomato productivity and quality in aquaponics: Comparison of three hydroponic methods. *Water*, *8*(11), p.533.

Seawright, D.E., Stickney, R.R. and Walker, R.B., 1998. Nutrient dynamics in integrated aquaculture–hydroponics systems. *Aquaculture*, *160*(3– 4), pp.215 –237.

Serabele, K., Chen, W., Tankeu, S., Combrinck, S., Veale, C.G., van Vuuren, S., Chaudhary, S.K. and Viljoen, A., 2021. Comparative chemical profiling and antimicrobial activity of two interchangeably used 'Imphepho'species (*Helichrysum odoratissimum* and *Helichrysum petiolare*). *South African Journal of Botany*, *137*, pp.117–132.

Shah, A.H. and Shah, S.H., 2009. Cultivation of lettuce in different strengths of the two nutrients solution recipes in a non-circulating hydroponics system. *Journal of Agronomy and Crop Science* , *25*, pp.419–428.

Shahidi, F., Janitha, P.K. and Wanasundara, P.D., 1992. Phenolic antioxidants. *Critical reviews in food science & nutrition*, *32*(1), pp.67–103.

Sheldon, J.W., Balick, M.J., Laird, S.A. and Milne, G.M., 1997. Medicinal plants: can utilization and conservation coexist? *Advances in economic botany*, *12*, p. 104.

Shete, A.P., Verma, A.K., Kohli, M.P.S., Dash, P. and Tandel, R., 2014. Optimization of stocking density for goldfish, *Carassius auratus* (Linnaeus, 1758) under aquaponic system. *The Israeli J Aquaculture–Bamidgeh IJA*,*65*, p.7.

Shete, A.P., Verma, A.K., Tandel, R.S., Prakash, C., Tiwari, V.K. and Hussain, T., 2013. Optimization of water circulation period for the culture of goldfish with spinach in aquaponic system. *Journal of Agricultural Science*, *5*(4), p.26.

Shettel, N.L. and Balke, N.E., 1983. Plant growth response to several allelopathic chemicals. *Weed Science*, *31*(3), pp.293–298.

Shipton, T., Tweddle, D. and Watts, M., 2008. ECDC 2008, Introduction of the Nile Tilapia (*Oreochromis niloticus*) into the Eastern Cape. *A report for the Eastern Cape Development Corporation*, p.20.

Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M., 1999. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in enzymology* , *299*, pp. 152–178). Academic press.

Stafford, G.I., Jäger, A.K. and Van Staden, J., 2005. Effect of storage on the chemical composition and biological activity of several popular South African medicinal plants. *Journal of Ethnopharmacology*, *97*(1), pp.107–115.

Street, R.A., Stirk, W.A. and Van Staden, J., 2008. South African traditional medicinal plant trade—challenges in regulating quality, safety and efficacy. *Journal of Ethnopharmacology*, *119*(3), pp.705 –710.

Surendran, U., Raja, P., Jayakumar, M. and Ramasubramoniam, S., 2021. Use of efficient water saving techniques for production of rice in India under climate change scenario: A critical review. *Journal of Cleaner Production*, p.127272.

Swelankomo, N., 2004. *Helichrysum odoratissimum* (L.) Sweet.(Asteraceae).

Tacon, AGJ and Metian, M. 2008. Global overview on the use of fish meal and fish oil in industrially compounded aquafeeds: trends and future prospects. *Aquaculture*, *285*, pp.146–158.

Teichert-Coddington, D.R., Popma, T.J. and Lovshin, L.L., 2017. Attributes of tropical pond-cultured fish. In *Dynamics of pond aquaculture*, pp. 183–198. CRC press.

Twilley, D., Moodley, D., Rolfes, H., Moodley, I., McGaw, L.J., Madikizela, B., Summers, B., Raaff, L.A., Lategan, M., Kgatuke, L. and Mabena, E.C., 2021. Ethanolic extracts of South

African plants, *Buddleja saligna* Willd. and *Helichrysum odoratissimum* (L.) Sweet, as multifunctional ingredients in sunscreen formulations. *South African Journal of Botany*, *137*, pp.171–182.

Van Puyvelde, L., De Kimpe, N., Costa, J., Munyjabo, V., Nyirankuliza, S., Hakizamungu, E. and Schamp, N., 1989. Isolation of flavonoids and a chalcone from *Helichrysum odoratissimum* and synthesis of helichrysetin. *Journal of Natural Products*, *52*(3), pp.629– 633.

Van Vuuren, S.F., Viljoen, A.M., van Zyl, R.L., Van Heerden, F.R. and Başer, K.H.C., 2006. The antimicrobial, antimalarial and toxicity profiles of helihumulone, leaf essential oil and extracts of *Helichrysum cymosum* (L.) D. Don *subsp*. *cymosum*. *South African Journal of Botany*, *72*(2), pp.287–290.

Van Wyk, B.E., Oudtshoorn, B.V. and Gericke, N., 1997. *Medicinal Plants of South Africa*. *Briza*, pp. 304.

Vasisht, K., Sharma, N. and Karan, M., 2016. Current perspective in the international trade of medicinal plants material: an update. *Current pharmaceutical design*, *22*(27), pp.4288–4336.

Vines, G., 2004. Herbal harvests with a future. *Towards Sustainable Sources for Medicinal Plants. Plantlife International, Salisbury, UK*.

Walters, K.J. and Lopez, R.G., 2022. Hydroponic Basil Production: Temperature Influences Volatile Organic Compound Profile, but Not Overall Consumer Preference. *Horticulturae*, *8*(1), p.76.

Watson, C.A., Hill, J.E. and Pouder, D.B., 2004. *Species profile: Koi and goldfish*. Southern Regional Aquaculture Center. *Southern Regional Aquaculture Center*, *7201*(7201), pp. 1– 6.

Wiersum, K.F., Dold, A.P., Husselman, M. and Cocks, M., 2006. Cultivation of medicinal plants as a tool for biodiversity conservation and poverty alleviation in the Amatola region, South Africa. *Frontis*, pp.43–57.

World Health Organization, 2004. WHO guidelines on safety monitoring of herbal medicines in pharmacovigilance systems. *World Health Organization*, p.82.

Xego, S., 2017. *Hydroponic propagation of Siphonochilus aethiopicus: an endangered medicinal plant* (Master's dissertation, Cape Peninsula University of Technology).

Xego, S., Kambizi, L. and Nchu, F., 2017. Effects of different hydroponic substrate combinations and watering regimes on physiological and anti-fungal properties of Siphonochilus aethiopicus. *African Journal of Traditional, Complementary and Alternative Medicines*, *14*(3), pp.89–104.

Yousif, H.A. and Köhler, S., 1990. A Fortran code for the scattering of EM plane waves by two

cylinders at normal incidence. *Computer physics communications*, *59*(2), pp.371–385.

Youssef, M.M.A. and Eissa, M.F.M., 2014. Biofertilizers and their role in management of plant parasitic nematodes. A review. *Journal of Biotechnology and Pharmaceutical Research*, *5*(1), pp.1– 6.

Zenze, K., 2012. *Helichrysum cymosum* (L.) D. Don subsp *cymosum* (Asteraceae).

Chapter 3

Comparing Crop Yield, Secondary Metabolite Contents, and Antifungal Activity of Extracts of *Helichrysum odoratissimum* Cultivated in Aquaponic, Hydroponic, and Field Systems

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Abstract

The overexploitation of wild plants for medicinal uses and conventional agricultural farming methods, which use high amounts of water, fertilizers, and pesticides, have had devastating environ- mental consequences. This study aimed to evaluate the prospects of using aquaponics and hydroponics as alternative approaches to soil cultivation by comparing the crop yields, secondary metabolite contents, and the antifungal activities of ethanol extracts of *Helichrysum odoratissimum* (L.) Sweet, a widely used medicinal plant species in Southern Africa. Six-week-old *H. odoratissimum* seedlings were grown in aquaponic and hydroponic systems. The growth parameters, secondary metabolite contents, and antifungal activity against *Fusarium oxysporum* were assessed. The results for crop yield (plant height, fresh and dry weights) and the tissue nutrient contents did not change substantially ($p > 0.05$) between aquaponic and hydroponic treatments. Gas chromatography–mass spectrometry (GC–MS) analysis showed that monoterpenes and sesquiterpenes were the most abundant compounds in *H. odoratissimum*; however, no statistical difference was observed among the field, hydroponic, and aquaponic plants (DF = 2; $\chi^2 = 2.67$; $p > 0.05$). While there was no significant difference in polyphenol contents among the three treatments, remarkably, the

flavonol contents in the leaves varied significantly (DF = 2; χ^2 = 6.23; p < 0.05) among the three treatments. A higher flavonol content occurred in leaves from the hydroponic system than in leaves from the aquaponic (p < 0.05) and field (p > 0.05) systems. The MIC results showed that the ethanolic extract of *H. odoratissimum* was fungistatic against *F. oxysporum*; however, this effect was more prominent in the ethanol extracts of plants grown in the aquaponic system, with a mean MIC value of 0.37 ± 0.00 mg/mL The key findings of this study are that aquaponically cultivated plants exhibited the best antifungal activity, while higher total flavonol contents occurred in the hydroponically cultivated plants. In conclusion, aquaponics and hydroponics performed better or similar to field cultivation and are viable alternative methods for cultivating *H. odoratissimum* plants.

Keywords: *H. odoratissimum*; Asteraceae; secondary metabolites; antioxidants; aquaponics; hydroponics

3.1. Introduction

The world's population has grown rapidly in the last century and is predicted to reach 10 billion by 2050 (Barea, 2015; Chen *et al*., 2020). This rapid population growth severely strains natural resources, including plants, water, and land. These resources are already overexploited (Hanjra and Qureshi, 2010; Goddek *et al*., 2020; Surendran *et al*., 2021). Anthropogenic factors, including conventional agricultural practices, are significant drivers of environmental degradation (Chen *et al*., 2020). Traditional (soil-based) cultivation is often associated with high inputs of heavy inorganic fertilizers, synthetic pesticides, and water (Santos *et al*., 2012). Lately, there has been an appreciation of the need to adopt environmentally friendly crop cultivation methods to mitigate environmental degradation. Aquaponics and hydroponics are increasingly used to cultivate vegetables; however, using these systems to grow medicinal plants is novel. Both cultivation technologies could limit the exploitation of some endangered medicinal plants from the wild, reduce water wastage during crop cultivation, and enhance the commercialization of medicinal plants (Nchu *et al*., 2018). Cultivating fish and medicinal plants in aquaponics is feasible and ecologically friendly and integrates hydroponics and aquaculture. Aquaponics is regarded as one of the most resource-efficient production systems (Stadler *et al*., 2015), where wastewater is recycled and reused, minimizing contamination of adjacent water bodies (Nuwansi *et al*., 2021).

In hydroponics, another type of soilless cultivation, plants are grown in a sterile nutrient solution (water culture) and inert substrate (Pardossi *et al*., 2006). Cultivating medicinal plants under

precise and controlled environmental conditions in a hydroponic system improves quality, bioactivity, and biomass output on a commercial scale (Polycarpou *et al*., 200; Jousse *et al*., 2010). This approach is beneficial in areas with persistent environmental stresses, such as extreme cold, heat, and drought (Polycarpou *et al*., 200). The prospect of using these cultivation systems to optimize the synthesis of secondary metabolites in highly valued medicinal plants is enticing (Léonhart *et al*., 2002; Maggini *et al*., 2014).

Secondary metabolites produced by plants play a crucial role in plants' defense, protection, and signaling systems (Griesser *et al*., 2015). Notably, many of the secondary metabolites produced by plants are valuable to the pharmaceutical industry (Pezzuto, 1995). Some bioactive compounds have pharmacological or toxicological effects on humans and animals (Bernhoft, 2010). Some plant-based compounds have cosmetics and nutritional uses; they produce drugs, dyes, fragrances, and dietary supplements (Li *et al*., 2020). Plant pathogens and predatory insects have been successfully controlled using plant extracts with high bioactive secondary metabolite contents (Stock *et al*., 201; Lahlali *et al*., 2022; Ntobela *et al*., 2022). Therefore, developing cultivation protocols to optimize the secondary metabolite content, and thus, the medicinal value of plants, is certainly a worthy research venture.

Helichrysum odoratissimum (L.) Sweet belongs to the Asteraceae family, and is a spreading perennial shrub with linear, oblong leaves that are greyish-white and woolly on both sides (Serabele *et al*., 2021). The flowers are pale golden yellow, with tiny flower heads borne in clusters at the terminals of the branches, woody at the base, erect, and up to 50 cm high, and bloom all year (Matrose *et al*., 2021). It is an aromatic species, and it is widely distributed throughout intertropical and Southern Africa (Van Puyvelde *et al*., 1989). *Helichrysum odoratissimum* is one of the most harvested and traded plants in South Africa (Serabele *et al*., 2021). Many studies have validated its traditional uses in traditional medicinal treatment of abdominal pains, heartburn, fever, catarrh, headache, menstrual problems, urinary tract infections, and wounds (Serabele *et al*., 2021; Van Puyvelde *et al*., 1989).

Numerous compounds, including chalcone, helichrysetin, 3,5-dihydroxy-6,7,8-trimethoxy flavone, 3-0-methylquercetin, and 3′,4′,3,5-tetrahydroxy-7-methoxyflavone, are found in the flowers of *H. odoratissimum* (Van Puyvelde *et al*., 1989; Legoale *et al*., 2013). The crude extracts and isolated compounds from *H. odoratissimum* exhibit pharmacological effects such as antibacterial, antimycobacterial (Lall and Meyer,1999; Seaman, 2006), antifungal (Boily and Van Puyvelde,1989; Van Puyvelde *et al*., 1989; Mathekga, 2004; Seaman, 2006; Reddy, 2007), anti-inflammatory (Frum and Viljoen, 2006), and antioxidant (Lall and Meyer, 1999) effects, as well as cytotoxicity and toxicity (Maroyi, 2017).

One of the most significant problems that impedes optimal crop production is the spread of

pathogens in plants. *Fusarium* wilt (*Fusarium oxysporum*) is one of the most common pests; it is widespread in nature and capable of causing significant crop and economic losses (Lahlali *et al*., 2022). Aquaponically and hydroponically cultivated *H. odoratissimum* could be a source of active plant extracts against *Fusarium oxysporum* (Stock *et al*., 2017).

This study aimed to evaluate the prospects of using aquaponics and hydroponics as alternative approaches to soil cultivation by comparing the crop yields, secondary metabolite contents, and the antifungal activities of *H. odoratissimum* extracts cultivated in aquaponic and hydroponic systems.

3.2. Materials and Methods

3.2.1 Research Design

Four-week-old, rooted cuttings of *H. odoratissimum* were grown using two cultivation systems (hydroponic and aquaponic), representing two treatments. Data on plant growth, secondary metabolite contents, and antifungal activity were obtained at the end of the experiment. The secondary metabolite contents and antifungal activities of plants obtained from aquaponics and hydroponic systems were compared with field-cultivated plants. *Helichrysum odoratissimum* seedlings were acquired from Shadowlands Wholesale Nursery Pty. Ltd. in Zevenwacht Link Road, Kuilsriver 7580, Western Cape. Plant specimens were mounted and deposited in the Herbarium of the Department of Horticultural Sciences, Cape Peninsula University of Technology (CPUT), Bellville campus, Cape Town. The roots were carefully cleaned and separated to eliminate potting soil debris. The plants were laid out on a cement floor and arranged in a completely randomized design inside the research greenhouse, where they were exposed to natural sunlight entering through the polycarbonate ceiling of the greenhouse.

3.2.2 Greenhouse Experiment

The experiment was conducted in a greenhouse on the Bellville campus of the Cape Peninsula University of Technology (CPUT). Plants were cultivated using aquaponic as presented in (Figure 3.1) and hydroponic techniques (Figure 3.2). For the hydroponic system, 4-week-old rooted seedlings of *H. odoratissimum* were transplanted into 23 cm diameter pots containing a substrate mix of two parts pine bark, one part perlite, and one part vermiculite. Fifteen replicates of *H. odoratissimum* seedlings were used. The plants were watered daily using 400 mL of deionized water and supplied with Nutrifeed fertilizer (Starke Ayres Pty. Ltd., Cape Town, South Africa), consisting of the following ingredients: nitrogen (65 mg kg^{-1}), phosphorus (27 mg kg^{-1}), potassium (130 mg kg^{-1}), calcium (70 mg kg^{-1}), copper (20 mg kg^{-1}), sulfur (75 mg kg^{-1}), boron (240 mg kg^{-1}), magnesium (240 mg kg^{-1}), and zinc (240 mg kg^{-1}). The fertilizer was mixed with deionized water at a dosage of 10 g/5 L. Each plant received 100 mL of the nutritional solution fortnightly, with a pH of 6.5 and an EC of 2 mS cm^{-1}, using Milwaukee EC 50 and pH 55 kits supplied by Spraytech Pty. Ltd., Cape Town, South Africa). A recirculating aquaponic system was used in the aquaponic system. The system consisted of a fish tank containing a pump and plant grow beds (four black 50 L plastic containers with perforated lids to fit the net pots). The aquaponic system had four fish tanks containing 400 L of water each and a submersible pump that pumped the wastewater (nutrient-rich water) from the tank to a grow bed through a PVC pipe. The grow bed had a deep culture design. It consisted of two black 50 L plastic containers with a perforated lid to fit the net pots. Fifteen *Helichrysum* seedlings were transplanted into net pots containing a mixture of perlite and coco coir (50:50 ratio) as substrate. The plants were watered from the bottom through the drain holes in the net pots immersed in the nutrient-rich water pumped from the fish tank. Recycled water from the grow-bed returned to the fish tank. Thus, recycling of the nutrient water was continuous. An air pump (Regent 7500) connected to an air stone using tubing was used to improve dissolved oxygen at 150 L/H in the fish tank. Each grow bed had 15 seedlings fed from the same fish tank. Ten-to-fifteen-centimeter Goldfish fingerlings (*Carassius auratus*) and fish food (Koi and Goldfish powder, small pellets) supplied by Stodels Nursery Pty. Ltd., Doncaster Road, Kenilworth 7708, Western Cape, South Africa, were used in this study. Twenty Goldfish (*Carassius auratus*) were placed in each tank (1000 L capacity). The constituents of the fish meal were maize, rice, wheat, wheat germ, dehulled soybean meal, lysine, methionine, lime, dicalcium phosphate, vitamins (A, D, E, K, BI, B2, B6, B12), biotin, folic acid, inositol, minerals, colorants, spirulina, immune stimulants, vegetable fats, natural antioxidants, and betaine. The fish were fed twice daily at 08:30 am and 4:00 pm. The aquaponic setup was replicated four times. The experiment lasted for 6 weeks. At the end of the experiment, plant height (cm) and fresh and dry weights (g) of aquaponically and hydroponically produced plants were recorded.

The growth parameters of field plants were not assessed because the plants were already cultivated and established on the premises of the Bellville campus of CPUT before the commencement of the study. The harvested plant materials were used for tissue nutrient and secondary metabolite content analysis and were screened for antifungal activities. The leaves of randomly selected field *H. odoratissimum* plants were harvested, secondary metabolites were characterized, and antifungal activity was assayed. The greenhouse conditions were 15–26 ℃ and 74% RH. The EC level of the nutrient solution in the fish tank was 0.8 mS cm^{-1} and the pH was 6.3. The secondary metabolite contents and antifungal activity of plants obtained from the aquaponic, and hydroponic systems were compared with the field-cultivated plants.

