Hydroponic propagation of *Siphonochilus aethiopicus*: an endangered medicinal plant

by

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Thesis submitted in fulfilment of the requirements for the degree

Master of Technology: Horticultural Sciences

in the Faculty of Applied Sciences at the

CAPE PENINSULA UNIVERSITY OF TECHNOLOGY

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Bellville

2016

DECLARATION

I, Sibusiso Xego, declare that the contents of this thesis represent my own unaided work, and that the 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**

DEDICATION

I dedicate this work to my grandmother Tazana Regina Sidumo, my mother Bulelwa Sylvia Xego and my father Mbuyiseli Eric Xego. To Sidumo family, thank you for your love, support and prayers throughout my academic career. You will always have a special place in my heart.

ACKNOWLEDGEMENTS

First and foremost I would like to thank God, the Almighty for his blessings, I thank him for the gift of life, health and for the strength he has given me throughout my life. "With man this is impossible, but with God all things are possible" Matthew 19:26.

A special thanks to Prof. L. Kambizi (supervisor) for his patience, support and understanding throughout this study. You have been an inspiration to me and source of strength, my co-supervisor Prof. F. Nchu thank you for your time, supervision and motivation. Thank you for your valuable input and guidance during the duration of my studies.

I also wish to express my sincere gratitude to my family my mother Bulelwa Xego, my father Mbuyiseli Xego and my dearest grandmother Tazana Regina Sidumo I knew I could always depend and count on you through thick and thin, your teachings, prayers and love made me who I am today. My aunts Nikelwa Sidumo and Sindiswa Sidumo thank you for your time and advice, ndiyabulela kakhulu uThixo anigcine. I wish to thank my brothers and sisters Aphiwe Sidumo, Phumeza Sidumo, Asavela Kema, Ayabonga Kema, Ntsikelelo Xego, Yanga Xego and other family members for their unconditional love; they were constantly there when I needed emotional and social support.

My thanks and appreciation also goes to my friends and associates who have enthusiastically helped me throughout my studies with their abilities Yonela Matanzima, Sinovuyo Ncuku, Mvuyisi Mtshisazwe, Loyiso Mzini, Dr. Simphiwe Nelani, Asanda Mlungwana, Yonela Payi, Deborah Erasmus and St Williams Circuit.

This study was financially supported by Cape Peninsula University of Technology through the CPUT Bursary and NRF (National Research Foundation). The authors would like to give an exceptional thanks to Dr. Marietjie Stander at the Central Analytical Facilities, University of Stellenbosch, for helping with the LC-MS analysis.

May God bless you all copiously!!!

TABLE OF CONTENTS

Declaration i

Dedication ii

Acknowledgements iii

Table of Contents iv

List of Acronyms ix

List of Tables xi

List of Figures xii

Abstract xiii

Chapter One 1

Introduction, background to the research problem and literature review

1.1 General introduction 1

1.2 Structure of the thesis 3

1.3 Background to the research problem 4

1.4 Statement of the research problem 6

1.5 Literature review 7

1.5.1 *Siphonochilus aethiopicus* 7

1.5.2 Geographical distribution of *S. aethiopicus* 7

1.5.3 Morphological characteristics of *S. aethiopicus* 8

1.5.4 Growth requirements of *S. aethiopicus* 9

1.5.5 Propagation 10

1.5.6 Current status of *S. aethiopicus* 10

1.5.7 Some of the medicinal and traditional uses of *S. aethiopicus* 12

1.5.8 Some of the phytochemicals and antimicrobial activity of *S. aethiopicus* 12

1.6 Challenges allied with soil cultivation of medicinal plants 13
1.7 Role of biotechnology for protecting medicinal plants 14
1.7.1 Tissue culture (micropropagation) 14
1.7.2 Hydroponic technology (Soilless culture) 15
1.8 Growing substrates and substrate mixes 17
1.8.1 Growing substrates 17
1.8.1.1 Coconut fiber (coir) 17
1.8.1.2 Perlite 18
1.8.1.3 Vermiculite 19
1.8.1.4 Bark 19
1.8.2 Substrate mixes 20
1.8.3 Importance of substrate (growing media) water holding capacity 21
1.9 Use of improved irrigation technology (watering regimes) to enhance water use efficiency 22
1.10 *Fusarium oxysporum* (Hypocreales) 24
1.11 Hypothesis 25
1.12 The overall aim of the study 25
1.13 Specific objectives of the research 25
1.14 References 27

Chapter Two 44

Optimizing water holding capacity using inert substrate mixes under different watering regimes for hydroponic cultivation of *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt (Zingiberaceae).