Figure 3.1: Experimental setup of aquaponic system with two 400L of fish tanks

Figure3.2: Hydroponics set-up with *H.odoratissimum* plants

3.2.3 Plant Tissue Analysis

Fresh aerial plant materials (leaves) obtained from the aquaponic, and hydroponic systems were sent to a certified commercial laboratory (Bemlab Pty. Ltd. in Somerset West, South Africa) for the analysis of macroelements and microelements. Aerial parts (leaves) of plants were washed with Teepol solution, rinsed in deionized water, and dried in an oven at 70 ℃ overnight. The dried leaves were then powdered and ashed at 480 ℃ for extraction using filter paper in a 50:50 HCl solution (Campell and Plank, 1998). The concentrations of potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), sodium (Na), manganese (Mn), iron (Fe), copper (Cu), zinc (Z), and boron (B) were measured in the extracts (Campbell and Plank, 1998 and Miller, 1998). Total combustion in a Leco N analyzer was used to determine the total nitrogen contents of the leaves. A conversion factor of 10,000 was used to convert the amounts of N, P, K, Ca, and Mg from percentages to mg/kg (Xego *et al*., 2017). Three replicates from each treatment were analyzed.

3.2.4 In Vitro Fungal Screening Using Microdilution Method

The microdilution method was used to assess the extracts' minimum inhibitory con- centration (MIC), as described by Eloff (1998) and Nchu *et al*. (2010). Five grams of milled *H. odoratissimum* leaf materials from three replicates were extracted with 25 mL ethanol overnight, and then filtered, and the filtrate was evaporated under a fan. To produce a starting concentration of 6 mg/mL, the extracts were diluted in ethanol and transferred to the first row of a 96-well microplate with wells containing 100 µL of sterile distilled water. Thereafter, the extracts were serially diluted twofold. A *Fusarium oxysporum* strain (UPFC no. 21) maintained at CPUT's Department of Horticultural Sciences was used in the microdilution assay. Fungal conidia obtained from stock agar plates were transferred to Nutrient Broth (Merck Pty. Ltd., Cape Town, South Africa) and incubated at 25 ℃ for 4 h. One hundred microliters of conidial suspension (10^5 conidia/mL) were added to each of the 96 wells of the microplates containing the plant extract. Dithane (Stodels Nursery Pty. Ltd., Garden Centre, South Africa) (200 mg/25 mL) was used as a positive control, and the negative control was the solvent blank (ethanol). Each microplate well was filled with 40 µL of 0.2 mg/mL p-iodonitrotetrazolium chloride (INT) (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa) diluted in sterile distilled water, sealed in a plastic bag, and incubated at 37 ℃ and 100% RH. In the presence of fungus development, the colorless tetrazolium salt was reduced to a red-colored formazan product.

At 18 h of incubation of the microtiter plates, the MIC values were recorded by visually comparing the pink color of the wells. The antifungal bioassay (MIC) included three replicates of each treatment.

3.2.5 Determination of Antioxidant Activities (FRAP, ABTS and DPPH)

3.2.5.1 Ferric Reducing Antioxidant Powder (FRAP)

The FRAP assay was carried out according to Benzie and Strain's procedure (1996). In a 96-well microplate, 10 µL of the crude extract was combined with 300 µL FRAP reagent (0.3 µM acetate buffer, pH 3.6) (Saarchem, South Africa), 10 mM 2,4,6-tripyridyl-s- triazine (TPTZ) in 0.1 µM HCl (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa), 20 mM iron (III) chloride hexahydrate (FeCl, 593 nm). As a standard, L-ascorbic acid (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa) was employed at concentrations ranging from 0 to 1000 µM. The absorbance was determined. The results were represented in milligrams of ascorbic acid equivalent per gram of dry weight (milligrams of AAE/g DW). Three replicates from each treatment were analyzed.

3.2.5.2 Antioxidant Capacity of 2,2'-diphenylpicrylhydrazyl (DPPH) Radicals

The DPPH free radical scavenging activities of the samples were determined according to Katalinic´ *et al*. (2004). A solution of 0.135 mM DPPH produced in a dark container was used to create the DPPH radical Olatunji and Afolayan, 2019). Approximately 300 µL of DPPH solution was combined with 25 µL of the crude extract and graded concentrations (0 and 500 µM) of Trolox standard (6-hydroxy-2,5,7,8-tetramethylchroman-2-20 carboxylic acid). After a 30 min incubation period, the absorbance at 517 nM was determined as µM/Trolox equivalent per gram of dry weight (µM TE/g DW).

3.2.5.3 2,2'-azino-bis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS) Antioxidant Capacity

The ABTS assay was carried out using the method described by Re *et al*. (1999). Stock solutions of 7 mM ABTS and a 140 mM potassium peroxodisulfate ($K_2S_2O_8$) (Merck, South Africa) were used. The working solution was then produced by mixing 88 µL of $K_2S_2O_8$ with 5 µL of ABTS solution. The two solutions were thoroughly mixed and left to react at room temperature in the dark for 24 h. The standard was Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-20 carboxylic acid) at concentrations ranging from 0 to 500 µM. The crude extracts (25 µL) were allowed to react with 300 µL of ABTS at room temperature for 30 min before being read in a plate reader (Multiskan Thermo Scientific, version 1.00.40, Vantaa, Finland) at 734 nm at 25 ℃. The results were represented as µM/Trolox equivalent per gram of dry weight (µM TE/g DW).

3.2.6 Secondary Metabolite Contents

3.2.6.1 Determination of Total Polyphenol and Flavonol Contents

The total polyphenol contents of dried *H. odoratissimum* samples (leaves) were de- termined using the Folin–Ciocalteu procedure (Singleton *et al*., 1999). Twenty-five microliters of aqueous extracts were mixed with 125 µL of Folin–Ciocalteu reagent (Merck Pty. Ltd., Cape Town, South Africa) in a 96-well microplate and diluted 1:10 with distilled water in a 96-well microplate. The well was filled with 100 µL of aqueous Na_2CO_3 (7.5%) after 5 min (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa). The plates were incubated for 2 hour at room temperature before being examined at 765 nm with a Multiskan plate reader (Thermo Electron Corporation, Waltham, Massachusetts, USA). The results are represented as mg

gallic acid equivalents per gram of dry weight (mg GAE/g DW) using 0, 20, 50, 100, 250, and 500 mg/L gallic acid in 10% ethanol (Singleton *et al*., 1999; Espinoza *et al*., 2019).

The total flavonol content of dried leaves of *H. odoratissimum* plants was evaluated using a standard of quercetin 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa). A volume of 12.5 µL of crude aqueous extracts was combined with 12.5 µL of 0.1% HCl (Merck Pty. Ltd., Cape Town, South Africa) in 95% ethanol and 225 µL of 2% HCl in the sample wells, which were incubated at room temperature for 30 min. At a temperature of 25 ℃, the absorbance was measured at 360 nm. The results are represented in milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW) (Espinoza *et al*., 2019). Three replicates from each treatment were analyzed.

3.2.7 GC/MS Analysis (Headspace) and Secondary Metabolite Analysis

3.2.7.1 Sample Preparation

Fresh plant materials (leaves) were freeze dried overnight at –80 ℃. After that, 1 g was weighed into a solid phase microextraction (SPME) vial, along with 2 mL of 12% ethanol. solution at pH3.5 and 3 mL of 20% NaCl solution. A divinylbenzene/carboxen/polydimethylsiloxane (DVB/CAR/PDMS) SPME fiber was used to analyze the headspace of all the samples (grey). Three replicates from each treatment were analyzed.

3.2.7.2 Chromatographic Separation

To determine the relative abundance of secondary metabolites, a method reported by Matrose *et al*. (2021) was used in the separation of volatile compounds using gas chromatography (6890N, Agilent Technologies Network) coupled to an Agilent Technologies Inert XL/CI Mass Selective Detector Analytics PAL autosampler. The separation of volatiles present in the samples was achieved using a polar ZB-WAX (30 m, 0.25 mm ID) at a flow rate of 1 mL/min, and helium was used as the carrier gas. With a 5:1 ratio, the injector temperature was kept at 250 ℃. The temperature of the oven was programmed as follows: 35 ℃ for 6 min, then 3 ℃/min to 70 ℃ for 5 min, then 4 ◦C/min to 120 ℃ for 1 min, and lastly, 20 ℃/min to 240 ℃, and maintained for 2.89 min. The Mass Selective Detector (MSD) was in full scan mode when the incident occurred. Volatile compounds exhibiting a match quality of at least 90% with the mass spectral library were identified and reported.

3.2.7.3 Statistical Analysis

The experimental data for the plant growth parameters (plant height, fresh and dry weight), tissue nutrient content, and secondary metabolite contents were analyzed using the Kruskal–Wallis test at $p < 0.05$ level of significance. Furthermore, multiple comparisons of the means were carried out using the Mann–Whitney test. PAST was used to carry out these computations (Hammer *et al*., 2001), and the number of volatiles in the aquaponics, hydroponics and field plants were compared using Pearson's chi-square test.

3.3 Results

3.3.1 Plant Height

The results show that the heights of aquaponically and hydroponically cultivated *H. odoratissimum* plants did not vary significantly (DF = 1; χ^2 = 1.128; $p > 0.05$) (Table 1). However, it was observed that aquaponic plants had considerably longer mean shoot lengths (17.06 cm) than hydroponic plants (15.93 cm) (Table 3.1).

Table 3.1: Growth parameters (mean ± SE) of *H. odoratissimum* grown in aquaponic and hydroponic systems for six weeks under greenhouse conditions

Treatments	Plant Height (cm)	Fresh Weight (g)	Dry Weight (g)
T1	17.06 ± 0.48a	9.83 ± 1.85a	4.98 ± 1.06a
T2	15.93 ± 0.45a	5.57 ± 0.53a	2.53 ± 0.28b

Values shown are mean S.E. Means followed by the same lowercase letters in the same column are not significantly different ($p < 0.05$) following comparison using the Mann–Whitney test for aquaponic (T1) and hydroponic systems (T2).

3.3.2 Fresh and Dry Weight

When the weights of plants grown in aquaponic and hydroponic systems were com- pared six weeks after treatment, there was a significant difference between the *H. odoratissimum* plants cultivated in aquaponic and hydroponic treatments (DF = 1; χ^2 = 4.84; $p = 0.02$; $p < 0.05$); the fresh weight of aquaponic plants was greater than that of hydroponic plants (Table 3.1). Aquaponic and hydroponic treatments did not significantly differ in the dry weights of *H. odoratissimum* (DF = 1; χ^2= 4.41; $p < 0.05$). Nevertheless, plants produced in the aquaponic system were heavier than plants cultivated in the hydroponic system.

3.3.3 Tissue Analysis

3.3.3.1 Macronutrients

The macronutrient contents, in terms of carbon, potassium, and calcium, in *H. odoratissimum* did not differ significantly between aquaponic and hydroponic treatments: C (DF = 1; χ^2 = 2.68; $p > 0.05$), K (DF = 1; χ^2 = 2.14; $p > 0.05$), and Ca (DF = 1; χ^2= 2.24; $p > 0.05$) (Table 3.2). However, the macronutrient contents of N, P, and Mg, on the other hand, were significantly

different ($p < 0.05$) among aquaponic and hydroponic plants. The highest mean tissue nutrient contents in plants were obtained in plants grown in the hydroponic system: N (16,000 mg/kg), P (3400 mg/kg), and Mg (1950 mg/kg). Broadly, plants grown in the hydroponic system had higher tissue contents of macronutrients than aquaponically cultivated plants.

Table 3.2: Tissue nutrient contents of shoots (mean ± SE) of *H. odoratissimum* grown in aquaponic and hydroponic systems for six weeks under greenhouse conditions

Treatments	**Quantity (mg/kg)**					
	C	**N**	**P**	**K**	**Ca**	**Mg**
T1	462300 ± 2655.81a	11350 ± 317.54a	1650 ± 28.87a	6600 ± 115.47a	6850 ± 433.01a	747.5± 28.33a
T2	454350 ± 4070.32a	16000 ± 1096.96b	3400 ± 346.41b	22850 ± 11113.10a	8000 ± 635.08a	1950 ± 86.60b

Means followed by the same lowercase letters in the same column are not significantly different ($p > 0.05$) following the Mann–Whitney test comparison. T1, aquaponic; T2, hydroponic.

3.3.3.2 Micronutrients

Plants grown in the aquaponic system had significantly higher leaf tissue micronutrient contents for the majority of elements, Mn (DF = 1; χ^2 = 8.54; $p < 0.05$), Cu (DF = 1; χ^2 = 185.41; $p < 0.05$), and Zn (DF = 1; χ^2 = 71.12; $p < 0.05$), analyzed than those in the hydroponic system, which, on the other hand, yielded significantly higher (DF = 1; $p < 0.05$) tissue contents for B and Fe (Table 3.3).

Table 3.3: Tissue nutrient contents of shoots (mean ± SE) of *H. odoratissimum* grown in aquaponic and hydroponic systems for six weeks under greenhouse conditions

Treatments	**Quantity (mg/kg)**					
	Na	**Mn**	**Fe**	**Cu**	**Zn**	**B**
T1	14445± 707.25a	103.8± 8.78a	147.67± 25.85a	17.1± 0.75a	116± 6.93a	15.43± 0.60a
T2	4570 ± 300.22b	78.15 ± 0.09b	168.5± 22.81a	5.1± 0.46b	44.4± 4.91b	48.07± 2.63b

Means followed by the same lowercase letters in the same column are not significantly different ($p > 0.05$) following the Mann–Whitney test comparison. T1, aquaponic; T2, hydroponic.

3.3.4 Secondary Metabolites (Polyphenols and Flavonol)

When the three cultivation methods were evaluated, there was no significant difference (DF = 2; χ^2 = 4.25; p = 0.07) in total polyphenol contents (mg GAE/g) in the leaves of *H. odoratissimum* (Table 3.4). However, plants cultivated via hydroponics had higher polyphenol contents (592.98 ±76.88 mg GAE/g) when compared with the aquaponic and field- collected plants. The flavonol contents in the leaves varied significantly (DF = 2; χ^2 = 6.23; p < 0.05) among the three treatments. Hydroponic cultivation produced a significantly higher flavonol capacity compared to aquaponic (Table 3.4).

Table 3.4: Mean ± SE total polyphenol (mg GAE/g) and flavonol (mg GAE/g) contents of leaves of *H. odoratissimum* grown using different cultivation methods

Treatments	Polyphenols (mg GAE/g)	Flavonols (mg QE/g)
T1	434.46± 27.67a	102.42± 10.27a
T2	592.98± 76.88a	172.85± 19.07b
T3	358.15± 58.75a	147.91± 12.01ab

Means followed by the same lowercase letters in the same column are not significantly different (p > 0.05) following the Mann–Whitney test comparison. T1, aquaponic; T2, hydroponic; T3, field

3.3.5 Antioxidant Capacity (FRAP, ABTS, and DPPH)

The FRAP analysis of *H. odoratissimum* revealed no significant effect (DF = 2; χ^2 = 2.69; p = 0.14) of treatment on the antioxidant capacity of the plant extracts from the three cultivation techniques (Table 3.5). However, plants grown via hydroponics produced a higher mean value in the FRAP bioassay (3078.55 ± 355.44 µmol AAE/g) than aquaponic (2350.46 ± 200.18 µmol AAE/g) and field-collected plants (2196.50 ± 284.01a µmol AAE/g). In the DPPH test, which is

based on the ability of an antioxidant to donate a hydrogen atom to the DPPH free radical, the extracts from the hydroponically cultivated plants displayed higher antioxidant activity; nevertheless, the DPPH contents (mol TE/g) did not vary substantially among the three cultivation methods (DF = 2; χ^2 = 0.91; p = 0.4). Similarly, the ABTS capacity (µmol TE/g) results indicate that the hydroponic plants produced a significantly higher value com- pared to aquaponic and field-collected plants (DF = 2; χ^2 = 8.44; p = 0.01) (Table 3.5). Broadly, *H. odoratissimum* plants cultivated in hydroponics had the best antioxidant activity.

Table 3.5: Mean ± SE of FRAP (µmol AAE/g), ABTS (µmol TE/g), and DPPH (µmol TE/g) contents of leaves of *H. odoratissimum* grown using different cultivation methods

Treatments	FRAP (umol AAE/g)	ABTS (umol TE/g)	DPPH (umol TE/g)
T1	2350.46± 200.18a	3163.67± 209.76ab	1639.13± 50.86a
T2	3078.55± 355.44a	4163.4± 344.29b	1907.1± 230.34a
T3	2196.50± 284.01a	2836.2± 86.15b	1902.7± 146.29a

Means followed by the same lowercase letters in the same column are not significantly different (p > 0.05) following the Mann–Whitney test comparison. T1, aquaponic; T2, hydroponic; T3, field

3.3.6 Analysis of Volatile Compounds

The number of volatile compounds produced by plants did not differ substantially when the treatments (hydroponic, aquaponic, and field) were compared using the chi- square test (DF = 2; χ^2 = 2.67; p = 0.26) (Table 3.6). The total number of compounds detected was 116 for all three cultivation methods. However, when the individual compounds were compared among treatments, the concentrations of alpha-terpinene, styrene, beta-ocimene, and cyclohexanone were significantly higher (DF = 2; p < 0.05) in the hydroponic plants (Table 3.6). Some

compounds were substantially higher (DF = 2; $p < 0.05$) in aquaponic plants compared to hydroponic and field-collected plants, which included alpha-phellandrene, o-ethyltoluene, tetradecane, alpha-terpineol, alpha-curcumene, and palustrol.