2.1 Introduction 44
2.2 Materials and Methods 46
2.2.1 Plant material 46
2.2.2 Substrates preparation 47
2.2.3 Experimental design 47
2.2.4 Watering regimes 49

2.2.5 Determination of water holding capacity (WHC) 49
2.2.6 Plant growth parameters 49
2.2.7 Calculating quantity of irrigation water used 50
2.2.8 Statistical analysis 50
2.3 Results 50
2.3.1 Water holding capacity 50
2.3.2 Plant emergence 51
2.3.3 Growth parameters 52
2.3.3.1 Number of leaves 52
2.3.3.2 Stem length 52
2.3.3.3 Leaf length 53
2.3.3.4 Number of rhizome eyes 53
2.3.3.5 New shoots 54
2.3.3.6 Plant total weight 54
2.3.3.7 Rhizome length (fresh) 54
2.3.3.8 Dry weight (aerial parts) 55
2.3.3.9 New shoots total weight 55
2.3.3.10 Fresh weight (aerial parts) 55
2.3.3.11 Rhizome length (old) 56
2.3.3.12 Rhizome weight 56
2.3.3.13 Root length 57
2.3.3.14 Root weight 57
2.3.3.15 Number of roots 57
2.3.4 Quantity of irrigation water used 61
2.4 Discussion 62
2.4.1 Plant emergence 62
2.4.2 Water holding capacity 62
2.4.3 Growth parameters 63
2.4.4 Quantity of irrigation water used 65
2.5 References 67

Chapter Three **75**

Effect of different substrate combinations and watering regimes on nutrient uptake, anti-*Fusarium oxysporum* (Hypocreales) activity and secondary metabolite profile of foliage and rhizome extracts of hydroponically cultivated *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt (Zingiberaceae)

3.1 Introduction **75**

3.2 Methods and Materials **78**

3.2.1 Plant material 78

3.2.2 Substrates preparation 78

3.2.3 Experimental design 79

3.2.4 Watering regimes 80

3.2.5 Tissue analysis 81

3.2.6 Extraction of plant material 81

3.2.7 Antimicrobial activities of extracts 81

3.2.8 Total activity (TA) 82

3.2.9 Preliminary analysis 82

3.2.10 Liquid Chromatography- Mass Spectrometry (LC-MS) 82

3.2.11 Statistical analysis 84

3.3 Results **84**

3.3.1 Tissue analysis 84

3.3.1.1 Macronutrients 84

3.3.1.2 Micronutrients 84

3.3.2 Yield, Minimum inhibitory concentration (MIC) and Total activity (TA) 88

3.3.2.1 Yield 88

3.3.2.2 Minimum inhibitory concentration (MIC) 88

3.3.2.3 Total activity (TA) 90

3.3.3 LC-MS 95

3.4 Discussion **101**
3.4.1 Tissue analysis 101
3.4.2 Antimicrobial activity and total activity 102
3.4.3 LC-MS 104
3.5 Conclusion **106**
3.6 References **107**
Chapter Four **117**
General discussion, conclusions and recommendations
4.1 General discussion **117**
4.2 Conclusions and recommendations **118**
4.3 Appendices **120**

LIST OF ACRONYMS

°C	degrees Celsius
ANOVA	Analysis of Variance
B	Boron
Ca	Calcium
Cu	Copper
CPUT	Cape Peninsula University of Technology
EC	Electrical Conductivity
ESI	Electrospray Ionization
Fe	Iron
HCl	Hydrogen chloride
HSD	Honest Significant Difference
INT	*p*-iodonitrotetrazolium
IUCN	International Union for Conservation of Nature
K	Potassium
LC-MS	Liquid Chromatography-Mass Spectrometry
L/h	Liters per hour
MIC	Minimum Inhibitory Concentration
Mg	Magnesium
Min	Minute
Mn	Manganese

N	Nitrogen
Na	Sodium
NH_4^-	Ammonium
NO_3^-	Nitrate
RH	Relative Humidity
S	Sulphur
SANBI	South African National Biodiversity Institute
TA	Total Activity
T_R	Retention Time
P	Phosphorus
WHC	Water Holding Capacity
WHO	World Health Organization
Zn	Zinc

LIST OF TABLES

Table 1.1: Top five most frequently used and high valued medicinal plants. 11

Table 1.2: Chemical and physical properties of substrates used in this study. 20

Table 2.1: Composition of different substrate blends used in the study. 47

Table 2.2a: Effect of substrate combinations and watering regimes on growth parameters of *S. aethiopicus* at 9 weeks after planting. 59

Table 2.2b: Effect of substrate combinations and watering regimes on growth parameters of *S. aethiopicus* at 9 weeks after planting. 60

Table 2.3a: Total amount of irrigation water applied per plant during the experimental period. 61

Table 2.3b: Total amount of irrigation water applied per watering regime during the experimental period. 61

Table 3.1: Composition of different substrate blends used in the study. 79

Table 3.2: Effect of different substrate combinations and watering regimes on macronutrient uptake of *S. aethiopicus* aerial parts. 86

Table 3.3: Effect of different substrate combinations and watering regimes on micronutrient uptake of *S. aethiopicus* aerial parts. 87

Table 3.4: Results on yield, minimum inhibitory concentration and total activities of acetone extracts obtained from aerial parts of hydroponically-cultivated *S. aethiopicus* following exposure to various substrate combinations and watering regimes. 91

Table 3.5: Results on yield, minimum inhibitory concentration and total activities of acetone extracts obtained from rhizomes of hydroponically-cultivated *S. aethiopicus* following exposure to various substrate combinations and watering regimes. 92

Table 3.6: Preliminary results on yield, minimum inhibitory concentration and total activities of acetone extracts obtained from rhizome of tissue culture- cultivated *S. aethiopicus*. 93

Table 3.7: Effect of watering regimes on phenolic compounds of *S. aethiopicus* aerial parts. 97

Table 3.8: Effect of watering regimes on phenolic compounds of *S. aethiopicus* rhizomes. 97

LIST OF FIGURES

Figure 1.1: Geographical distribution map of *S. aethiopicus* 8

Figure 1.2: (A) *S. aethiopicus* bright pink flowers to white with a yellow centre 9

(B) Cone-shaped rhizomes and the aromatic roots of *S. aethiopicus* 9

Figure 1.3: Major water use sectors 23

Figure 2.1: (A) The experimental arrangement (setup) 48

(B) Table represents watering regimes (3 and 5-days interval); three gutters with different treatments and one gutter without plants used to determine water holding capacity. 48

Figure 2.2: Water holding capacity of four different substrate combinations 51

Figure 2.3: *S. aethiopicus* flowers appearing directly from the rhizome, independently of the leaves 52

Figure 3.1: Photograph showing anti-*Fusarium* activity of acetone extracts of *S. aethiopicus* 94

Figure 3.2: LC-MS profiles (TIC = total ion chromatogram) of acetone extract of *S. aethiopicus* aerial parts subjected to different watering regimes (3 and 5-days interval) 98

Figure 3.3: LC-MS profiles (TIC = total ion chromatogram) of acetone extract of *S. aethiopicus* rhizomes subjected to different watering regimes (3 and 5-days interval) 99

Figure 3.4: Chemical structures of the nine phenolic compounds isolated from *S. aethiopicus* 100