Table 3.6: Volatile compounds in *H. odoratissimum* plants grown in aquaponic, hydroponic, and field systems

Compounds	**Aquaponics Peak Area in the chromatogram**	**Hydroponics Peak Area in the chromatogram**	**Field plants Peak Area in the chromatogram**	**Retention times**
decane	0.88 ± 0.02a	1.28 ± 0.33a	0.92 ± 0.01a	5.48
alpha-pinene	3.31 ± 0.00b	24.33 ± 6.09a	20.06 ± 0.78a	5.79
nonadecane	0.21 ± 0.10a	0.61 ± 0.14a	0.42 ± 0.07a	6.68
camphene	0.21 ± 0.12a	1.15 ± 0.48a	0.37 ± 0.01a	7.01
4-methyl-octane	0.60 ± 0.30a	2.69 ± 0.94a	1.03± 0.11a	7.38
beta-pinene	0.79 ±0.06a	2.68 ± 0.93a	1.03 ± 0.11a	8.13
undecane	0.53 ± 0.05a	0.43 ± 0.00a	0.42 ± 0.00a (8.34)	8.34
alpha-phellandrene	2.26 ± 1.11a	0.31 ± 0.15a	0.48 ± 0.04a	8.47
myrcene	0.77 ± 0.38a	3.22 ± 1.03a	1.38 ± 0.09a	9.59
beta-phellandrene	0.33 ± 0.03b	0.50 ± 0.04a	0.51 ± 0.01a	12.28
1,8-cineole - (eucalyptol)	4.33 ± 0.04a	6.90 ± 0.36a	5.83 ± 1.71a	12.9
o-Ethyltoluene	2.13 ± 1.17a	0.31 ± 0.03a	0.18 ± 0.10a	12.19

ocimene	1.40 ± 0.50a	6.54 ± 2.15a	2.53± 0.28a	14.17
gamma-terpinene	1.42 ± 0.57a	6.443 ± 2.05a	2.79 ± 0.37a	11.87
styrene	0.22 ± 0.10a	0.67 ± 0.13b	0.45 ± 0.02ab	14.72
beta-ocimene	0.24±0.09a	0.66 ± 0.13a	0.44 ± 0.03a	14.72
para- cymene	5.18 ± 1.86a	14.76 ± 4.69a	9.16 ± 0.87a	15.24
1,2,3-trimethylbenzene	0.01 ± 0.01a	5.91 ± 0.92b	–	
alpha-fenchene	0.22± 0.12a	1.38 ± 0.58a	0.32 ±0 .10a	15.66
alpha-terpinolene	0.89 ± 0.20a	2.44 ± 0.46b	1.29 ± 0.13ab	15.76
cyclohexanone	2.37 ± 0.32b	1.07 ± 0.08a	4.00 ± 0.06c	15.76
2,6,6-trimethylcyclohexanone	0.01 ± 0.01a	0.10 ± 0.00b	0.16 ± 0.01c	15.99
3-hexenyl-acetate	1.72 ± 0.86a	3.07 ± 0.56a	1.47 ± 0.43a	14.15
2-heptenal	1.56 ± 0.56a	3.19 ± 0.45a	1.52 ± 0.41a	11.6
6-methyl-5-hepten-2-one	1.07 ± 0.21a	1.74 ± 0.23bc	2.31 ± 0.13c	17.41
allo-ocimene	2.77 ± 0.05a	3.33 ± 0.47a	3.19 ± 1.84a	19.19
octenyl -acetate	2.32 ± 1.09a	1.67 ± 1.67a	4.98 ± 0.62a	16.27
3-hexenol	0.21 ± 0.11a	0.49 ± 0.05a	0.62 ± 0.15a	19.74
4-methyl-1,5-heptadiene	0.28 ± 0.05b	0.04 ± 0.01a	0.09 ± 0.01a	20

3-octanol-istd	–	–	–	
3-ethyl-o-xylene	0.48 ± 0.16a	0.62 ± 0.24a	0.20 ± 0.00a	20.83
para-cymenyl	0.90 ± 0.09a	0.96 ± 0.06a	0.96 ± 0.10a	20.96
tetradecane	2.67 ± 0.46a	1.49 ± 0.85a	1.03 ± 0.12a	21.05
1-octen-3-ol	4.07 ± 0.78a	14.15 ± 1.71b	7.59 ± 1.01a	21.74
beta-fencyl-acetate	3.79 ± 0.79a	13.77 ± 1.69b	3.77 ± 0.03a	21.9
6-methyl-5-hepten-2-ol	0.30 ± 0.00a	2.67 ± 0.38b	4.59 ± 0.05c	22.12
2,5-dimethyl-p-xylene	0.51 ± 0.03a	2.17 ± 1.16ab	4.67 ± 0.07b	22.28
alpha-ylangene	6.25 ± 0.01a	13.29 ± 3.98a	8.15 ± 0.63a	22.63
italicene	2.93 ± 0.65a	4.64 ± 1.08a	23.43 ± 0.85b	22.99
benzaldehyde	0.23 ± 0.09a	1.15 ± 0.26a	26.18 ± 0.78b	23.16
allyl-isopentanoate	10.08 ± 1.34a	12.70 ± 1.05a	5.24 ± 0.39b	23.66
gamma-curcumene	4.83 ± 0.20a	2.83 ± 1.23a	22.09 ± 2.19b	20.05
l-linalool	6.71 ± 0.68a	5.18 ± 2.09a	52.53 ± 6.80b	24.32
alpha-copaene	7.54 ± 0.91a	13.07 ± 3.84a	55.57 ± 8.76b	24.37
cis-sabinene-hydrate	0.01 ± 0.01a	0.01 ± 0.00a	1.08 ± 0.04b	24.63

e,e-alpha-farnesene	2.29 ± 0.17a	4.67 ± 0.87b	3.45 ± 0.06ab	24.96
fenchol	1.40 ± 0.14a	2.03 ± 0.60a	2.28 ± 0.01a	25.13
beta-caryophyllene	115.48 ± 24.27a	114.71 ± 37.55a	102.57 ± 20.13a	25.71
(+)-aromadendrene	4.00 ± 0.46a	49.89 ± 25.87ab	97.52 ± 12.59b	25.8
delta-elemene	0.66 ± 0.23a	2.37 ± 0.61b	2.06 ± 0.02ab	25.84
(-)-Isoledene	1.50 ± 0.31a	2.35 ± 0.44a	2.15 ± 0.19a	26.59
ethyl-caprate	1.05 ± 0.10a	2.23 ± 0.45a	1.52 ± 0.11a	26.87
pinocarveol	0.63 ± 0.01a	2.15 ± 0.34b	0.59 ± 0.03a	27.04
gamma-elemene	0.09 ± 0.03a	0.14 ± 0.07a	0.38 ± 0.04b	27.11
alpha-humulene	63.10 ± 1.97a	110.20 ± 28.20a	83.32 ± 7.45a	27.74
linalyl-propanoate	27.80 ± 15.17a	100.91 ± 23.74b	7.35 ± 0.41a	27.81
alpha-humulene	57.50 ± 3.19a	99.98 ± 24.27a	74.61 ± 9.23a	20.23
acoradiene	28.411 ± 15.58a	101.85 ± 23.19b	6.4 8± 0.37a	27.91
1,8-menthadien-4-ol	4.11 ± 0.05a	5.25 ± 0.66a	12.61 ± 2.79b	28.09

beta-himachalene	–	0.73 ± 0.21b	0.44± 0.04ab	28.14
beta-himachalene	71.11 ± 35.55a	78.89 ± 39.44a	–	–
alpha-terpineol	140.01 ± 19.25a	114.10 ± 2.44a	103.51 ± 2.56a	19.05
ledene	250.73 ± 112.38a	232.02 ± 65.63a	343.67 ± 40.25a	28.65
(+)-2-carene	369.88 ± 2.36b	538.97 ± 44.64a	508.44 ± 21.85a	28.73
valencene	181.80 ± 104.35a	302.16 ± 173.95a	0.88± 0.08a	24.09
alpha-gurjunene	1.02 ± 0.39a	1.19 ± 0.25a	0.36 ± 0.03a	29.08
eremophilene	1.02 ± 0.39a	1.19 ± 0.25a	0.36 ± 0.04a	29.11
beta-selinene	28.68 ± 2.82a	44.48 ± 10.06a	32.62 ± 3.45a	29.11
neryl-acetate	27.44 ± 2.10a	45.58 ± 10.74a	32.62 ± 3.45a	29.93

alphabisabonele	8.84 ± 1.15a	16.43 ± 3.69a	8.63 ± 0.95a	29.31
beta-bisabonele	6.76 ± 0.57a	12.93 ± 2.74a	8.58 ± 1.22a	29.37
alpha-cedrene	5.89± 1.10a	15.72 ± 4.63a	10.33 ± 1.58a	29.54
7-epi-alpha-selinene	17.24 ± 2.03a	25.47 ± 4.27a	22.90± 2.07a	29.93
delta-cadinene	17.24 ± 2.03a	25.37 ± 4.33a	22.90 ± 2.07a	30.24
alpha-curcumene	176.39 ± 24.57a	100.28 ± 41.98a	112.44 ± 50.42	30.24
ar-curcumene	180.67 ± 26.09a	213.66 ± 20.00a	192.62 ± 5.33a	30.57
gamma-selinene	2.59 ± 0.63a	62.51 ± 33.73a	6.42 ± 0.15a	30.62
alpha-cadinene	1.68 ± 0.21a	2.63 ± 0.67a	1.92 ± 0.30a	30.95
nerol	0.33 ± 0.03a	0.91 ± 0.16b	0.58 ± 0.06ab	31.06
2-phenylethyl-acetate	4.54 ± 0.01ab	5.93 ± 1.30b	2.32 ± 0.40a	31.18

isogeraniol	0.42 ± 0.03a	2.26 ± 0.41b	0.83 ± 0.04a	31.29
beta-damascenone	0.42 ± 0.03a	2.26 ± 0.41b	0.79 ± 0.06a	31.29
1s-calamenene	5.48 ± 0.34b	0.34 ± 0.33a	4.13 ± 1.59ab	31.5
carveol	5.64 ± 0.38a	8.72 ± 1.68a	6.16 ± 0.36a	31.61
p-cymen-8-ol	1.93 ± 0.66a	0.92 ± 0.15a	8.06 ± 0.55b	31.85
4-phenyl-2-btanone	0.83 ± 0.04a	0.75 ± 0.09a	0.61 ± 0.07a	31.87
ethyl-laurate	2.04 ± 1.14a	5.39 ± 1.75a	2.97 ± 1.36a	31.98
(e)-geranyl-acetone	2.91 ± 0.73a	5.52 ± 1.83a	5.00 ± 0.35a	32.02
ascaridole	2.68 ± 0.78a	5.57 ± 1.84a	4.87 ± 0.32a	32.02

benzyl-alcohol	0.72 ± 0.07a	1.42 ± 0.26a	1.20 ± 0.03a	32.24
4-ethyl-o-xylene	2.08 ± 0.22a	3.21 ± 0.70a	2.49 ± 0.37a	32.24
ethyl-3-phenylpropionate	2.15 ± 0.25a	3.24 ± 0.73a	2.69 ± 0.37a	32.4
phenylethyl_Alcohol	4.80 ± 0.72a	6.57 ± 1.13a	3.19 ± 0.70a	32.4
alpha-calacorene	12.86 ± 1.61a	21.67 ± 5.27a	17.73 ± 1.19a	32.8
palustrol	3.38 ± 0.08a	3.20 ± 0.40a	0.82 ± 0.05b	32.92
alpha-cubene	2.70 ± 0.30a	3.47 ± 0.74a	2.80 ± 0.31a	33.23
caryophyllene_Oxide	2.54 ± 0.01a	2.71 ± 0.52a	3.02 ± 0.20a	33.91
(+)-ledol	2.42 ± 0.10a	2.01 ± 0.24a	2.02 ± 0.12a	34.53

alpha-caryophyllene-alcohol	0.74 ± 0.03a	0.71 ± 0.19a	0.89 ± 0.01a	34.82
fonenol	14.12 ± 1.15a	15.36 ± 1.07a	13.65 ± 1.81a	34.83
longifolenaldehyde	12.54 ± 0.98a	13.88 ± 0.68a	11.63 ± 1.76a	34.87
n-benzylidenecyclohexylamine	3.49 ± 0.35a	3.47 ± 0.41a	4.47 ± 0.38a	35.01
cyclooctanone	4.74 ± 0.01a	7.54 ± 1.97a	6.95 ± 0.72a	35.16
caryophyll-5-En-2-beta-ol	1.97 ± 0.13a	1.54 ± 0.21a	3.36 ± 0.07b	35.78
t-cadinol	1.57 ± 0.01a	1.54 ± 0.21a	1.52 ± 0.14a	35.78
eugenol	12.06 ± 0.95a	12.31 ± 1.69a	17.22 ± 1.52a	35.94
(+)-calarene	1.20 ± 0.04a	1.76 ± 0.17b	1.36 ± 0.11ab	36.13

eudesm-7(11)-en-4-ol-(juniper-camphor)	1.24 ± 0.09a	1.98 ± 0.24b	1.27 ± 0.08a	36.13
beta-cadinene	1.39 ± 0.11a	1.55 ± 0.19a	1.41 ± 0.05a	36.19
epi-bicyclosesquiphellandrene	1.74 ± 0.44a	1.41 ± 0.14a	2.27 ± 0.18a	36.3
carvacrolok	2.95 ± 0.14a	3.47 ± 0.55a	3.61 ± 0.21a	36.46
alpha-eudesmol	6.33 ± 0.61a	0.62 ± 0.09b	8.06 ± 0.60a	36.62
beta-Eudesmol	2.27± 0.15a	2.48 ± 0.25a	2.22 ± 0.18a	36.73
decanoic_acid	2.37 ± 0.09a	4.03 ± 0.95a	3.00 ± 0.53a	37.08
(-)-phyllocladene	7.55 ± 0.76a	9.58 ± 0.61a	6.46 ± 1.03a	38.44
2,7-dimethyl-1,6-octadiene	5.83 ± 0.34a	11.11 ± 3.55a	8.23 ± 1.75a	38.57
xanthorrhizol	1.95 ± 0.29a	3.34 ± 0.64a	3.27 ± 0.38a	40.9

Total number of compounds	116	116	116

* Volatile compounds having a match quality of at least 90% with the mass spectral library were identified and reported. Means followed by the same lowercase letters in the same column are not significantly different ($p > 0.05$) following comparison using Pearson's chi-square test.

3.3.7 *In Vitro* Fungal Activity Using the Microdilution Assay

The Minimum Inhibitory Concentration of *H. odorattisimum*

There was a significant difference in the minimum inhibitory concentrations among the three cultivation methods when the ethanol extracts of *H. odorattisimum* (DF = 2; $\chi^2 = 7.5$; $p = 0.03$) were screened against *F. oxysporum* at 18 h of incubation (Table 3.7). Treatment one (aquaponics) yielded the best fungistatic results, with an MIC value of 0.37 mg/mL, followed by hydroponics (0.56 ± 0.18 mg/mL). Treatment three (field-collected plants) yielded the least activity among all the treatments tested, with a MIC value of 0.75 mg/mL; this is equivalent to the synthetic fungicide (Dithane) used as a positive control, with a MIC value of 0.75 mg/mL (Table 3.7).

Table 3.7: Anti-*F. oxysporum* activity (mean MIC ± SE) of ethanol extracts of *H. odorattisimum* plants that were cultivated in aquaponic, hydroponic, and field systems

Treatments	**MIC ± SE (mg/ml)**
	18hours
T1	0.37± 0.00b
T2	0.56± 0.18a
T3	0.75± 0.00b
Dithane (Positive control)	0.75 ± 0.00a
Negative control	0a

Values shown are mean S.E. Means followed by the same lowercase letters in the same column are not significantly different ($p > 0.05$) following the Mann–Whitney test comparison. T1, aquaponic; T2, hydroponic; T3, field

3.4 Discussion

Many previous studies have demonstrated the promising prospects of using aquaponics and hydroponics to cultivate vegetables sustainably (Graber and Junge, 2009; Pantanella *et al*., 2010; Li *et al.*, 2018; Obirikorang *et al*., 2021) in addition, the current results suggest that aquaponics and hydroponics can also be used to produce high-quality medicinal plants (Bakiu and Julian, 2014). This study's findings show that aquaponics slightly outperformed hydroponics in plant growth (plant height, fresh and dry weight), although there were no significant differences between the two cultivation methods. These results agree with a previous study, which showed that the dry weights of *Solanum lycopersicum L.* did not significantly differ between organic and aquaponic cultivation methods (Braglia *et al*., 2022). Earlier, Ranawade *et al*. (2017) studied spinach yields in hydroponic, aquaponic, and traditional (soil-based cultivation) systems and found that aquaponically grown spinach had a higher yield than hydroponically and traditionally cultivated spinach. Additionally, Schmautz *et al*. (2016) used tomatoes to assess whether the mineral contents and nutritional quality in plants grown in aquaponically and conventionally grown tomatoes were similar; the aquaponically grown plants were equivalent or superior to conventionally grown tomatoes. Graber and Junge (2009) also reported that the plant yield of an aquaponic system was similar to conventional hydroponic production systems for three crops, aubergine, tomato, and cucumber. However, in an earlier study, Roosta and Ghorbani (2011) reported that hydroponics outperformed aquaponics in many of the assessed growth parameters for two species (*Mentha sativa* and *Mentha piperita*). These authors suggested the growth of plants in aquaponic systems was likely slowed down compared to hydroponic systems because of lower concentrations of critical nutrients, such as Mn and Mg, in *Mentha sativa* shoots, and N, P, Mg, and Mn in *Mentha piperita*.

In this study, we found no clear association between the tissue nutrient contents and the plant growth parameters. The tissue nutrient contents of C, Ka, and Ca were not significantly different between hydroponic and aquaponic plant leaves; however, the tissue macronutrient contents of N, P, and Mg were significantly higher in the hydroponic plants. Previous reports suggest that despite lower concentrations of most tissue nutrients in the aquaculture water, aquaponic plant growth results were comparable to those of hydroponics, and production can be even better than in soil cultivation (Graber and Junge 2009; Goddek *et al*., 2015; Bittsanszky *et al*., 2016; Delaide *et al*., 2016). In a recent study, researchers found that plants grown in

aquaponic systems grow at the same rate or even faster than conventionally or field-grown plants (Nuwansi *et al*., 2019). This result is not unexpected given that plants have optimal thresholds for each nutrient, beyond which growth may not be positively affected. Tissue macronutrient levels in *H. odoratissimum* plants in the hydroponic system were higher for N, P, and Mg. However, this was expected because hydroponic systems have readily available nutrients in the nutrient-rich medium, unlike aquaponic systems. However, concerning aquaponic performance in terms of tissue macronutrient contents in plants, it has been proven that aquaponic systems can generate comparable concentrations of K, Ca, and Mg (Pantanella *et al*., 2010). These elements are essential elements for plant growth (Pantanella *et al*., 2010).

The aquaponic system outperformed the hydroponic system in many of the plant leaf tissues' essential micronutrients (Mn, Cu, and Zn) (Table 3.3). Perhaps the high concentrations of these nutrients in the aquaponics plants could be due to the high concentration of these elements in fish feed, which is routinely dissolved in the water, and then absorbed by the plants. According to Palm *et al*. (2018), at least 50% of the nutrients in an aquaponic system come from uneaten fish feed and solid and soluble fish excretions; hence, monitoring the nutrient concentrations available for plant absorption is complex but critical. Future studies should continuously monitor the concentrations of minerals in the aquaponics system to establish the efficiency of nutrient cycling.

When compared with aquaponic and field-collected plants, hydroponic plants had higher polyphenol content; however, there were no significant differences among the treatments. Plants in the hydroponics yielded considerably higher total flavonol content than the aquaponic plants and field plants, but they were also not significantly different. Broadly, these results demonstrate that aquaponics and hydroponics perform equally or even better in the case of hydroponics, than field-cultivated plants. Research has revealed that nutrient availability can significantly influence plants' secondary metabolism and antioxidant activity (Yang *et al*., 2018). While in the current study, the high levels of Cu, Zn, and Mn in aquaponics did not positively influence the secondary metabolites, the hydroponic plants had higher N, P, and Mg, and higher flavonol contents. Previously, it has been demonstrated that these macronutrients in higher levels influence secondary metabolite production. For example, Ibrahim *et al*. (2010) reported that nitrogen levels significantly affected the production of total phenolics and flavonoids in *Labisia pumila* Benth; total phenolics and flavonoids were reduced with increased concentration increased progressively.

The essential oil profiles of *H. odoratissimum* have been studied extensively, revealing that these species produce a complex bouquet of vegetative and floral volatiles (Asekun *et al*., 2007). Several essential oil products derived from Helichrysum sp. are available for medical and non-medicinal uses on the market commercially. Although there was no statistical difference in the total number of volatile constituents in the three cultivation strategies, it is

worth mentioning that alpha-terpineol, a potent antioxidant and antifungal agent, occurred in higher concentrations in the aquaponic plants (Kuiate *et al*., 1999; Baldissera *et al.*, 2016). Alpha-terpineol can cause leakage of the cytoplasm and serious hyphae distortions and spore disruptions in *Aspergillus ochraceus* (Khaleel *et al*., 2018; Kong *et al*., 2019). They are used as a pesticide substitute in plants because of their safety and efficiency. Other important compounds that occurred in significantly higher levels in aquaponic plants than in the hydroponic and field-collected plants included alpha-phellandrene, o-ethyltoluene, tetradecane, alpha-curcumene, and palustrol. Alpha-curcumene, isolated from the fresh aerial parts of *Senecio selloi* Spreng. D.C had high antifungal activity against Enterobacter cloacae (Silva *et al*., 2021). In previous research, monoterpenes and sesquiterpenes. Generally, monoterpene and sesquiterpene hydrocarbons dominated the essential oil of *H. odoratissimum*. Monoterpenes, sesquiterpenes, and diterpenes are some of the broad groups of compounds present in the essential oils of most plants, including the Helichrysum genus; these compounds are primarily responsible for the reported antifungal, antibacterial, antidiabetic, anti-inflammatory, antiulcer, anticancer, antioxidant, antinociceptive, and antispasmodic properties associated with these plants (Judzentiene and Butkiene, 2006). The beauty and pharmaceutical industries extensively use alpha-terpineol (-terpineol), a monoterpenoid alcohol which was also confirmed in this study (Zhang *et al*., 2019). Another significant sesquiterpene found in a variety of plant essential oils known as beta-caryophyllene has been linked to several significant pharmacological effects, including immune-modulating, anti-inflammatory, anti- cancer, cardioprotective, hepatoprotective, gastroprotective, and renal protective effects (Machado *et al*., 2018). Interestingly, the same compound was confirmed in the current study.