Abstract

The increasing demand for medicinal plants has led into serious over-harvesting of wild populations and presents an opportunity for potential profitable cultivation. Production of medicinal plants in controlled environments particularly hydroponic technology provides opportunities for high quality biomass accumulation and optimizes production of secondary metabolites. Water availability and supplies are becoming scarce, thus search for innovative irrigation practices is desirable and vital. The proper irrigation interval and growing media can play a major role in increasing the water use efficiency. Thus, *Siphonochilus aethiopicus* was cultivated by means of the hydroponic technique, under various substrate combinations and watering regimes. This study aimed to determine optimal growth conditions for enhanced plant biomass accumulation and improve quality of *S. aethiopicus* bioactive compounds. In Chapter 1, the conceptual background and scientific rationalizations of the study are presented. In Chapter 2, the research objective was to evaluate the effect of different substrate combinations and watering regimes on growth parameters of *S. aethiopicus*. Six weeks old seedlings of *S. aethiopicus* were cultivated using different substrate combinations and watering regimes. Coconut fiber (coir) was used as the main component for the preparation of media in different proportions and combinations; T1 (Coir + vermiculite + perlite + bark), T2 (Coir + bark), T3 (Coir + perlite) and T4 (Coir + vermiculite). Plants in different treatments were grown under two watering regimes: 3-days and 5-days watering intervals. Observations on the following parameters were recorded: water holding capacity (WHC), stem length, number of leaves, leaf length, number of rhizome eyes, new shoots, plant total weight, newly developed rhizome length; old rhizome length, dry weight of aerial parts, fresh weight of aerial parts, new shoots total weight, root length, root weight and number of roots. Quantity of irrigation water used per watering regime during the experimental period was also calculated. Results concerning WHC showed significant difference ($P < 0.05$) among treatments. The WHC of treatments ranged from 174% to 365%; maximum WHC (337.86%) was found in T3 while T2 recorded minimum WHC (201.06%). Furthermore, there was no significant difference ($P > 0.05$) among the treatments and watering regimes with respect to growth parameters; except for the number of roots, new shoots and stem length. Plants grown

in T4 showed a marked increase in number of roots (75.83 ± 4.35) and new shoots (4.16 ± 0.6) under 3-days interval while the tallest stem length was obtained in T2 (23.25 ± 1.07 cm) under 5-days interval watering. The highest mean values due to watering regimes were recorded with plants that received water every third day (3-days interval watering). Results from the quantity of irrigation water used per watering regime showed that 3-days interval received the highest application of water 695.52 L whereas; 5-days interval total amount of water applied throughout the study was 397.44 L. The 5-days interval reduced water application by 55% when compared to 3-days interval watering. The results demonstrated that *S. aethiopicus* may be cultivated hydroponically and 5-days interval watering for these substrate combinations has a potential to save water without any significant negative impact on plant growth.

In Chapter 3 experiments were conducted to assess the effects of substrate combinations and watering regimes on tissue nutrient content, antimicrobial and total activity of acetone extracts of *S. aethiopicus* (Zingiberaceae). At 9 weeks post treatment, plants (aerial parts) were harvested, oven dried and tissue nutrient content analysed. Nutrients were evaluated into two groups as macronutrients (N, P, K, Ca, Mg and Na) and micronutrients (Mn, Cu, Fe, B, Zn, NO_3^- and NH_4^-). The results showed that substrate combinations did not have any significant effect ($P > 0.05$) on the uptake of Ca, Na, Mn, Zn and NO_3^- in both watering regimes. Significant differences were observed in P, K, N, Mg, Fe, Cu, B and NH_4^-. The highest uptake of P (6266.6 ± 88.19 mg/kg) and B (73.33 ± 5.89 mg/kg) was obtained in T3; K (63000 ± 763.76 mg/kg) in T2; Fe (85.3 ± 2.18 mg/kg) in T1 while Cu (3.3 ± 0.33 mg/kg) and NH_4^- (4646.6 ± 140.1 mg/kg) highest uptake was recorded in T4 under 3-days watering interval. Furthermore, N uptake (33400 ± 360.55 mg/kg) best result was observed in T4 whereas, Mg (3233.3 ± 66.66 mg/kg) highest uptake was found in T1 under 5-days interval. Generally, the highest mean values for most nutrients were obtained in treatments under 3-days interval watering except Na, Mn, B and Cu which were highly enhanced by 5-days interval.

Powdered aerial parts and rhizomes (3 g) of *S. aethiopicus* obtained from different treatments were extracted with 60 ml of acetone. Substrate combinations and

watering regimes did not significantly affect the yield of crude extracts of *S. aethiopicus* aerial parts and rhizomes; aerial parts ranged from 104 ± 47.47 mg (T4) to 179 ± 10.17 mg (T3) while rhizomes yield ranged from 100 ± 0 (T4 and T3) to 166 ± 33.3 (T2). Acetone extracts of plants were screened for antifungal activity against *Fusarium oxysporum* using minimum inhibitory concentration (MIC) method. A preliminary study which examined antifungal activities of tissue culture grown rhizome was also conducted. The MIC values of acetone extracts of *S. aethiopicus* aerial parts and rhizomes were not statistically different ($P > 0.05$) among treatments and watering regimes. However, in both watering regimes the MIC values obtained with acetone extracts of plants (aerial parts) grown in T3 (3-days interval [0.5 ± .125 mg/ml] and 5-days interval [0.313 ± 0.06 and 0.375 ± 0 mg/ml]) were lower compared to the MICs of other treatments (T1, T2 and T4) at 12 and 18 hours respectively. Similarly, MIC value (0.078 ± 0.02 and 0.25 ± 0.06 mg/ml) of acetone extracts of *S. aethiopicus* rhizomes grown in T3 under 3-days interval watering was lower compared to the MICs of other treatments at 12 and 18 hours respectively. Conversely, in 5-days interval T3 and T4 (0.1875 ± 0 mg/ml) obtained the lowest MIC value at 18 hours. Additionally, the MIC value of the tissue culture grown rhizome (0.3125 ± 0.06 mg/ml) at 18 hours was not significantly different compared to hydroponically cultivated rhizomes. Generally, acetone extracts of *S. aethiopicus* plants that were grown in 5-days interval watering were the most bioactive against *F. oxysporum.* The MIC values obtained in the current study are relatively higher for the rhizomes ranged from 0.078-0.3125 mg/ml but very low in the leaves (0.375-0.75 mg/ml). Moreover, the total activities of the acetone extracts from the rhizomes and aerial parts of *S. aethiopicus* obtained among substrate combinations and watering regimes were not statistically different at 12 or 18 hours ($P > 0.05$); aerial parts TA ranged from 52.86 ± 27.2 to 203.2 ± 49.99 ml/g and rhizomes ranged from 118.5 ± 29.6 to 591 ± 118.2 ml/g. LC-MS analysis of acetone extracts of *S. aethiopicus* plants obtained from the treatments revealed the presence of phytochemicals such as caffeic acid, quercetin, p-hydroxybenzoic acid, rutin, kaempferol, epicatechin, naringenin, hesperetin and protocatechuic acid. The results revealed that rutin was the main compound in the aerial parts, while p-hydroxybenzoic acid was the main compound in the rhizome.

These results suggest that *S. aethiopicus* may be cultivated hydroponically. The antimicrobial activity and/or the phytochemical profile of the crude extracts are affected by nutrient content and watering regimes. Renewable plant parts such as leaves can be used instead of rhizomes to ensure more sustainable use of *S. aethiopicus*. The results confirm the use of the plant in traditional medicine, supported by the presence of the above mentioned polyphenolic components.

CHAPTER ONE

Introduction, background to the research problem and literature review

1.1. General introduction

South Africa has a very rich plant biodiversity, many of which are medicinally valuable (Afolayan & Adebola, 2004). It is one of only two countries in the world whose borders contain three globally recognised hotspots of biodiversity (Mittermeier et al., 2005). An estimated three million people in South Africa are currently using indigenous, traditional plant medicine for primary health care purposes (Van Wyk & Gericke, 2000). The supply of wild medicinal plant stocks is declining and highly valued species are becoming inaccessible due to extinctions and rapidly rising market prices (Mander et al., 2007). At least 40 South African plant species on the IUCN Red List are threatened in part by international trade (Moeng, 2010).