The antioxidant capacity of leaves from *H. odoratissimum* cultivated via hydroponics was significantly higher ($p < 0.05$; ABTS) than plants grown in the field. Although the higher antioxidant activities were produced by hydroponic plants than aquaponic plants, the differences were not significant. Flavonols are important antioxidants in reducing oxidative damage and have potent radical scavenging abilities (Hossain *et al*., 2006; Makris *et al*., 2008; Martínez-Lüscher *et al*., 2019). Many studies on *Helichrysum* species have reported the association between flavonol and antioxidant activities (Lourens *et al*., 2008). The relationship between flavonol and antioxidant activities is well known; for example, Braglia *et al*. 2021 reported that both total phenolic content (7.25 versus 6.11 mg GAEq g^{-1} DW) and antioxidant capacity (28.04 versus 20.33 mol AAEq g^{-1} DW) were significantly higher ($p < 0.001$) in aquaponic basil compared to organic soil-grown crops. On the antifungal activity, the MIC findings of this research demonstrated that aquaponic plants had significantly higher inhibition of *F. oxysporum* growth after 18 h of incubation. The reason for the higher antifungal activity in aquaponic plants' extracts is unclear, given the lower polyphenol and flavonol contents obtained in this study. However, it is worth noting that higher concentrations of volatile

compounds, such as alpha-curcumene and alpha- terpineol, with proven antifungal activity, occurred in the aquaponics plants. Additionally, these findings corroborate those from a comprehensive study of tea leaves that evaluated the link between tissue nutrient contents and secondary metabolite contents and concluded that increases in N, P, or K beyond a target value resulted in decreases in secondary metabolite concentrations (Sun *et al*., 2019).

3.5 Conclusions

The key findings of this study reveal that *H. odoratissimum* plants cultivated via aquaponics exhibited the best antifungal activity, while hydroponically cultivated plants yielded the highest total flavonol content and antioxidant activities of the plant extracts. The results also showed that the tissue nutrient contents varied with cultivation method. Lastly, based on the chemicals identified from GC–MS analysis, aquaponic, hydroponic, and field plants yielded the same number of compounds. Based on these findings, aquaponics and hydroponics are viable alternative methods for cultivating medicinal plants. Future studies should include the economic viability of cultivating medicinal plants using these two methods.

3.6 References

Asekun, O.T., Grierson, D.S., Afolayan, A.J., 2007. Characterization of Essential Oils from *Helichrysum odoratissimum* Using Different Drying Methods. *Journal of Applied Sciences*, *7*, pp.1005–1008.

Bakiu, R. and Shehu, J., 2014. Aquaponic systems as excellent agricultural research instruments in Albania. *Albanian Journal of Agricultural Sciences*, p.385.

Baldissera, M.D., Souza, C.F., Grando, T.H., Sagrillo, M.R., De Brum, G.F., Nascimento, K., Peres, D.S., Maciel, M.F., Silveira, S.O., Da Luz, S.C. and Doleski, P.H., 2016. Memory deficit, toxic effects and activity of Na+, K+-ATPase and NTPDase in brain of Wistar rats submitted to orally treatment with alpha-terpinene. *Environmental Toxicology and Pharmacology*, *46*, pp.1–8.

Barea, J.M., 2015. Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. *Journal of soil science and plant nutrition*, *15*(2), pp.261–282.

Benzie, I.F. and Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1), pp.70–76.

Bernhoft, A., 2010. A brief review on bioactive compounds in plants. *Bioactive compounds in plants-benefits and risks for man and animals*, *50*, pp.11–17.

Bittsanszky, A., Uzinger, N., Gyulai, G., Mathis, A., Junge, R., Villarroel, M., Kotzen, B. and Kőmíves, T., 2016. Nutrient supply of plants in aquaponic systems. *Ecocycles*, *2*(2), pp.17–20.

Boily, Y. and Van Puyvelde, L., 1986. Screening of medicinal plants of Rwanda (Central Africa) for antimicrobial activity. *Journal of Ethnopharmacology*, *16*(1), pp.1–13.

Braglia, R., Costa, P., Di Marco, G., D'Agostino, A., Redi, E.L., Scuderi, F., Gismondi, A. and Canini, A., 2022. Phytochemicals and quality level of food plants grown in an aquaponics system. *Journal of the Science of Food and Agriculture*, *102*(2), pp.844–850.

Campbell, C.R. and Plank, C.O., 1998. Preparation of plant tissue for laboratory analysis. *Methods for Plant Analysis*, *37*.

Chen, P., Zhu, G., Kim, H.J., Brown, P.B. and Huang, J.Y., 2020. Comparative life cycle assessment of aquaponics and hydroponics in the Midwestern United States. *Journal of Cleaner Production*, *275*, p.122888.

Delaide, B., Goddek, S., Gott, J., Soyeurt, H. and Jijakli, M.H., 2016. Lettuce (*Lactuca sativa* L. var. *Sucrine*) growth performance in complemented aquaponic solution outperforms hydroponics. *Water*, *8*(10), p.467.

Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimal

inhibitory concentration of plant extracts for bacteria. *Planta medica*, *64*(08), pp.711–713.

Espinoza, F., Vidal, S., Rautenbach, F., Lewu, F. and Nchu, F., 2019. Effects of *Beauveria bassiana* (*Hypocreales*) on plant growth and secondary metabolites of extracts of hydroponically cultivated chive (*Allium schoenoprasum* L. [*Amaryllidaceae*]). *Heliyon*, *5*(12), p.e03038.

Frum, Y. and Viljoen, A.M., 2006. In vitro 5-lipoxygenase activity of three indigenous South African aromatic plants used in traditional healing and the stereospecific activity of limonene in the 5-lipoxygenase assay. *Journal of Essential Oil Research*, *18*(sup1), pp.85– 88.

Goddek, S., Delaide, B., Mankasingh, U., Ragnarsdottir, K.V., Jijakli, H., Thorarinsdottir, R., 2015. Challenges of sustainable and commercial aquaponics. *Sustainability*, *7*, pp.4199– 4224.

Goddek, S., Joyce, A., Kotzen, B. and Burnell, G.M., 2020. Correction to: Aquaponics Food Production Systems. In *Aquaponics Food Production Systems* (pp.C1–C1). Springer, Cham.

Graber, A. and Junge, R., 2009. Aquaponic Systems: Nutrient recycling from fish wastewater by vegetable production. *Desalination*, *246*(1–3), pp.147–156.

Griesser, M., Weingart, G., Schoedl-Hummel, K., Neumann, N., Becker, M., Varmuza, K., Liebner, F., Schuhmacher, R. and Forneck, A., 2015. Severe drought stress is affecting selected primary metabolites, polyphenols, and volatile metabolites in grapevine leaves (*Vitis vinifera cv. Pinot noir*). *Plant Physiology and Biochemistry*, *88*, pp.17–26.

Hammer, Ø., Harper, D.A. and Ryan, P.D., 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia electronica*, *4*(1), p.9.

Hanjra, M.A. and Qureshi, M.E., 2010. Global water crisis and future food security in an era of climate change. *Food policy*, *35*(5), pp.365 –377.

Hossain, M.A., Salehuddin, S.M. and Ismail, Z., 2006. Rosmarinic acid and methyl rosmarinate from *Orthosiphon stamineus* Benth. *Journal of Bangladesh Academy of Sciences*, *30*(2), p.167.

Ibrahim, M.H., Jaafar, H.Z., Rahmat, A. and Rahman, Z.A., 2010. The relationship between phenolics and flavonoids production with total non-structural carbohydrate and photosynthetic rate in *Labisia pumila* Benth. under high CO2 and nitrogen fertilization. *Molecules*, *16*(1), pp.162–174.

Jousse, C., Vu, T.D., Le Minh Tran, T., Al Balkhi, M.H., Molinié, R., Boitel-Conti, M., Pilard, S., Mathiron, D., Hehn, A., Bourgaud, F. and Gontier, E., 2010. Tropane alkaloid profiling of hydroponic *Datura innoxia* Mill. plants inoculated with Agrobacterium rhizogenes. Phytochemical Analysis*: An International Journal of Plant Chemical and Biochemical Techniques*, *21*(1), pp.118–127.

Judzentiene, A. and Butkiene, R., 2006. Chemical composition of the essential oils of wild *Helichrysum arenarium* (L.) with differently colored inflorescences from Eastern Lithuania. *Journal of essential oil Research*, *18*(1), pp.80– 83.

Khaleel, C., Tabanca, N. and Buchbauer, G., 2018. α-Terpineol, a natural monoterpene: A review of its biological properties. *Open Chemistry*, *16*(1), pp.349– 361.

Kong, Q., Zhang, L., An, P., Qi, J., Yu, X., Lu, J. and Ren, X., 2019. Antifungal mechanisms of α-terpineol and terpene-4-alcohol as the critical components of Melaleuca alternifolia oil in the inhibition of rot disease caused by *Aspergillus ochraceus* in postharvest grapes. *Journal of applied microbiology*, *126*(4), pp.1161–1174.

Kuiate, J.R., Amvam Zollo, P.H., Nguefa, E.H., Bessière, J.M., Lamaty, G. and Menut, C., 1999. Composition of the essential oils from the leaves of Microglossa pyrifolia (Lam.) O. Kuntze and *Helichrysum odoratissimum* (L.) Less. growing in Cameroon. *Flavour and fragrance journal*, *14*(2), pp.82–84.

Lahlali, R., Ezrari, S., Radouane, N., Kenfaoui, J., Esmaeel, Q., El Hamss, H., Belabess, Z. and Barka, E.A., 2022. Biological control of plant pathogens: A global perspective. *Microorganisms*, *10*(3), p.596.

Lall, N. and Meyer, J.J.M., 1999. In vitro inhibition of drug-resistant and drug-sensitive strains of Mycobacterium tuberculosis by ethnobotanically selected South African plants. *Journal of Ethnopharmacology*, *66*(3), pp.347–354.

Legoale, P.B., Mashimbye, M.J., van Ree, T., 2013. Antiinflammatory and antioxidant flavonoids from *Helichrysum kraussii* and *H. odoratissimum* flowers. *Natural Product. Communications*, *8*, pp.1403–1404.

Léonhart, S., Pedneault, K., Gosselin, A., Angers, P., Papadopoulos, A.P. and Dorais, M., 2000, November. Diversification of greenhouse crop production under supplemental lighting by the use of new cultures with high economic potential. In *IV International ISHS Symposium on Artificial Lighting 580* (pp. 249–254).

Li, Y., Kong, D., Fu, Y., Sussman, M.R. and Wu, H., 2020. The effect of developmental and environmental factors on secondary metabolites in medicinal plants. *Plant Physiology and Biochemistry*, *148*, pp.80– 89.

Lourens, A.C.U., Viljoen, A.M. and Van Heerden, F.R., 2008. South African *Helichrysum* species: a review of the traditional uses, biological activity and phytochemistry. *Journal of Ethnopharmacology*, *119*(3), pp.630– 652.

Machado, K.D.C., Islam, M.T., Ali, E.S., Rouf, R., Uddin, S.J., Dev, S., Shilpi, J.A., Shill, M.C., Reza, H.M., Das, A.K. and Shaw, S., 2018. A systematic review on the neuroprotective perspectives of beta-caryophyllene. *Phytotherapy Research*, *32*(12), pp.2376– 2388.

Maggini, R., Kiferle, C. and Pardossi, A., 2014. Hydroponic production of medicinal plants. *Medicinal plants: Antioxidant properties, traditional uses and conservation strategies. Nova Science Publishers, Inc., Hauppauge, NY*, pp.91–116.

Makris, D.P., Kallithraka, S. and Kefalas, P., 2006. Flavonols in grapes, grape products and wines: Burden, profile and influential parameters. *Journal of Food Composition and Analysis*, *19*(5), pp.396– 404.

Maroyi, A., 2017. Diversity of use and local knowledge of wild and cultivated plants in the Eastern Cape province, South Africa. *Journal of Ethnobiology and Ethnomedicine*, *13*(1), pp.1–16.

Martínez-Lüscher, J., Brillante, L. and Kurtural, S.K., 2019. Flavonol profile is a reliable indicator to assess canopy architecture and the exposure of red wine grapes to solar radiation. *Frontiers in plant science*, *10*, p.10.Mathekga, A.D.M., 2004. Antimicrobial Activity of *Helichrysum* Species and the Isolation of a New Phloroglucinol from *Helichrysum caespititium*. Ph.D. Dissertation, University of Pretoria, Pretoria, South Africa.

Matrose, N.A., Obikeze, K., Belay, Z.A. and Caleb, O.J., 2021. Impact of spatial variation and extraction solvents on bioactive compounds, secondary metabolites and antifungal efficacy of South African Impepho [*Helichrysum odoratissimum* (L.) Sweet]. *Food Bioscience*, *42*, p.101139.

Matrose, N.A., Obikeze, K., Belay, Z.A., Caleb, O.J., 2021. Plant extracts and other natural compounds as alternatives for post-harvest management of fruit fungal pathogens: A review. *Food Biosciences*, *41*, p.100840. Miller, N.J., Rice-Evans, C., Davies, M.J., 1993. A new method for measuring antioxidant activity. *Biochemical Society Transaction*, *21*, p.95.

Nchu, F., Aderogba, M.A., Mdee, L.K. and Eloff, J.N., 2010. Isolation of anti-Candida albicans compounds from Markhamia obtusifolia (Baker) Sprague (*Bignoniaceae*). *South African Journal of Botany*, *76*(1), pp.54 –57.

Nchu, F., Matanzima, Y. and Laubscher, C.P., 2018. Prospects of N fertilization in medicinal plants cultivation. *Nitrogen in Agriculture-Updates*, pp.209 –222.Ntobela, B., Oguntibeju, O.O., Rautenbach, F., Etsassala, N.G., Akinpelu, E.A. and Nchu, F., 2022. Evaluating the effect of 40% and 0% shading levels on the secondary metabolites, antifungal and anti-insect activities of extracts of *Allium porrum* cultivated hydroponically under greenhouse conditions. *Journal of Medicinal Plants for Economic Development*, *6*(1), p.9.

Nuwansi, K.K.T., Verma, A.K., Chandrakant, M.H., Prabhath, G.P.W.A. and Peter, R.M., 2021. Optimization of stocking density of koi carp (*Cyprinus carpio var. koi*) with gotukola (*Centella asiatica*) in an aquaponic system using phytoremediated aquaculture wastewater. *Aquaculture*, *532*, p.735993.

Nuwansi, K.K.T., Verma, A.K., Rathore, G., Prakash, C., Chandrakant, M.H. and Prabhath, G.P.W.A., 2019. Utilization of phytoremediated aquaculture wastewater for production of koi carp (*Cyprinus carpio* var. koi) and gotukola (*Centella asiatica*) in an aquaponics. *Aquaculture*, *507*, pp.361–369.Obirikorang, K.A., Sekey, W., Gyampoh, B.A., Ashiagbor, G., Asante, W., 2021. Aquaponics for improved food security in Africa: A review. *Frontiers in Sustainable Food Systems*, *5*, p.705549.

Olatunji, T.L. and Afolayan, A.J., 2019. Comparative quantitative study on phytochemical contents and antioxidant activities of *Capsicum annuum* L. and *Capsicum frutescens* L. *The Scientific World Journal*, *2019*.

Palm, H.W., Knaus, U., Appelbaum, S., Goddek, S., Strauch, S.M., Vermeulen, T., Haïssam Jijakli, M. and Kotzen, B., 2018. Towards commercial aquaponics: a review of systems, designs, scales and nomenclature. *Aquaculture international*, *26*(3), pp.813– 842.

Pantanella, E., Cardarelli, M., Colla, G., Rea, E. and Marcucci, A., 2010, August. Aquaponics vs. hydroponics: production and quality of lettuce crop. In *XXVIII International Horticultural Congress on Science and Horticulture for People (IHC2010): International Symposium on 927* (pp. 887–893).

Pardossi, A., Malorgio, F., Incrocci, L., Tognoni, F., 2006. Hydroponic technologies for greenhouse crops. In *Crops: Quality, Growth and Biotechnology*; Dris, R., Ed.; WFL Publisher, pp. 360 –378.

Pezzuto, J.M., 1995. Natural product cancer chemopreventive agents. In *Phytochemistry of medicinal plants* (pp. 19– 45). Springer, Boston, MA.

Polycarpou, P., Neokleous, D., Chimonidou, D. and Papadopoulos, I., 2005. A closed system for soil less culture adapted to the Cyprus conditions. *F. El Gamal, AN Lamaddalen, C. Bogliotti, and R. Guelloubi. Nonconventional water use*, pp.237–241.

Ranawade, P.S., Tidke, S.D. and Kate, A.K., 2017. Comparative cultivation and biochemical analysis of spinacia oleraceae grown in aquaponics, hydroponics and field conditions. *International Journal of Current Microbiology and Applied Science*, *6*(4), pp.1007–1013.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9–10), pp.1231–1237.Reddy, D., 2007. The Phytochemistry and Antimicrobial Activity of Selected Indigenous *Helichrysum* species. Ph.D. Dissertation, University of the Witwatersrand, Braamfontein, South Africa.

Roosta, H.R. and Ghorbani, F., 2011. Investigation of the growth and development, essential oil and minerals content in two species of mint in hydroponics and aquaponics. *Journal of Science and Technology of Greenhouse Culture*, *2*(7), pp.19– 28.

Santos, V.B., Araújo, A.S., Leite, L.F., Nunes, L.A. and Melo, W.J., 2012. Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems. *Geoderma*, *170*, pp.227–231.Schmautz, Z., Loeu, F., Liebisch, F., Graber, A., Mathis, A., Griessler Bulc, T. and Junge, R., 2016. Tomato productivity and quality in aquaponics: Comparison of three hydroponic methods. *Water*, *8*(11), p.533.

Seaman, T., 2006. The Antimicrobial and Antimycobacterial Activity of Plants Used for the Treatment of Respiratory Ailments in Southern Africa and the Isolation of Anacardic Acid from *Ozoroa paniculosa*. Ph.D. Dissertation, University of the Witwatersrand, Johannesburg,

South Africa.Serabele, K., Chen, W., Tankeu, S., Combrinck, S., Veale, C.G., van Vuuren, S., Chaudhary, S.K. and Viljoen, A., 2021. Comparative chemical profiling and antimicrobial activity of two interchangeably used 'Imphepho'species (*Helichrysum odoratissimum* and *Helichrysum petiolare*). *South African Journal of Botany*, *137,* pp.117–132.

Silva, G.N.S.D., Pozzatti, P., Rigatti, F., Hörner, R., Alves, S.H., Mallmann, C.A. and Heinzmann, B.M., 2021. Antimicrobial evaluation of sesquiterpene α-curcumene and its synergism with imipenem. *Journal of Microbiology, Biotechnology and Food Sciences*, *2021*, pp.434436.Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M., 1999. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. In *Methods in enzymology* ,*299*, pp. 152–178. Academic press.

Stadler, M.M., Baganz, D., Vermeulen, T. and Keesman, K.J., 2015. Circular economy and economic viability of aquaponic systems: Comparing urban, rural and peri-urban scenarios under Dutch conditions. *ICESC2015: Hydroponics and Aquaponics at the Gold Coast 1176*, pp.101–114.Stock, S.P., Kusakabe, A. and Orozco, R.A., 2017. Secondary Metabolites Produced by Symbionts and Their Application in Agriculture: What We Know and What to Do Next. J*ournal of Nematology, 49*(4), pp.373– 383.

Sun, L., Liu, Y., Wu, L. and Liao, H., 2019. Comprehensive analysis revealed the close relationship between N/P/K status and secondary metabolites in tea leaves. *Acs Omega*, *4*(1), pp.176–184.

Surendran, U., Raja, P., Jayakumar, M. and Subramoniam, S.R., 2021. Use of efficient water saving techniques for production of rice in India under climate change scenario: A critical review. *Journal of Cleaner Production*, *309*, p.127272.

Unuofin, J.O., Otunola, G.A. and Afolayan, A.J., 2017. Phytochemical screening and in vitro evaluation of antioxidant and antimicrobial activities of *Kedrostis africana* (L.) Cogn. *Asian Pacific journal of tropical biomedicine*, *7*(10), pp.901–908.

Van Puyvelde, L., De Kimpe, N., Costa, J., Munyjabo, V., Nyirankuliza, S., Hakizamungu, E. and Schamp, N., 1989. Isolation of flavonoids and a chalcone from *Helichrysum odoratissimum* and synthesis of helichrysetin. *Journal of Natural Products*, *52*(3), pp.629–633.Xego, S., Kambizi, L. and Nchu, F., 2017. Effects of different hydroponic substrate combinations and watering regimes on physiological and anti-fungal properties of *Siphonochilus aethiopicus. African Journal of Traditional, Complementary and Alternative Medicines*, *14*(3), pp.89–104.

Yang, L., Wen, K.S., Ruan, X., Zhao, Y.X., Wei, F. and Wang, Q., 2018. Response of plant secondary metabolites to environmental factors. *Molecules*, *23*(4), p.762.

Zhang, C., Li, M., Zhao, G.R. and Lu, W., 2019. Alpha-Terpineol production from an engineered Saccharomyces cerevisiae cell factory. *Microbial cell factories*, *18*(1), pp.1–9.

Chapter 4

Bioactivities of *Helichrysum cymosum* cultivated in aquaponic, hydroponic and field systems

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Abstract

Current reliance on wild harvesting of medicinal plants and intensive agriculture systems, which rely heavily on fertilizer and pesticide inputs, are unsustainable. Hence, three cultivation techniques, aquaponic, hydroponic, and field-harvested plants, were evaluated for crop yields, secondary metabolite contents and antifungal activities. The results showed that there was no significant difference between aquaponics and hydroponics treatments on crop yields (plant height, fresh and dry weight). Interestingly, there were significant differences (DF = 2; χ^2 = 19.76; p = 0.00) in total polyphenol contents between the three cultivation techniques; however, the field-collected plants yielded higher polyphenol contents (452.10 ± 53.37 mg GAE/g) than hydroponics (433.49 ± 11.95 mg GAE/g) followed by aquaponics (136.46 ± 42.09 mg GAE/g). The flavonol contents differed significantly between the three cultivation techniques (DF = 2; χ^2 = 6.31; p = 0.03), with higher flavonol contents in the field-collected plants (250.62 ± 58.12 mg QE/g), followed by hydroponics (164.05 ± 14.89 mg QE/g) and aquaponics (71.60 ± 14.45 mg QE/g). The volatile chemical contents did not differ significantly according to the gas chromatography-mass spectrometry (GC-MS) analysis (DF = 2; χ^2 = 3.53; p = 0.17; p > 0.05) among the three treatments. However, plants grown in aquaponics had a higher number of compounds (104) than those grown in hydroponics (102) or field plants (101). The antifungal bioassay showed that the ethanol extracts of *H. cymosum* harvested from the

field also had a higher fungistatic activity against *F. oxysporum*. The highest antioxidant capacity was obtained in plants cultivated in hydroponics followed by field-collected plants although there was no statistical difference among the treatments. The results of the present study suggest that commercial cultivation of *H. cymosum* using hydroponic and aquaponic systems may be feasible.

Key words: *Helichrysum cymosum*, *Asteraceae*, secondary metabolites, aquaponics, semi hydroponics.

4.1 Introduction

A growing number of citizens in developing countries including South Africa rely on medicinal plants for treating many diseases (Taylor *et al*., 2001). Many wild medicinal plants are under severe pressure due to over-harvesting and habitat degradation. The risks of extinction of many species are at an all-time high, warranting urgent interventions to achieve sustainable utilization of medicinal plant resources (Chen *et al*., 2016). Soilless cultivation is an innovative approach, which could limit the exploitation of some endangered medicinal plants from the wild. Numerous opportunities exist for the commercialization of medicinal plants in aquaponic and hydroponic systems (Nchu *et al*., 2018). As a production method that combines hydroponics and aquaculture, aquaponics is one of the most resource-effective plant cultivation methods (Stadler *et al*., 2015). Hydroponics is increasingly used to substitute typical agricultural soil cultivation (Pardossi *et al*., 2005). In hydroponics, plants are grown in a pure nutrient solution or substrate culture. Both aquaponics and hydroponics provide opportunities to manipulate nutrient availability, crop yield and quality.

Previous research studies have shown that cultivating medicinal plants under controlled conditions in aeroponic or hydroponic systems improve quality, bioactivity, and biomass output on a commercial scale (Polycarpou *et al*., 2005; Jousse *et al*., 2010). These approaches are particularly useful in areas where environmental stresses (cold, heat, desert) are significant (Polycarpou *et al*., 2005). They are also compatible with a variety of medicinal plant species and the viability, and advantages of these cultivation systems for the synthesis of secondary metabolites have been proven (Léonhart *et al*., 2000; Papadopoulos *et al*., 2001; Hyden, 2006; Maggini *et al*., 2014). However, few studies have compared the cultivation of indigenous medicinal plants in aquaponic, hydroponic and field systems.

The genus *Helichrysum* Mill. belongs to the *Asteraceae* family, which consists of roughly 600 different species, of which 250 are found in South Africa (Lourens *et al*., 2008). *Helichrysum cymosym* (L*)* D. Don is native to South Africa and is a member of the *Asteraceae* family. It is among the country's most sought-after medicinal species. The leaves are small, elliptic-oblong or linear-oblong in shape, with an acute, sometimes acuminate, apex, mucronate, somewhat

constricted, and clasping base (Heyman, 2013). *H. cymosum* is used to treat pain, coughs, colds, fever, headache, menstrual pains, wound dressing, and infection prevention (Hayman, 2013; Maroyi, 2019). Other ethnomedicinal uses of *H. cymosum* include treatment of a blocked nose, cardiovascular problems, diarrhea, dizziness, eye problems, flatulence, kidney problems, menstrual pain, pertussis, pulmonary problems, skin infections, urinary problems, varicose veins, vomiting, and weak bones, and boosting immune system (Hayman, 2013). The leaves, stems, and twigs of *H. cymosum* are sold as herbal medicines in the informal herbal medicine markets in the Gauteng and the Western Cape provinces in South Africa (Maroyi, 2019). The fragrance of the plant has long been used to treat respiratory and wound infections (Kutluk *et al*., 2018).

Essential oils from *H. cymosum* have antibacterial characteristics and could be used to treat tropical diseases such as malaria (Van Vuuren, 2006). Crude extracts of *H. cymosum* and compounds isolated from the species have been found to have antibacterial (Sindambiwe *et al*., 1999; Stafford and Van Staden, 2005; Maroyi, 2019; Van Vuuren *et al*., 2006; Van Vuuren, 2007), antioxidant (Tchoumbougnang, 2010), antifungal (Vuuren *et al*.,2006; Van Vuuren, 2007; Tchoumbougnang, 2010; Runyoro *et al*., 2010); antiviral (Sindambiwe *et al*., 1999), anti-HIV and cytotoxic (Heyman, 2009, Heyman *et al*., 2015), anti-inflammatory (Stafford and Van Staden, 2005), and antimalarial (Vuuren *et al*., 2006) effects. The antimicrobial properties of *H. cymosum* have made the plant to be one of South Africa's most sought-after medicinal plant species. Numerous compounds have been isolated from the alcoholic extract of the leaves and roots of *H. cymosum* including sesquiterpenes and chalcones (Jakupovic *et al*., 1989; Van Vuuren *et al*., 2006; Popoola *et al*., 2015). Several other classes of compounds have been discovered in *Helichrysum* species, including phenolics such as flavonoids, phthalides, -pyron derivatives, terpenoids, and fatty acids (Czinner *et al*., 2001).

This is the first study to compare the effects of cultivation methods, aquaponics, hydroponics and field methods on the growth, secondary metabolite contents and antifungal activity of *H. cymosum*.

4.2 Materials and methods

4.2.1 Research design

Four-week-old, rooted cuttings of *H. cymosum* were grown using two cultivation systems (hydroponic and aquaponic), demonstrating two treatments. The data on plant growth, secondary metabolite contents, and antifungal activities were obtained at the end of the experiment. The secondary metabolite contents and antifungal activities of plants obtained

from aquaponic, and hydroponic systems were compared with field-cultivated plants. *Helichrysum* seedlings were acquired from Shadowlands Wholesale Nursery Pty. Ltd. in Zevenwacht Link Road, Kuilsriver 7580, Western Cape. The plant specimens were mounted and placed in the Herbarium of the Department of Horticultural Sciences, Cape Peninsula University of Technology (CPUT), Bellville campus, Cape Town, South Africa. The roots were carefully cleaned and separated to eliminate potting soil debris. The plants were laid out on a cement floor and arranged in a completely randomized design inside the research greenhouse, where they were exposed to natural sunlight entering through the polycarbonate ceiling of the greenhouse.

4.2.2 Greenhouse experiment

The experiment was carried out in a greenhouse on the Bellville campus of the Cape Peninsula University of Technology (CPUT). Plants were cultivated using aquaponic as presented in Figure (4.1) and hydroponic techniques (Figure 4.2). For the hydroponic system, 4-week-old rooted seedlings of *H. cymosum* were transplanted into 23 cm diameter pots containing a substrate mix of two parts pine bark, one part perlite, and one part vermiculite. Fifteen replicates of *H. cymosum* seedlings were used. The plants were watered daily using 400 mL of deionized water and supplied with Nutrifeed fertilizer (Starke Ayres Pty. Ltd., Cape Town, South Africa), consisting of the following ingredients: Nitrogen (65 mg kg^{-1}), phosphorus (27 mg kg^{-1}), potassium (130 mg kg^{-1}), calcium (70 mg kg^{-1}), copper (20 mg kg^{-1}), sulfur (75 mg kg^{-1}), boron (240 mg kg^{-1}), magnesium (240 mg kg^{-1}), and zinc (240 mg kg^{-1}). The fertilizer was mixed with deionized water at a dosage of 10 g/5 L. Each plant received a volume of 100 mL of the nutritional solution fortnightly, with a pH of 6.5 and an EC value of 2 mS cm^{-1}, using Milwaukee EC 50 and pH 55 kits supplied by Spraytech Pty. Ltd., Cape Town, South Africa). A recirculating aquaponic system was used in the aquaponic system. The system consisted of a fish tank containing a pump and plant grow beds (four black 50 L plastic containers with perforated lids to fit the net pots). The aquaponic system had four fish tanks containing 400 L of water each and a submersible pump that pumped the wastewater (nutrient-rich water) from the tank to a grow bed through a PVC pipe. The grow bed had a deep culture design, which consisted of two black 50 L plastic containers with a perforated lid to fit the net pots. Fifteen *Helichrysum* seedlings were transplanted into net pots containing a mixture of perlite and coco coir (50:50 ratio) as substrate. The plants were watered from the bottom through the drain holes in the net pots immersed in the nutrient-rich water pumped from the fish tank. Recycled water from the grow bed returned to the fish tank. Thus, recycling of the nutrient water was continuous. An air pump (Regent 7500) connected to an air stone using tubing was used to improve dissolved oxygen at 150 L/H in the fish tank. Each grow bed had 15 seedlings fed from the same fish tank. Ten-to-fifteen-centimeter Goldfish fingerlings (*Carassius auratus*) and fish food (Koi and Goldfish powder, small pellets) supplied by Stodels Nursery Pty. Ltd.,

Doncaster Road, Kenilworth 7708, Western Cape, South Africa, were used in this study. Twenty Goldfish (*Carassius auratus*) were placed in each tank (1000 L capacity). The components of the fish meal were maize, rice, wheat, wheat germ, dehulled soybean meal, lysine, methionine, lime, dicalcium phosphate, vitamins (A, D, E, K, BI, B2, B6, B12), biotin, folic acid, inositol, minerals, colorants, spirulina, immune stimulants, vegetable fats, natural antioxidants, and betaine. The fish were fed twice daily at 08:30 am and 4:00 pm. The aquaponic setup was replicated four times. The experiment lasted for 6 weeks and at the end of the experiment, plant height (cm) and fresh and dry weights (g) of aquaponically and hydroponically produced plants were recorded and compared. However, the growth parameters of field plants were not assessed because the plants were already cultivated and established on the premises of the Bellville campus of CPUT before the commencement of the study. The harvested plant materials were used for tissue nutrient and secondary metabolite content analysis and were screened for antifungal activities. The leaves of randomly selected field *H. cymosum* plants were harvested, secondary metabolites were characterized, and antifungal activity was assayed. The greenhouse conditions were 15–26 °C and 74% RH. The EC level of the nutrient solution in the fish tank was 0.8 mS cm^{-1} and the pH was 6.3. The secondary metabolite contents and antifungal activity of plants obtained from the aquaponic, and hydroponic systems were compared with the field-cultivated plants.

Figure 4.1: Experimental setup of aquaponic system with two 400L of fish tanks

Figure 4.2: Hydroponics set-up with *H.cymosum* plants

4.2.3 Plant tissue analysis

Fresh aerial plant materials (leaves) obtained from the aquaponic, and hydroponic systems were sent to a certified commercial laboratory (Bemlab [Pty] Ltd in Somerset West, South Africa) for the analysis of macroand micro-elements. The aerial parts (leaves) of *H. cymocum* were washed with Teepol solution, rinsed with de-ionized water, and dried in an oven at 70 ℃ overnight. The dried leaves were then powdered and ashed at 480 ℃ for extraction using filter paper in a 50:50 HCl solution (Campbell and Plank, 1998). The concentrations of potassium (K), phosphorus (P), calcium (Ca), magnesium (Mg), Sodium (Na), manganese (Mn), iron (Fe), copper (Cu), zinc (Z), and boron (B) were measured in the extracts (Campbell and Plank, 1998 and Miller, 1998. Total combustion in a Leco N analyzer was used to determine the total nitrogen contents of the leaves. A conversion factor of 10000 was used to convert the amounts of N, P, K, Ca, and Mg from percentages to mg/kg (Xego *et al*.,2017). Three replicates from each treatment were analysed.

4.2.4 *In vitro* fungal screening using micro-dilution method

The microdilution method was used to assess the extracts' minimum inhibitory concentration (MIC) as described by Eloff (1998) and Nchu *et al*. (2010). Five grams of milled *H. cymosum* leaf materials from three replicates were extracted with 25 mL ethanol (analytical grade 99%) overnight, then filtered and evaporated and reformulated to produce a starting concentration

of 6 mg/mL. The extracts were diluted in ethanol and transferred to the first row of a 96-well microplate with wells containing 100 µL of sterile distilled water. Thereafter, the extracts were serially diluted twofold. A *Fusarium oxysporum* strain (UPFC no. 21) maintained at CPUT's Department of Horticultural Sciences was used in the microdilution assay. Fungal conidia obtained from stock agar plates were transferred to Nutrient Broth (Merck Pty. Ltd, Cape Town, South Africa) and incubated at 25 ℃ for four hours. One hundred microliters (100 µL) of conidial suspension (10^5 conidia/mL) were added to each of the 96 wells of the microplates containing the plant extract. Dithane (Stodels Nursery Pty. Ltd, Garden Centre, South Africa) (200 mg/25mL) was used as a positive control and the solvent (ethanol) as a negative control. Each microplate well was filled with 40 microliters (40 µL) of 0.2 mg/mL p-iodonitrotetrazolium chloride (INT) (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa) diluted in sterile distilled water, sealed in a plastic bag, and incubated at 37 ℃ and 100% RH. In the presence of fungus development, the colorless tetrazolium salt was reduced to a red- colored formazan product.

At 18 hours of incubation of the microtiter plates, the MIC values were recorded by visually comparing the pink colour of the wells. Three replicates of each treatment were used in the antifungal bioassay (MIC).

4.2.5 Determination of antioxidant activities (FRAP, ABTS and DPPH)

4.2.5.1 Ferric Reducing Antioxidant Powder (FRAP)

The FRAP assay was carried out according to Benzie and Strain's procedure (1996). In a 96-well microplate, 10 µL of the crude extract was combined with 300 µL FRAP reagent [0.3 µM acetate buffer, pH 3.6] (Saarchem, South Africa), 10 mM 2,4,6-tripyridyl-s-triazine (TPTZ) in 0.1 µM HCl (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa), 20 mM Iron (III) chloride hexahydrate (FeCl At 593 nm. As a standard, L-ascorbic acid (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa) was employed at concentrations ranging from 0 to 1000 µM. The absorbance was determined. The results were represented in milligrams of ascorbic acid equivalent per gram of dry weight (milligrams of AAE/g DW). Three replicates from each treatment were analysed.

4.2.5.2 2,2'-diphenylpicrylhydrazyl (DPPH) assay

The DPPH free radical scavenging activity of the tested samples was determined according to Katalinić *et al* (2004). A solution of 0.135 mM DPPH produced in a dark container was used to

create the DPPH radical (Olatunji and Afolayan, 2019). About 300 µL of DPPH solution were combined with 25 µL of the crude extract and graded concentrations (0 and 500 µM) of Trolox standard (6-Hydrox-2,5,7,8- tetramethylchroman-2- 20 carboxylic acid). After a 30-minutes of the incubation period, the absorbance at 517 nM was determined as µM/Trolox equivalent per gram dry weight (µM TE/g DW) to express the results.

4.2.5.3 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) ABTS Antioxidant capacity

The ABTS assay was carried out using the method described by Re *et al.* (1999). The solutions of 7 mM ABTS and 140 mM potassium–peroxodisulphate ($K_2S_2O_8$) (Merck, South Africa) were used as stock solutions. The working solution was then made by mixing 88 µL of $K_2S_2O_8$ with 5 µL of ABTS solution. The two solutions were thoroughly mixed and left to react at room temperature in the dark for 24 hours. The standard was Trolox (6-Hydrox-2,5,7,8-tetramethylchroman-2-20 carboxylic acid) at concentrations ranging from 0 to 500 µM. The crude extracts (25 µL) were allowed to react with 300 L of ABTS at room temperature for 30 minutes before being read in a plate reader at 734 nm at 25 ℃. The results were represented as µM/Trolox equivalent per gram dry weight (µM TE/g DW).

4.2.6 Secondary metabolite contents

4.2.6.1 Determination of total polyphenol and flavonol contents

The total polyphenol contents of dried *H. cymosum* samples (leaves) was determined using the Folin-Ciocalteu procedure (Singleton *et al*., 1999). Twenty-five microliters of aqueous extracts were mixed with 125 µL of Folin-Ciocalteu reagent (Merck Pty. Ltd., Cape Town, South Africa) in a 96-well microplate and diluted 1:10 with distilled water in a 96-well microplate. The well was filled with 100 µL of aqueous Na_2CO_3 (7.5%) after 5 min (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa). The plates were incubated for 2 hrs. at room temperature before being examined at 765 nm with a Multiskan plate reader (Thermo Electron Corporation, Waltham, Massachusetts, USA). The results were represented as mg gallic acid equivalents per gram dry weight (mg GAE/g DW) using 0, 20, 50, 100, 250, and 500 mg/L gallic acid in 10% ethanol (Singleton *et al*., 1999; Espinoza *et al*., 2019).

The total flavonol contents of dried leaves of *H. cymosum* plants was evaluated using a standard of quercetin 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol (Sigma Aldrich SA Pty. Ltd., Kempton Park, South Africa). A volume of 12.5 µL of crude aqueous extracts were combined

with 12.5 µL of 0.1 %HCl (Merck Pty. Ltd., Cape Town, South Africa) in 95% ethanol and 225 µL of 2% HCl in the sample wells, which were incubated at room temperature for 30 min. At a temperature of 25 ℃, the absorbance was measured at 360 nm. The results were represented in milligrams of quercetin equivalent per gram of dry weight (mg QE/g DW) (Espinoza *et al*., 2019). Three replicates from each treatment were analysed.

4.2.7 GC/MS analysis (Headspace) and secondary metabolite analysis

4.2.7.1 Sample preparation (Headspace of the extract)

Fresh plant materials (leaves) were taken off from both species and freeze-dried overnight at an -80 ℃ temperature. After that, 1 g was weighed into a solid phase micro-extraction (SPME) vial, along with 2 mL of 12% ethanol solution at pH 3.5 and 3 mL of 20 % NaCl solution. A Divinylbenzene/Carboxen/Polydimethylsiloxane (DVB/CAR/PDMS) SPME fibre was used to analyse the headspace of all the samples (grey). Leaf samples from three plants from each treatment were analysed.

4.2.7.2 Chromatographic separation

To determine the relative abundance of secondary metabolites, a method reported by Matrose *et al*. (2021) was used in the separation of volatile compounds using a gas chromatography (6890N, Agilent Technologies Network) coupled to an Agilent Technologies Inert XL/CI Mass Selective Detector Analytics PAL autosampler, and the separation of volatiles present in the samples was achieved using a polar ZB-WAX (30 m, 0.25 mm ID, at a flow rate of 1mL/min, helium was used as the carrier gas. With a 5:1 ratio, the injector temperature was kept at 250 ℃. The temperature of the oven was programmed as follows: 35 ℃ for 6 min, then 3 ℃/min to 70 ℃ for 5 min, then 4 ℃/min to 120 ℃ for 1 min, and lastly 20 ℃/min to 240 ℃ and maintained for 2.89 min. The Mass Selective Detector (MSD) was in full scan mode when the incident occurred.

4.2.8 Statistical analysis

The experimental data for the plant growth parameters (plant height, fresh and dry weight) tissue nutrient contents and secondary metabolite contents were analysed using the Kruskal Wallis test, at a significance level of $p < 0.05$. Furthermore, multiple comparisons of the means were carried out using the Mann-Whitney test. PAST was used to carry out these computations

(Hammer *et al*., 2001), and the number of volatiles in the aquaponics, hydroponics, and field plants were compared using the Pearson's Chi-Square test.

4.3 Results

4.3.1 Plant height

Aquaponics and hydroponics had no significant effect on the vegetative growth of *H. cymosum* plants (DF = 1; χ^2 = 1.63; p = 0.21) (Table 4.1). The results showed that the heights of plants grown in aquaponics and hydroponics did not differ substantially (DF = 1; 2 = 1.128; p > 0.05) (Table 4.1). However, the mean shoot lengths of hydroponic plants (26.46 cm) were higher than those of aquaponic plants (22.83 cm).

4.3.2 Fresh and dry weight

Plant height did not vary significantly between hydroponic and aquaponic plants (p > 0.05) (Table 4.1). There was a significant difference between the *H. cymosum* plants grown in aquaponics and hydroponics treatments, six weeks after treatment, when the fresh weights of the plants were compared (DF = 1; χ^2 = 3.85; p = 0.05). Hydroponics plants had significantly higher fresh weight (13.82 ± 0.7 g) than aquaponic plants (11.60 ± 0.80 g) (Table 4.1).

There were no significant differences (DF = 1; χ^2 = 3.75; p > 0.05) in dry weights between aquaponics and hydroponics grown *H. cymosum* plants, but the highest mean values were observed in hydroponics grown plants (7.23 ± 0.39g) (Table 4.1).

Table 4.1: Growth parameters of *H. cymosum* grown in aquaponics and hydroponics for six-weeks under greenhouse condition

Treatments	Plant height (cm)	Fresh weight (g)	Dry weight (g)
T1	22.83 ± 1.59a	11.60 ± 0.80a	6.04 ± 0.47a
T2	26.46 ± 0.94a	13.82 ± 0.71b	7.23 ± 0.39a

Values shown are mean ± S.E.

Means followed by the same lowercase letters in the same column are not significantly different (p > 0.05) following comparison using the Man Whitney test, T1, aquaponic; T2, hydroponic

4.3.3 Tissue analysis

4.3.3.1 Macronutrients

The plants cultivated in hydroponics had significantly higher macronutrient contents (P, K, and Mg) in the hydroponic plants than the aquaponic plants; P (DF = 1; χ^2 = 14.29; p = 0.01), K (DF = 1; χ^2 = 34.34; p < 0.05), and Mg (DF = 1; χ^2 = 34.68; p < 0.05) (Table 4.2). However, there were no significant differences in C, N, and Ca contents between aquaponics and semi-hydroponics plants (DF = 1; p > 0.05). In general, macronutrient intake was found to be higher in hydroponic (T2) plants (Table 4.2).

Table 4.2: Tissue nutrient contents of leaves of *H. cymosum* grown in aquaponics and hydroponics for six-weeks under greenhouse condition

Treatments	**Mean nutrient quantity ± SE (mg/kg)**					
	C	**N**	**P**	**K**	**Ca**	**Mg**
T1	445200 ± 13452.26a	14900 ± 1385.64a	2200 ± 230.94a	13150 ± 1991.86a	9800 ± 750.56a	1300 ± 115.47a
T2	447400 ± 3002.22a	14950 ± 259.80a	3100 ± 57.73b	24900 ± 230.94b	8950 ± 548.48a	2150 ± 86.60b

Values shown are mean ± S.E.

Means followed by the same lowercase letters in the same column are not significantly different ($p > 0.05$) following comparison using the Mann-Whitney test, T1, aquaponic; T2, hydroponic

4.3.3.2 Micronutrients

Tissue nutrient concentrations of Na (DF = 1; χ^2 = 21.65; p = 0.01), Cu: (DF = 1; χ^2 = 12.52; p = 0.02), Zn (DF = 1; χ^2 = 15.50; p= 0.01) varied significantly (Table 4.3), with higher levels occurring in aquaponic plants. In contrast, B was significantly lower (20.37 ± 2.51 mg/kg) in aquaponic grown plants. There was no significant difference in Mn and Fe uptake between aquaponics and hydroponics produced plants (p>0.05).

Table 4.3: Tissue nutrient contents of leaves of *H. cymosum* grown in aquaponics and hydroponics for six-weeks under greenhouse condition

Treatments	**Mean nutrient quantity ± SE (mg/kg)**					
	Na	**Mn**	**Fe**	**Cu**	**Zn**	**B**
T1	7425 ± 1082.53a	58.23 ± 4.19a	214 ± 30.02a	7.07 ± 0.66a	60.8 ± 1.27a	20.37 ± 2.51a
T2	2375 ± 77.94b	45.75 ± 3.20a	251.5 ± 23.96a	4.37 ± 0.38b	46.17 ± 3.49b	36.5 ± 2.60b

Values shown are mean ± S.E.

Means followed by the same lowercase letters in the same column are not significantly different ($p > 0.05$) following comparison using the Mann-Whitney test, T1, aquaponic; T2, hydroponic

4.3.4 Secondary metabolites (polyphenols and flavonols)

Plants harvested from the field and hydroponics had considerably higher total polyphenol contents in aerial part (leaves) of *H. cymosum* (DF = 2; χ^2 = 19.76; p = 0.00) (Table 4.4). Total flavonol contents varied significantly (DF = 2; χ^2 = 6.31; p = 0.03) between aquaponic and hydroponic treatments with higher contents occurring in field plants.

Table 4.4: Mean total polyphenol ± SE (mg GAE/g) and flavonol ± SE (mg GAE/g) contents of *H. cymosum* leaves cultivated in aquaponics, hydroponics and in the field at six weeks post-treatment

Treatments	Polyphenols (mg GAE/g)	Flavonols (mg QE/g)
T1	136.46 ± 42. 09a	71.60 ± 14. 45a
T2	433.49 ± 11.95ab	164.05 ± 14.89ab
T3	452.10 ± 53. 37b	250.62 ± 58. 12b

Values shown are mean ± S.E.

Means followed by the same lowercase letters in the same column are not significantly different (p > 0.05) following comparison using the Mann-Whitney test, T1, aquaponic; T2, hydroponic; and T3, field.

4.3.5 Analysis of volatile compounds

Extracts from *H. cymosum* shoots were analysed using gas chromatography-mass spectrometry. As shown in Table 4.5, compounds that matched the mass spectral library more than 90% were chosen, and a variety of volatile chemicals occurred in the species. Although more volatile chemicals (104) were found in aquaponics-grown plants than in field-grown (101) and hydroponics-grown (102) plants, the difference was not statistically significant using Pearson chi-square test (DF = 2; χ^2 = 3.53; p = 0.17). The compounds detected included some compounds that are known for antifungal and antioxidant activities such as nonadecane, 1-octen-3-ol, beta-fencyl acetate, benzaldehyde, alpha-humulene, linalyl propanoate, acoradiene, beta-himachalene, alpha-cedrene, alpha-ced 2,7-dimethyl-1,6-octadiene, trans-

(+)-carveol, (-)-phyllocladene, and cyclooctanone were abundant in aquaponic-produced plants when compared to hydroponic- and field-collected plants.

Table 4.5: Volatile compounds in *H. cymosum* plants grown under field, aquaponics and hydroponic.

Compounds	**Aquaponics** **Mean peak area in the chromatogram ± SE**	**hydroponics** **Mean peak area in the chromatogram ± SE**	**Field plants** **Mean peak area in the chromatogram ± SE**	**Retention times**
decane	1.26 ± 0.025a	0.83 ± 0.02b	1.17± 0.05a	5.48
alpha-pinene	12.53 ± 2.18a	24.33 ± 6.09a	20.06 ± 0.78a	5.79
nonadecane	0.51± 0.01b	0.19± 0.01a	0.20 ± 0.03a	6.68
camphene	0.35 ± 0.04a	0.11± 0.01b	0.25 ± 0.00a	7.01
4-methyl-octane	4.34 ± 0.08b	1.19 ± 0.43a	1.64 ± 0.08a	7.38
beta-pinene	4.34 ± 0.08b	1.22 ± 0.42a	1.64 ± 0.08a	8.13
undecane	0.55 ± 0.02a	0.57 ± 0.01a	0.41 ± 0.00b	8.34
alpha-phellandrene	0.51 ± 0.03a	0.36 ± 0.05a	0.00 ± 0.00b	8.47
myrcene	0.90 ± 0.02c	0.21± 0.05a	0.50 ± 0.03b	9.59

alpha-terpinene	0.77 ± 0.15a	0.52 ± 0.05a	0.41 ± 0.03a	10.9
limonene	2.77 ± 0.05a	0.90 ± 0.16b	2.49 ± 0.19a	11.9
beta-phellandrene	0.89 ± 0.03c	0.51 ± 0.07b	0.13 ± 0.00a	12.28
1,8-cineole_(eucalyptol)	57.177 ± 3.68b	31.28 ± 8.85a	31.28 ± 8.85ab	12.9
o-ethyltoluene	0.14 ± 0.01ab	0.00 ± 0.00a	0.24 ± 0.07b	12.19
cis-ocimene	6.49 ± 1.07b	2.01 ± 0.41a	4.86 ± 0.20ab	14.17
gamma-terpinene	2.45 ± 1.22a	0.02 ± 0.01a	1.41 ± 0.05a	11.87
styrene	2.65 ± 0.04c	0.89 ± 0.10a	2.09 ± 0.06b	14.72
trans-beta-ocimene	2.70 ± 0.06c	0.88 ± 0.10a	2.22 ± 0.12b	14.72
para-cymene	3.32 ± 0.16a	14.76 ± 4.69a	9.16 ± 0.87a	15.24
1,2,3-trimethylbenzene	–	–	0.62 ± 0.06b	9.13
alpha-fenchene	0.20 ± 0.05a	0.11 ± 0.01a	0.52 ± 0.06b	15.66
alpha-terpinolene	0.23 ± 0.12a	0.10 ± 0.03a	0.40 ± 0.02a	15.76
cyclohexanone	0.82 ± 0.02b	0.35 ± 0.03a	3.89 ± 0.12c	15.76

2,6,6-trimethylcyclohexanone	0.17± 0.09a	–	0.01± 0.00a	15.99
cis-3-hexenyl_acetate	0.44 ± 0.02b	–	–	
trans-2-heptenal	0.43 ± 0.01c	0.16 ± 0.01b	0.01 ± 0.01a	11.6
6-methyl-5-hepten-2-one	1.02 ± 0.01a	0.88 ± 0.15a	0.69 ± 0.02a	17.41
allo-ocimene	0.94 ± 0.08a	2.71 ± 0.15b	0.55 ± 0.06a	19.19
octenyl_acetate	–	–	–	–
cis-3-hexenol	0.16 ± 0.00c	0.08 ± 0.00b	0.04 ± 0.00a	19.74
4-methyl-1,5-heptadiene	2.09 ± 0.00c	0.83 ± 0.08b	0.19 ± 0.07a	20
3-octanol_Istd	– (20.19)	– (20.19)	– (20.19)	
3-ethyl-o-xylene	0.26 ± 0.03b	0.11 ± 0.00a	0.08 ± 0.01a	20.83
para-cymenyl	0.40 ± 0.08b	1.85 ± 0.17a	2.02 ± 0.23a	20.96
tetradecane	2.48 ± 0.26a	2.11 ± 0.22a	2.34 ± 0.30a	21.05
1-octen-3-ol	4.07 ± 0.79b	1.59 ± 0.16a	1.12 ± 0.07a	21.74
beta-fencyl_acetate	3.79 ± 0.79b	0.84 ± 0.44a	0.03 ± 0.01a	21.9

6-methyl-5-hepten-2-ol	0.30 ± 0.00a	0.27 ± 0.08a	0.31 ± 0.08a	22.12
2,5-dimethyl-p-xylene	0.51 ± 0.03a	0.26 ± 0.01b	0.41 ± 0.02a	22.28
alpha-ylangene	0.45 ± 0.03b	0.09 ± 0.04a	0.06 ± 0.00a	22.63
Italicene	0.01 ± 0.00b	0.00 ± 0.00a	0.00 ± 0.00a	22.99
benzaldehyde	1.19 ± 0.08b	0.70 ± 0.07a	0.48 ± 0.05a	23.16
allyl_isopentanoate	0.16 ± 0.02a	0.03 ± 0.01a	0.16 ± 0.09a	23.66
gamma-curcumene	0.43 ± 0.10b	– (20.05)	0.22 ± 0.03ab	20.05
l-linalool	0.14 ± 0.01a	0.05 ± 0.01b	0.14 ± 0.01a	24.32
alpha-copaene	0.37 ± 0.01b	0.07 ± 0.01a	0.05 ± 0.01a (24.37)	24.37
cis-sabinene_hydrate	0.28 ± 0.01c	0.08 ± 0.02b	0.00 ± 0.00a	24.63
e, e-alpha-farnesene	0.59 ± 0.17ab	0.56 ± 0.05a	1.13 ± 0.11b	24.96
fenchol	1.96 ± 0.04a	1.65 ± 0.07a	2.78 ± 0.30b	25.13
beta-caryophyllene	115.48 ± 24.27a	76.78 ± 7.70a	90.04 ± 13.21a	25.71
(+)-aromadendrene	1.49 ± 0.82a	32.11 ± 18.52a	0.23 ± 0.05a	25.8

delta-elemene	0.51 ± 0.08ab	0.78 ± 0.11b	0.34 ± 0.01a	25.84
(-)-Isoledene	0.05 ± 0.03a	0.05 ± 0.00a	0.20 ± 0.00a	26.59
ethyl-caprate	2.31 ± 0.94a	0.336 ± 0.07a	0.01 ± 0.00a	26.87
pinocarveol	0.62 ± 0.12a	0.36 ± 0.06a	0.78 ± 0.14a	27.04
gamma-elemene	0.64 ± 0.02a	0.22 ± 0.01a	5.44 ± 0.51b	27.11
alpha-humulene	8.50 ± 1.21b	2.44 ± 0.30a	5.23 ± 0.42 ab	27.74
linalyl-propanoate	1.85 ± 0.15b	0.75 ± 0.23a	1.42 ± 0.13ab	27.81
Alpha-humulene	6.11 ± 3.05a	0.23 ± 0.11a	–	20.23
acoradiene	0.32 ± 0.04b	0.05 ± 0.02a	0.03 ± 0.01a	27.91
1,8-menthadien-4-ol	0.00 ± 0.01a	0.00 ± 0.00a	0.00 ± 0.00a	28.09
beta-himachalene	19.27 ± 2.15b	10.87 ± 0.70a	5.79 ± 1.18a	28.14
beta-himachalene	18.98 ± 2.06b	10.59 ± 0.74a	5.64 ± 1.27a	23.75
alpha-terpineol	18.98 ± 2.06b	10.59 ± 0.74a	16.19 ± 1.01ab	19.05

ledene	8.45 ± 4.00ab	0.20 ± 0.02a	16.26 ± 1.18b	28.65
(+)-2-carene	0.37 ± 0.21a	–	0.00 ± 0.00a	28.73
valencene	3.61 ± 1.11a	1.09 ± 0.18a	1.00 ± 0.01a	24.09
alpha-Gurjunene	3.57 ± 1.14a	1.08 ± 0.18a	0.93 ± 0.01a	29.08
eremophilene	3.57 ± 1.14a	1.09 ± 0.19a	0.98 ± 0.03a	29.11
beta-Selinene	3.28 ± 1.07a	0.98 ± 0.15a	1.27 ± 0.27a	29.11
Neryl-acetate	15.71 ± 6.02a	4.56 ± 0.63a	1.62 ± 0.08a	29.93
cis-alphabisabonele	14.56 ± 5.68a	4.42 ± 0.58a	1.47 ± 0.17a	29.31
beta-bisabonele	1.13 ± 0.01a	0.36 ± 0.07b	1.22 ± 0.05a	29.37
alpha-cedrene	0.53 ± 0.01c	0.13 ± 0.02a	0.31 ± 0.03b	29.54
7-epi-alpha-selinene	15.11 ± 5.98a	4.69 ± 0.67a	1.82 ± 0.12a	29.93
delta-cadinene	15.11 ± 5.98a	4.68 ± 0.67a	1.88 ± 0.14a	30.24
alpha-curcumene	9.86 ± 1.46a	4.68 ± 0.42b	14.04 ± 0.72a	30.24
ar-curcumene	9.71 ± 1.18b	4.74 ± 0.37a	14.01 ± 0.61c	30.57

gamma-Selinene	10.01 ± 1.62a	4.73 ± 0.38b	14.43 ± 0.62a	30.62
alpha-cadinene	0.22 ± 0.08a	0.04 ± 0.00a	0.15 ± 0.06a	30.95
nerol	0.21 ± 0.09	0.19 ± 0.02	0.31 ± 0.02	31.06
2-phenylethyl-acetate	0.10 ± 0.05a	0.10 ± 0.04a	0.00 ± 0.00a	31.18
isogeraniol	0.11 ± 0.01b	0.18 ± 0.00c	0.03 ± 0.01a	31.29
trans-beta-damascenone	0.11 ± 0.00b	0.20 ± 0.00c	0.04 ± 0.00a	31.29
1s-(cis)-calamenene	0.39 ± 0.13a	0.38 ± 0.01a	1.17 ± 0.02b	31.5
trans- (+)-carveol	0.04 ± 0.01b	0.00 ± 0.00a	0.15 ± 0.01c	31.61
p-cymen-8-ol	0.48 ± 0.05a	0.20 ± 0.00b	0.55 ± 0.04a	31.85
4-phenyl-2-btanone	0.38 ± 0.00b	0.19 ± 0.01a	0.55 ± 0.02c	31.87
ethyl-laurate	3.700 ± 1.14b	2.02 ± 0.18ab	0.53 ± 0.03a	31.98
(e)-geranyl-acetone	4.92 ± 1.83b	2.30 ± 0.28ab	1.35 ± 1.35a	32.02
ascaridole	4.44 ± 1.57b	2.12 ± 0.24ab	1.34 ± 0.03a	32.02
benzyl-alcohol	0.61 ± 0.07b	0.38 ± 0.01a	0.28 ± 0.00a	32.24

4-ethyl-o-xylene	0.36 ± 0.06b	0.14 ± 0.01a	0.04 ± 0.01a	32.24
ethyl-3-phenylpropionate	0.38 ± 0.08b	0.16 ± 0.02a	0.02 ± 0.00a	32.4
phenylethyl-alcohol	0.55 ± 0.02b	0.24 ± 0.01a	0.26 ± 0.02a	32.4
alpha-calacorene	0.33 ± 0.04a	0.13 ± 0.00b	0.34 ± 0.03a	32.8
palustrol	0.11 ± 0.00a	0.06 ± 0.00a	0.27± 0.03a	32.92
alpha-cubene	0.16 ± 0.00a	0.05 ± 0.00a	0.31 ± 0.05b	33.23
caryophyllene-oxide	3.55 ± 0.18b	1.29 ± 0.04a	3.56 ± 0.20b	33.91
(+)-ledol	0.01 ± 0.00a	0.01 ± 0.00a	0.02 ± 0.00a	34.53
alpha-caryophyllene-alcohol	0.78 ± 0.05a	0.52 ± 0.02a	1.37 ± 0.09b	34.82
fonenol	2.20 ± 0.85b	0.65 ± 0.10a	1.13 ± 0.09a	34.83
longifolenaldehyde	0.04 ± 0.02a	0.05 ± 0.01a	1.15 ± 0.09b	34.87
n-benzylidenecyclohexylamine	0.02 ± 0.00a	0.01 ± 0.00a	0.16 ± 0.01b	35.01
cyclooctanone	0.43 ± 0.07b	0.09 ± 0.01a	0.07± 0.02a	35.16
caryophyll-5-en-2-beta-ol	0.44 ± 0.05a	0.32 ± 0.01a	0.77 ± 0.05b	35.78

t-cadinol	0.48 ± 0.03a	0.32 ± 0.01a	0.77 ± 0.05b	35.78
eugenol	0.06 ± 0.01a	0.1 ± 0.03a	0.44 ± 0.00b	35.94
(+)-calarene	10.97 ± 4.27a	5.92 ± 0.56a	1.13 ± 0.15a	36.13
eudesm-7(11)-en-4-ol_(juniper_camphor)	10.97 ± 4.27a	5.93 ± 0.56a	1.13 ± 0.16a	36.13
beta-cadinene	10.99 ± 4.28c	5.94 ± 0.56b	0.71 ± 0.40a	36.19
epi-bicyclosesquiphellandrene	0.43 ± 0.05ab	0.22 ± 0.00a	0.53 ± 0.07b	36.3
carvacrolok	0.16 ± 0.04a	0.07 ± 0.01a	0.11 ± 0.01a	36.46
alpha-eudesmol	0.42 ± 0.04a	0.41 ± 0.05a	2.23 ± 0.46b	36.62
beta-eudesmol	0.11 ± 0.05a	0.00 ± 0.00a	2.37 ± 0.47b	36.73
decanoic-acid	0.43 ± 0.23a	0.03 ± 0.00a	0.14 ± 0.06a	37.08
(-)-phyllocladene	0.05 ± 0.00b	0.02 ± 0.01ab	0.00 ± 0.00a	38.44
2,7-dimethyl-1,6-octadiene	0.01 ± 0.00a	0.00 ± 0.00a	0.01 ± 0.00a	38.57
xanthorrhizol	0.03 ± 0.00a	0.02 ± 0.00a	0.01 ± 0.00a	40.9

Total number of compounds	104	102	101

Volatile compounds having a match quality of at least 90% with the mass spectral library were identified and reported. Means followed by the same lowercase letters in the same column are not significantly different ($p > 0.05$) following comparison using Pearson's chi-square test.

4.3.6 Antioxidant capacity (FRAP, ABTS and DPPH)

Plants that were collected from the field (T3) showed significantly higher antioxidant capacity, with a mean FRAP value of 2719.42 ± 278.72 umol AAE/g in *H. cymosum* plants (DF = 2; χ^2 = F = 21.90; $p < 0.05$), than hydroponics and aquaponics cultivated plants (Table 4.6).

For the DPPH capacity, the results indicated that hydroponics plant extracts yielded a significantly higher mean value (DF = 2; $\chi^2 = 28.68$; $p < 0.05$) compared with aquaponics and field collected plant extracts while aquaponics had the least DPPH capacity (Table 4.6).

For the ABTS capacity (mol TE/g) results showed that field-collected and hydroponic plants produced significantly higher values compared with aquaponics and hydroponics plants (DF = 2; $\chi^2 = 16.25$; $p < 0.05$).

Table 4.6: Mean ± SE of FRAP (µmol AAE/g), ABTS (µmol TE/g) and DPPH (µmol TE/g) contents of *H. cymosum* leaves grown using different cultivation methods

Treatments	FRAP (umol AAE/g)	ABTS (umol TE/g)	DPPH (umol TE/g)
T1	1043.71± 189.81b	1402.77± 244.92b	539.13± 169.37b
T2	2657.30± 99.59a	3190.66± 41.80a	1764.97± 25.12a
T3	2719.42± 278.72a	3446.77± 408.85a	1651.2± 136.65a

Values shown are mean ± S.E.

Means followed by the same lowercase letters in the same column are not significantly different ($p > 0.05$) following comparison using the Man Whitney test, T1, aquaponic; T2, hydroponic; and T3, field

4.3.7 *In vitro* fungal activity using the micro-dilution assay

The minimum inhibitory concentration of *H. cymosum*

There was a significant difference in the minimum inhibitory concentrations among the three cultivation methods when the ethanol extracts of *H. cymosum* plants were assessed against *F. oxysporum* (DF = 2.5; χ^2 = 7.5; p = 0.03). Field plants (T3) were more bioactive at 18 h incubation period with a MIC value of 0.37 mg/mL, whereas aquaponics plants (T1) were least active with MIC value of 0.75 mg/mL, which was equivalent to the synthetic fungicide (Dithane) used as a positive control (Table 4.7). Generally, field-collected plants had the best fungistatic results.

Table 4.7: Anti- *F. oxysporum* activity (mean MIC ± SE) of ethanol extracts of *H. cymosum* plants that were cultivated under aquaponic, hydroponic and field systems

Treatments	**MIC ± SE (mg/ml)** **18hours**
T1	0.75 ± 0b
T2	0.56 ± 0.125ab
T3	0.37 ± 0.00a
Dithane (Positive control)	0.75 ± 0.00b
Ethanol	0

Values shown are mean ± S.E.

Means followed by the same lowercase letters in the same column are not significantly different (p > 0.05) following comparison using the Man Whitney test, T1, aquaponic; T2, hydroponic; and T3, field

4.4 Discussion

In this study, various effects of aquaponics and hydroponics on growth metrics, including plant height, dry and fresh weights were reported. Plants cultivated in hydroponic systems yielded higher fresh and dry weights and mean heights than aquaponic plants. However, there was no statistical difference ($p > 0.05$) between these two cultivation methods except for the fresh weight which was significantly higher in hydroponics plants. Interestingly, despite higher levels of macronutrients such as N, P, and Mg occurring in the hydroponic plants, these did not translate to increased growth of plants. These elements influence plant growth and development (Uchida, 2000; Yousaf *et al*., 2021; Nget *et al*., 2022). Previous research has shown that the availability of nitrogen in aquaponics medium, light intensity, root zone temperature, air temperature, nutrient availability, growth stage, and growth pace are factors that affect how well plants absorb nutrients (Buzby and Lin, 2014). Although aquaponics outperformed hydroponics in terms of plant micronutrient uptake, previous research has demonstrated that plant species may behave differently in the system (Ibáñez Otazua *et al*., 2022). Also, according to Delaide *et al*. (2016), adding mineral nutrients to an aquaponic solution to achieve the same nutrient concentrations as hydroponics can occasionally result in higher yields. The primary source of nitrogen (N) and phosphorus (P) in the system is fish feed. When the fish feed is introduced to the system, a sizable portion of it is consumed by the fish and either utilised for development and metabolism or eliminated as soluble and solid faeces.

Higher secondary metabolite concentrations (total phenolic and flavonol contents) in field-collected *H. cymosum,* obtained in this study*,* could, for example, be related to abiotic stresses such as exposure to drought and nutrient deficiency, insect and fungal exposure (Bennett and Wallsgrove, 1994; Akula and Ravishankar, 2011; Chalker-Scott and Fuchigami , 2018) The production of secondary metabolites is regarded to be a plant's mechanism for adjusting to unfavourable environmental changes (Edreva *et al*., 2008 ; Kosová, 2018). Through signalling mechanisms and pathways, this process involves the production of complex chemicals. Interestingly, higher production of bioactive secondary metabolites could translate to higher antifungal and antioxidant activities.

In the current study, the antioxidant capacity of the tested samples showed a strong correlation with the polyphenol and flavonol contents. Interestingly, hydroponics and field-collected plants were not significantly different in antioxidant capacity; however, they performed better than aquaponic-cultivated plants. Previous studies have reported that the secondary metabolite components of *Cantella asiatica* such asanthocyanins, flavonoids, and phenolic substances, are thought to be responsible for its antioxidant action (Shin *et al*., 2021). Additionally, it is believed that phenolic compounds play a significant role in the sensory qualities and antioxidant activity of wines (Pandeya et al., 2018). Recently, Ibarra-Cantún *et al*. (2020) found

that apple bagasse's antioxidant activity increased progressively with levels of polyphenols, which is consistent with results obtained in this study. The FRAP and ABTS bioassays showed that plants harvested from the field had higher antioxidant capacity than plants generated in aquaponics and hydroponic systems. These results suggest that plant extracts of *H. cymosum* have quite high antioxidant activity and higher phenol and flavonoid contents is an important source of natural antioxidants, justifying their use in treatment of ailments associated with oxidative stresses and disorders (Kripasana and Xavier, 2020).

The MIC findings of this research demonstrated that field-collected plants treatments had a higher significant effect on extracts of *H*. *cymosum* species tested, and these results correlated with the total polyphenol contents; It's worth noting that when plants are subjected to a variety of environmental situations, they acquire large amounts of bioactive compounds (Ncube *et al*., 2011). Several studies have been done on the investigation of antifungal activities in *Helichryum* species including *H. cymosum*. In a study by Van Vuuren *et al*. (2006), *H. cymosum* was tested against eleven pathogens, with MIC values ranging from 0.156 to 0.313 mg/mL from field-collected plants. Notably, these outcomes reflect those from the current study's anti-*F. oxysporum* bioassay, which revealed MIC ranges from 0.375 to 0.75 mg/mL. Matanzima, (2014) previously showed similar results with MIC values ranging from 0.078 to 03.31 mg/mL, but it's important to note that the plant materials used by Matanzima, (2014) were obtained from hydroponically grown plants, whereas the ones used in this study were obtained from plants grown in aquaponic, hydroponic, and field systems. This suggests that the variation in phytochemical profiles in extracts from plants in the different treatments might perhaps explains the differences in bioactivities.

Based on the gas chromatography-mass spectrometry (GC-MS) analysis, *H. cymosum* plants is a rich source of volatile compounds; the plants obtained from the three cultivation methods (aquaponics, hydroponics, and field cultivated plants) contained up to 106 compounds with 90% match with the mass spectra library. These compounds included nonadecane, 4-methyl-octane, beta-pinene, cis-ocimene, 1-octen-3-ol, beta-fencyl acetate, benzaldehyde, alpha-humulene, linalyl propanoate, acoradiene, beta-himachalene, alpha-cedrene, alpha-ced 2,7-dimethyl-1,6-octadiene, trans- (+)-carveol, (-)-phyllocladene, and cyclooctanone were among the compounds that were dominating in aquaponics produced plants when compared to hydroponics and field collected plants. Alpha-humulene and beta-pinene have been reported as one of the compounds with potent antifungal properties (Zuzarte *et al*., 2021; Ruiz-Vásquez *et al*., 2022). Aquaponic plants produced the highest number of volatile compounds (106). The three cultivation methods, however, did not differ statistically ($p > 0.05$). In general, plants taken from the field had the lowest overall number of volatiles (104). Trans-caryophyllene is a sesquiterpene found in the essential oils of many therapeutic plants of which sesquiterpene has been found as a class of major compounds in most members of the *Helichrysum* genus.

Many studies have documented its pharmacological effects, including its antibacterial (Moo *et al*., 2020), anti-helicobacter pylori (Woo *et al*., 2020), antioxidant and anti-inflammatory (Ames-Sibin *et al*., 2018), analgesic and anticancer potential, neuroprotective (Machado *et al*., 2018). Also, some of these chemicals, such as nanodecane, were previously found as aliphatic hydrocarbons in essential oil of *Helichrysum* plants by hydrodistillation (Radušienė, 2008). However, nanodecane, has also been discovered to be a plant secondary metabolite with antioxidant and antifungal properties in plants other than the *Helichrysum* genus (Ganesan and Raja, 2021). *H. cymosum* chemical constituents were previously determined using GC-MS (Van Vuuren *et al*., 2006). Remarkably, alpha-humulene, trans- (+)-carveol, 1-octen-3-ol, and beta-pinene are among the compounds discovered, which match with some of the substances reported in this study. By comparing the percentages of the compounds and their retention indices, Bougatsos *et al*. (2004) identified 65 phytochemicals as essential oil components, some of which were also detected in this study: Beta-pinene, cis-ocimene, 1-octen-3-ol, and trans- (+)-carveol. Also, although aquaponics had a higher quantity of volatile compounds than hydroponics and field collected plants, the chemical compositions of *H. cymosum* oils were very similar between the three growing systems. It is, however, worth-noting that a few compounds occurred in aquaponics but not in hydroponics or field-collected plants, such as (+)-2-carene; 1,2,3-trimethylbenzene; 2,6,6-trimethylcyclohexanone; cis-3-hexenyl-acetate; trans-2-heptenal; octenyl acetate cis-3-hexenol; 3-octanol-istd; gamma-curcumene.

4.5 Conclusion

The findings of the current study are that as aquaponic-cultivated *H. cymosum* yielded extracts with significantly lower antioxidant capacity and polyphenol contents than hydroponics and field-cultivated plants. Furthermore, there were no significant differences between field and hydroponic systems in terms of antioxidant capacities and polyphenol contents. The fungistatic activity of the ethanol extracts against *F. oxysporum* related to the cultivation of *H. cymosum* in aquaponics, hydroponics, and field-collected plants. Plant growth parameters did not significantly vary between aquaponics and hydroponics. It is advised that more of these trials including field trials for cultivating medicinal plants be carried out. The results of the present study suggest that commercial cultivation of *H. cymosum* using hydroponic and aquaponic systems may be feasible.

4.6 References

Akula, R. and Ravishankar, G.A., 2011. Influence of abiotic stress signals on secondary metabolites in plants. *Plant signaling & behavior*, *6*(11), pp.1720–1731.

Ames-Sibin, A.P., Barizão, C.L., Castro-Ghizoni, C.V., Silva, F.M., Sá-Nakanishi, A.B., Bracht, L., Bersani-Amado, C.A., Marçal-Natali, M.R., Bracht, A. and Comar, J.F., 2018. β-Caryophyllene, the major constituent of copaiba oil, reduces systemic inflammation and oxidative stress in arthritic rats. *Journal of Cellular Biochemistry*, *119*(12), pp.10262–10277.

Barea, J.M., 2015. Future challenges and perspectives for applying microbial biotechnology in sustainable agriculture based on a better understanding of plant-microbiome interactions. *Journal of soil science and plant nutrition*, *15*(2), pp.261–282.

Bennett, R.N. and Wallsgrove, R.M., 1994. Secondary metabolites in plant defence mechanisms. *New phytologist*, *127*(4), pp.617–633.

Benzie, I.F. and Strain, J.J., 1996. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay. *Analytical biochemistry*, *239*(1), pp.70–76.

Bittsanszky, A., Uzinger, N., Gyulai, G., Mathis, A., Junge, R., Villarroel, M., Kotzen, B. and Kőmíves, T., 2016. Nutrient supply of plants in aquaponic systems. *Ecocycles*, *2*(2), pp.17–20.

Bougatsos, C., Ngassapa, O., Runyoro, D.K. and Chinou, I.B., 2004. Chemical composition and in vitro antimicrobial activity of the essential oils of two *Helichrysum* species from Tanzania. *Zeitschrift für Naturforschung C*, *59*(5–6), pp.368–372.

Bruni, R. and Sacchetti, G., 2009. Factors affecting polyphenol biosynthesis in wild and field grown St. John's Wort (*Hypericum perforatum* L. *Hypericaceae/Guttiferae*). *Molecules*, *14*(2), pp.68–725.

Buhmann, A. and Papenbrock, J., 2013. Biofiltering of aquaculture effluents by halophytic plants: Basic principles, current uses and future perspectives. *Environmental and Experimental Botany*, *92*, pp.122133.

Buzby, K.M. and Lin, L.S., 2014. Scaling aquaponic systems: Balancing plant uptake with fish output. *Aquacultural Engineering*, *63*, pp.39–44.

Campbell, C.R. and Plank, C.O., 1998. Preparation of plant tissue for laboratory analysis. *Methods for Plant Analysis*, *37*.

Chalker-Scott, L., and Fuchigami, L.H., 2018. The role of phenolic compounds in plant stress responses. In *Low temperature stress physiology in crops*, pp. 67–80. CRC press.

Chen, P., Zhu, G., Kim, H.J., Brown, P.B. and Huang, J.Y., 2020. Comparative life cycle assessment of aquaponics and hydroponics in the Midwestern United States. *Journal of Cleaner Production*, *275*, p.122888.

Chen, S.L., Yu, H., Luo, H.M., Wu, Q., Li, C.F. and Steinmetz, A., 2016. Conservation and sustainable use of medicinal plants: problems, progress, and prospects. *Chinese medicine*, *11*(1), pp.1–10.

Czinner, E., Hagymasi, K., Blazovics, A., Kery, A., Szőke, É. and Lemberkovics, E., 2001. The in vitro effect of *Helichrysi flos* on microsomal lipid peroxidation. *Journal of ethnopharmacology*, *77*(1), pp.31–35.

Delaide, B., Goddek, S., Gott, J., Soyeurt, H. and Jijakli, H.M., 2016. Lettuce (*Lactuca sativa L. var. Sucrine*) growth performance in, complemented solution encourages the development of decoupled aquaponics. *Water*, *8*, p.467.

Edreva, A., Velikova, V., Tsonev, T., Dagnon, S., Gürel, A., Aktaş, L. and Gesheva, E., 2008. Stress-protective role of secondary metabolites: diversity of functions and mechanisms. *Gen Appl Plant Physiol*, *34*(1–2), pp.67–78.

Eloff, J.N., 1998. A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta medica*, *64*(08), pp.711–713.

Endut, A., Jusoh, A., Ali, N., Nik, W.W. and Hassan, A., 2010. A study on the optimal hydraulic loading rate and plant ratios in recirculation aquaponic system. *Bioresource technology*, *101*(5), pp.1511–1517.

Espinoza, F., Vidal, S., Rautenbach, F., Lewu, F. and Nchu, F., 2019. Effects of *Beauveria bassiana* (*Hypocreales*) on plant growth and secondary metabolites of extracts of hydroponically cultivated chive (*Allium schoenoprasum L.* [*Amaryllidaceae*]). *Heliyon*, *5*(12), p.e03038.

Ganesan, K. and Raja, A.B., 2021. Evaluation of phyto-compounds from *Dictyota dichotoma* extract using gas chromatographic and mass spectroscopic technique. *International Journal of Modern Agriculture*, *10*(2), pp.2544 –2548.

Goddek, S., Joyce, A., Kotzen, B. and Burnell, G.M., 2020. Correction to: Aquaponics Food Production Systems. In *Aquaponics Food Production Systems* (pp. C1–C1). Springer, Cham.

Hammer, Ø., Harper, D.A. and Ryan, P.D., 2001. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia electronica*, *4*(1), p.9.

Hanjra, M.A. and Qureshi, M.E., 2010. Global water crisis and future food security in an era of climate change. *Food policy*, *35*(5), pp.365–377.

Hayden, A.L., 2006. Aeroponic and hydroponic systems for medicinal herb, rhizome, and root crops. *HortScience*, *41*(3), pp.536–538.

Heyman, H.M., 2009. *Metabolomic comparison of selected Helichrysum species to predict their antiviral properties* (Doctoral dissertation, University of Pretoria).

Heyman, H.M., 2013. *Identification of anti-HIV compounds in Helichrysum species (Asteraceae) by means of NMR-based metabolomic guided fractionation* (Doctoral dissertation, University of Pretoria).

Heyman, H.M., Senejoux, F., Seibert, I., Klimkait, T., Maharaj, V.J. and Meyer, J.J.M., 2015. Identification of anti-HIV active dicaffeoylquinic-and tricaffeoylquinic acids in *Helichrysum populifolium* by NMR-based metabolomic guided fractionation. *Fitoterapia*, *103*, pp.155–164.

Hu, Z., Lee, J.W., Chandran, K., Kim, S., Brotto, A.C. and Khanal, S.K., 2015. Effect of plant species on nitrogen recovery in aquaponics. *Bioresource technology*, *188*, pp.92–98.

Ibáñez Otazua, N., Blázquez Sánchez, M., Ruiz Yarritu, O., Unzueta Balmaseda, I., Aboseif, A.M., Abou Shabana, N.M., Taha, M.K.S. and Goda, A.M.A., 2022. Integrated Multitrophic Aquaponics—A Promising Strategy for Cycling Plant Nutrients and Minimizing Water Consumption. In *Biology and Life Sciences Forum*, *16*(1), p. 28.

Ibarra-Cantún, D., Ramos-Cassellis, M.E., Marín-Castro, M.A. and Castelán-Vega, R.D.C., 2020. Secondary metabolites and antioxidant activity of the solid-state fermentation in apple (*Pirus malus* l.) and *Agave mezcalero* (*Agave angustifolia* H.) Bagasse. *Journal of Fungi*, *6*(3), p.137.

Jakupovic, J., Zdero, C., Grenz, M., Tsichritzis, F., Lehmann, L., Hashemi-Nejad, S.M. and Bohlmann, F., 1989. Twenty-one acylphloroglucinol derivatives and further constituents from South African *Helichrysum* species. *Phytochemistry*, *28*(4), pp.1119–1131.

Jousse, C., Vu, T.D., Le Minh Tran, T., Al Balkhi, M.H., Molinié, R., Boitel-Conti, M., Pilard, S., Mathiron, D., Hehn, A., Bourgaud, F. and Gontier, E., 2010. Tropane alkaloid profiling of hydroponic *Datura innoxia* Mill. plants inoculated with Agrobacterium rhizogenes. *Phytochemical Analysis: An International Journal of Plant Chemical and Biochemical Techniques*, *21*(1), pp.118–127.

Katalinić, V., Milos, M., Modun, D., Musić, I. and Boban, M., 2004. Antioxidant effectiveness of selected wines in comparison with (+)-catechin. *Food chemistry*, *86*(4), pp.593–600.

Kosová, K., Vítámvás, P., Urban, M.O., Prášil, I.T. and Renaut, J., 2018. Plant abiotic stress proteomics: the major factors determining alterations in cellular proteome. *Frontiers in plant science*, *9*, p.122.

Kripasana, K. and Xavier, J., 2020. Phytochemical analysis and antioxidant activity of leaf extracts of some selected plants of the family *Acanthaceae*. *Plant Science Today*, *7*(2), pp.264–274.

Kutluk, I., Aslan, M., Orhan, I.E., and Özçelik, B., 2018. Antibacterial, antifungal, and antiviral bioactivities of selected *Helichrysum* species. *South African Journal of Botany*, *119*, pp.252–257.

Lam, S.S., Ma, N.L., Jusoh, A. and Ambak, M.A., 2015. Biological nutrient removal by recirculating aquaponic system: Optimization of the dimension ratio between the hydroponic & rearing tank components. *International Biodeterioration & Biodegradation*, *102*, pp.107–115.

Lee, S.E., Hwang, H.J., Ha, J.S., Jeong, H.S. and Kim, J.H., 2003. Screening of medicinal plant extracts for antioxidant activity. *Life sciences*, *73*(2), pp.167–179.

Léonhart, S., Pedneault, K., Gosselin, A., Angers, P., Papadopoulos, A.P. and Dorais, M., 2000, November. Diversification of greenhouse crop production under supplemental lighting by the use of new cultures with high economic potential. In *IV International ISHS Symposium on Artificial Lighting 580*, pp. 249–54).

Liu, F. and Ng, T.B., 2000. Antioxidative and free radical scavenging activities of selected medicinal herbs. *Life sciences*, *66*(8), pp.725–735.

Lourens, A.C.U., Viljoen, A.M. and Van Heerden, F.R., 2008. South African Helichrysum species: a review of the traditional uses, biological activity and phytochemistry. *Journal of Ethnopharmacology*, *119*(3), pp.630– 652.

Lubbe, A. and Verpoorte, R., 2011. Cultivation of medicinal and aromatic plants for specialty industrial materials. *Industrial crops and products*, *34*(1), pp.785– 801.

Machado, K.D.C., Islam, M.T., Ali, E.S., Rouf, R., Uddin, S.J., Dev, S., Shilpi, J.A., Shill, M.C., Reza, H.M., Das, A.K. and Shaw, S., 2018. A systematic review on the neuroprotective perspectives of beta-caryophyllene. *Phytotherapy Research*, *32*(12), pp.2376–2388.

Maggini, R., Kiferle, C. and Pardossi, A., 2014. Hydroponic production of medicinal plants. *Medicinal plants: Antioxidant properties, traditional uses and conservation strategies. Nova Science Publishers, Inc., Hauppauge, NY*, pp.91–116.

Maroyi, A., 2019. *Helichrysum caespititium* (DC.) Harv.: Review of its medicinal uses, phytochemistry and biological activities. *Journal of Applied Pharmaceutical Science*, *9*(6), pp.111–118.

Maroyi, A.L.F.R.E.D., 2019. *Helichrysum cymosum* (L.) D. Don (*Asteraceae*): Medicinal uses, chemistry, and biological activities. *Asian Journal of Pharmaceutical Research*, *12*, pp.19–26.

Matanzima, Y., 2014. *Quantitative and qualitative optimization of antimicrobial bioactive constituents of Helichrysum cymosum using hydroponics technology* (Master's dissertation, Cape Peninsula University of Technology).

Matrose, N.A., Obikeze, K., Belay, Z.A. and Caleb, O.J., 2021. Impact of spatial variation and extraction solvents on bioactive compounds, secondary metabolites, and antifungal efficacy of South African Impepho [*Helichrysum odoratissimum* (L.) Sweet]. *Food Bioscience*, p.101139.

Miller, N.J., Rice-Evans, C., Davies, M.J., Gopinathan, V. and Milner, A., 1993. A novel method for measuring antioxidant capacity and its application to monitoring the antioxidant status in premature neonates. *Clinical science (London, England: 1979)*, *84*(4), pp.407– 412.

Moo, C.L., Yang, S.K., Osman, M.A., Yuswan, M.H., Loh, J.Y., Lim, W.M., SWEE-HUA-ERIN, L.I.M. and Lai, K.S., 2020. Antibacterial Activity and Mode of Action of β-caryophyllene on. *Polish journal of microbiology*, *69*(1), pp.49 –54.

Munguia-Fragozo, P., Alatorre-Jacome, O., Rico-Garcia, E., Torres-Pacheco, I., Cruz-Hernandez, A., Ocampo-Velazquez, R.V., Garcia-Trejo, J.F. and Guevara-Gonzalez, R.G., 2015. Perspective for aquaponic systems: "omic" technologies for microbial community analysis. *BioMed Research International*, *2015*, pp.1–10.

Nchu, F., Aderogba, M.A., Mdee, L.K. and Eloff, J.N., 2010. Isolation of anti-Candida albicans compounds from Markhamia obtusifolia (Baker) Sprague (*Bignoniaceae*). *South African Journal of Botany*, *76*(1), pp.54 –57.

Nchu, F., Matanzima, Y. and Laubscher, C.P., 2018. Prospects of N fertilization in medicinal plants cultivation. *Nitrogen in Agriculture-Updates*, pp.209 –222.

Ncube, B., Ngunge, V.N.P., Finnie, J.F. and Van Staden, J., 2011. A comparative study of the antimicrobial and phytochemical properties between outdoor grown and micropropagated *Tulbaghia violacea* Harv. plants. *Journal of Ethnopharmacology*, *134*(3), pp.775 –780.

Nget, R., Aguilar, E.A., Cruz, P.C.S., Reaño, C.E., Sanchez, P.B., Reyes, M.R. and Prasad, P.V., 2022. Responses of Soybean Genotypes to Different Nitrogen and Phosphorus Sources: Impacts on yield Components, Seed Yield, and Seed Protein. *Plants, 11*(3), p.298.

Nuwansi, K.K.T., Verma, A.K., Chandrakant, M.H., Prabhath, G.P.W.A. and Peter, R.M., 2021. Optimization of stocking density of koi carp (*Cyprinus carpio* var. koi) with gotukola (*Centella asiatica*) in an aquaponic system using phytoremediated aquaculture wastewater. *Aquaculture*, *532*, p.735993.

Olatunji, T.L. and Afolayan, A.J., 2019. Comparative quantitative study on phytochemical contents and antioxidant activities of *Capsicum annuum* L. and *Capsicum frutescens* L. *The Scientific World Journal*, *2019*.

Pandeya, A., Rayamajhi, S., Pokhrel, P. and Giri, B., 2018. Evaluation of secondary metabolites, antioxidant activity, and color parameters of Nepali wines. *Food Science & Nutrition*, *6*(8), pp.2252–2263.

Papadopoulos, A.P., Luo, X., Leonhart, S., Gosselin, A., Pedneault, K., Angers, P., Gaudreau, L. and Dorais, M., 2000, May. Soilless greenhouse production of medicinal plants in North-Eastern Canada. In *World Congress on Soilless Culture: Agriculture in the Coming Millennium 554*, pp. 297–304.

Pardossi, A., Malorgio, F., Incrocci, L. and Tognoni, F., 2005. Hydroponic technologies for greenhouse crops, pp. 360– 378.

Polycarpou, P., Neokleous, D., Chimonidou, D. and Papadopoulos, I., 2005. A closed system for soil-less culture adapted to the Cyprus conditions. *F. El Gamal, AN Lamaddalen, C. Bogliotti, and R. Guelloubi. Non-conventional water use*, pp.237–241.

Ponisio, L.C., M'Gonigle, L.K., Mace, K.C., Palomino, J., De Valpine, P. and Kremen, C., 2015. Diversification practices reduce organic to conventional yield gap. *Proceedings of the Royal Society B: Biological Sciences*, *282*(1799), p.20141396.

Popoola, O.K., Marnewick, J.L., Rautenbach, F., Ameer, F., Iwuoha, E.I. and Hussein, A.A., 2015. Inhibition of oxidative stress and skin aging-related enzymes by prenylated chalcones and other flavonoids from *Helichrysum teretifolium*. *Molecules*, *20*(4), pp.7143 –7155.

Radušienė, J. and Judžentienė, A., 2008. Volatile composition of *Helichrysum arenarium* field accessions with differently coloured inflorescences. *Biologija*, *54*(2), pp.116–120.

Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free radical biology and medicine*, *26*(9 –10), pp.1231–1237.

Ruiz-Vásquez, L., Ruiz Mesia, L., Caballero Ceferino, H.D., Ruiz Mesia, W., Andrés, M.F., Díaz, C.E. and Gonzalez-Coloma, A., 2022. Antifungal and Herbicidal Potential of Piper Essential Oils from the Peruvian Amazonia. *Plants*, *11*(14), p.1793.

Runyoro, D.K.B., Ngassapa, O., Kachali, L., Obare, V. and Lyamuya, E.F., 2010. Biological activities of essential oils from plants growing in Tanzania. *East and Central African Journal of Pharmaceutical Sciences*, *13*(3), pp. 85–91.

Santos, V.B., Araújo, A.S., Leite, L.F., Nunes, L.A. and Melo, W.J., 2012. Soil microbial biomass and organic matter fractions during transition from conventional to organic farming systems. *Geoderma*, *170*, pp.227–231.

Shin, H.Y., Kim, H., Jung, S., Jeong, E.J., Lee, K.H., Bae, Y.J., Suh, H.J., Jang, K.I. and Yu, K.W., 2021. Interrelationship between secondary metabolites and antioxidant capacities of

Centella asiatica using bivariate and multivariate correlation analyses. *Applied Biological Chemistry*, *64*(1), pp.1–10.

Sindambiwe, J.B., Calomme, M., Cos, P., Totte, J., Pieters, L., Vlietinck, A. and Berghe, D.V., 1999. Screening of seven selected Rwandan medicinal plants for antimicrobial and antiviral activities. *Journal of Ethnopharmacology*, *65*(1), pp.71 –77.

Singleton, V.L., Orthofer, R. and Lamuela-Raventós, R.M., 1999. [14] Analysis of total phenols and other oxidation substrates and antioxidants by means of folin-ciocalteu reagent. *Methods in enzymology*, *299*, pp.152–178.

Stadler, M.M., Baganz, D., Vermeulen, T. and Keesman, K.J., 2015. Circular economy and economic viability of aquaponic systems: Comparing urban, rural and peri-urban scenarios under Dutch conditions. *ICESC2015: Hydroponics and Aquaponics at the Gold Coast 1176*, pp.101–114.

Stafford, G.I., Jäger, A.K. and Van Staden, J., 2005. Effect of storage on the chemical composition and biological activity of several popular South African medicinal plants. *Journal of Ethnopharmacology*, *97*(1), pp.107–115.

Surendran, U., Raja, P., Jayakumar, M. and Ramasubramoniam, S., 2021. Use of efficient water saving techniques for production of rice in India under climate change scenario: A critical review. *Journal of Cleaner Production*, p.127272.

Taylor, J.L.S., Rabe, T., McGaw, L.J., Jäger, A.K. and Van Staden, J., 2001. Towards the scientific validation of traditional medicinal plants. *Plant growth regulation*, *34*(1), pp.23 –37.

Tchoumbougnang, F.O., Sameza, M.L., Jazet, D.P.M., Nkouaya, M.E.G., Fekam, B.F., Ngoko, Z., Amvam, Z.P.H. and Menut, C., 2010. Composition, radical scavenging and antifungal activities of essential oils from 3 *Helichrysum* species growing in Cameroon against *Penicillium oxalicum* a yam rot fungi. *African Journal of Agricultural Research*, *5*(2), pp.121–127.

Uchida, R., 2000. Essential nutrients for plant growth: nutrient functions and deficiency symptoms. *Plant nutrient management in Hawaii's soils*, *4*, pp.31 – 55.

Van Vuuren, S.F., 2007. *The antimicrobial activity and essential oil composition of medicinal aromatic plants used in African traditional healing* (Doctoral dissertation, University of the Witwatersrand).

Van Vuuren, S.F., Viljoen, A.M., van Zyl, R.L., Van Heerden, F.R. and Başer, K.H.C., 2006. The antimicrobial, antimalarial and toxicity profiles of helihumulone, leaf essential oil and extracts of *Helichrysum cymosum* (L.) D. Don *subsp. cymosum*. *South African Journal of Botany*, *72*(2), pp.287–290.

Woo, H.J., Yang, J.Y., Lee, M.H., Kim, H.W., Kwon, H.J., Park, M., Kim, S.K., Park, S.Y., Kim, S.H. and Kim, J.B., 2020. Inhibitory effects of β-caryophyllene on Helicobacter pylori infection in vitro and in vivo. *International journal of molecular sciences*, *21*(3), p.1008.

Wortman, S.E., 2015. Crop physiological response to nutrient solution electrical conductivity and pH in an ebb-and-flow hydroponic system. *Scientia Horticulturae*, *194*, pp.34–42.

Xego, S., Kambizi, L. and Nchu, F., 2017. Effects of different hydroponic substrate combinations and watering regimes on physiological and anti-fungal properties of *Siphonochilus aethiopicus*. *African Journal of Traditional, Complementary and Alternative Medicines*, *14*(3), pp.89–104.

Yousaf, M., Bashir, S., Raza, H., Shah, A.N., Iqbal, J., Arif, M., Bukhari, M.A., Muhammad, S., Hashim, S., Alkahtani, J. and Alwahibi, M.S., 2021. Role of nitrogen and magnesium for growth, yield and nutritional quality of radish. *Saudi Journal of Biological Sciences, 28*(5), pp.3021–3030.

Zuzarte, M., Correia, P.M., Alves-Silva, J.M., Gonçalves, M.J., Cavaleiro, C., Cruz, T. and Salgueiro, L., 2021. Antifungal and Anti-Inflammatory Potential of *Bupleurum rigidum* subsp. *paniculatum* (Brot.) *H. Wolff* Essential Oil. *Antibiotics*, *10*(5), p.592.

Chapter 5

General discussion, recommendation, and conclusion

There has been a rise in medicinal plant research in recent years to better understand the mechanism plants produce, the various compounds that are used for the treatment of various illnesses in humans. This study demonstrated for the first time that it is feasible to cultivate medicinal plants, in particular *Helichrysum* species, in aquaponic and hydroponic systems and obtain high-quality medicinal extracts. This study opens new opportunities for commercial and precision cultivation of *Helicrysum* species in South Africa. Further, the study also demonstrated that species in the same genus respond differently to the aquaponic and hydropnic systems. For example, *H. odoratissimum* produced best antifungal activity in aquaponics, while *H cymosum* yielded higher antioxidant and antifungal in soil medium than in aquaponics.

In the current study, a comparison of extracts of *H. odoratissimum* and *H. cymosum* grown in aquaponic and hydroponic systems was conducted in terms of crop yields, secondary metabolite contents, and antifungal activity. The current study, which focused on both *Helichrysum* species, showed that the cultivation strategies varied in crop yields, with aquaponics slightly outperforming hydroponics in terms of plant development (plant height, fresh weight, and dry weight in *H.odoratissimum*. For *H. cymosum*, however, the situation was the inverse, with hydroponic plants performing better than aquaponic ones. Although these findings are consistent with prior research, they were expected since various plants behave differently in these two systems, as was discussed in the preceding chapters of the current study. This study demonstrated that *H. odoratissimum* plants grown using aquaponics had the best antifungal activity, while plants grown using hydroponics had the highest levels of total flavonols and antioxidant activity in the plant extracts. Whereas *H. cymosum* yielded extracts with significantly lower antioxidant capacity and polyphenol contents than hydroponics and field-cultivated plants. The current study further demonstrated that the tissue nutrient contents varied with cultivation method in *H. odoratissimum* plants.

Recommendations

The use of aquaponics and hydroponics to cultivate *Helichrysum* species have been demonstrated as potentially viable alternative methods for cultivating medicinal plants. Additionally, these two methods offer environmentally safe approach for meeting the growing demand for high quality and quantity of medicinal materials and food while using less

freshwater, managing waste more effectively, and recycling nutrients. The economic viability of growing medicinal plants using these two techniques must be taken into consideration to accomplish the sustainable usage of aquaponics and hydroponics in the future. It is also suggested that more of these studies, including outdoor cultivation trials for medicinal plants, be conducted.

Because the plant species vary in bioactivities in aquaponics and hydroponics, more studies should be carried out for the different *Helicrysum spp* is recommended.

Although there is limited information on medicinal plant cultivation in aquaponics and hydroponics, many studies have suggested that medicinal plants should be tested for longer periods of time in experimental and commercial systems to obtain sufficient data to ascertain determine the feasibility for large-scale medicinal plant cultivation. Finally, to optimize plant development, nutrient availability and uptake by plants in aquaponics need to be improved. Even though much research has been done in this area, more work is needed to completely appreciate nutrient cycling in aquaponics, as it has a significant impact on plant development.