Cunningham (1998) and Liphadzi (2013) reported that several plant species, such as *Siphonochilus aethiopicus* have been exploited to such an extent that they are seldom found in unprotected areas in South Africa (Moeng, 2010). Williams et al. (2001) indicated that *S. aethiopicus* is already extinct from natural areas of Kwa-Zulu Natal, and possibly with time this will also happen in the Limpopo Province as the species has been recorded as the most used, most traded and the most scarce medicinal plant in the *muthi* market and communal lands around the province. *Siphonochilus aethiopicus* have several applications, ranging from treatments of flu and colds to being utilized as magic charm and for protection to treating ulcers and makgoma (Moeng, 2010). A study undertaken by Fouche et al. (2011) revealed that the plant has anti-inflammatory and anti-allergic activities anecdotal its effectiveness against asthma, sinusitis, cold and flu. The plant is now so scarce, nonetheless demand continues unabated and the plant's price continues to increase (Diederichs, 2006).

Medicinal plants are mostly used for their bioactive compounds and the high demand for ‘natural’ medicines has enthused cultivation of medicinal plant species. At present, most medicinal plants are grown under field conditions, but it is difficult to

control growing conditions and maintain constituent product yields under such circumstances (Simeunovic, 2002). Therefore, for this reason hydroponic production of medicinal plants can overcome these cultivation difficulties. Hydroponics is an ideal growing method for producing culinary and medicinal herbs (Smith, 2013). Plants grown hydroponically can be manipulated to optimize production of secondary metabolites (Hayden, 2006) because environmental factors such as temperature, light intensity and nutrient blends can be controlled (Matanzima, 2014). Experiments on many spices, herbs and medicinal plants have been successfully conducted using hydroponic systems instead of soil cultivation in order to eliminate possible inaccurate results that may occur in soil cultivation due to unmanageable variables (Soudek et al., 2006; Sgherri et al., 2010; Maggini et al., 2012). Hydroponic systems can be a solution to over-harvesting of medicinal plants and prevent medicinal resources from depletion. Cultivation of plant material in hydroponics offers many advantages such as good quality, all year round production, minimized use of water and high productivity.

Soilless culture is the modern cultivation system of plants that use either inert organic or inorganic substrate through nutrient solution nourishment (Asaduzzaman et al., 2013). Several substrates have evolved due to their unique properties for holding moisture, aeration, leaching or capillary action, and reuse potentiality (Bilderback et al., 2005; Mastouri et al., 2005). Mixture of different substrates has been used for higher growth and yield of several crops around the world (Seymour, 1993; Donnan, 1998). Individual components of mixed substrates are often chosen considering their properties so that they complement each other and the resultant medium possesses most of the desirable attributes for good plant growth and production (Raviv & Lieth, 2008). Substrates make irrigation management easier. Previous studies have shown that soilless culture can provide more efficient use of water and fertilizers (Schwarz, 1995; Jensen, 1997).

Sufficient amount of water in growing substrates is one of the most critical factors for plant growth and development (Beardsell et al., 1979). Water availability and supplies are becoming inadequate as a result of climate change and growing

competition between domestic and industry uses in many parts of the world, nursery industries are being encouraged to reduce the amount of irrigation water (Raviv & Blom, 2001; Cameron et al., 2004). The proper irrigation interval can play a major role in increasing the water use efficiency and the productivity by applying the required amount of water when it is needed (Ismail & Ozawa, 2009). Watson (2014) stated that secondary metabolites under different irrigation regimes has not been widely studied, therefore further studies in this direction are required to help growers to understand the balance between enhancing phytochemical content by regulating irrigation.

Thus, this study was carried out to investigate the influence of various substrate combinations and watering regimes on growth, quality of antifungal components and secondary metabolites of *S. aethiopicus*. Furthermore, the outcome of this research will be a bench mark that will recommend the best substrate blend that has high water retention capacity that could be used by communities and farmers with less water to cultivate *S. aethiopicus*.

1.2. Structure of the thesis

The study is divided into four chapters, which are concisely discussed.

Chapter One: Introduction, background to the research problem and literature review.
Chapter One comprises of conceptual background and scientific justification of the study, overview information on what problem rationalizes the undertaking of the work contained within this thesis and the aims and specific objectives of the study.

Chapter Two: Optimizing water holding capacity using inert substrate mixes under different watering regimes for hydroponic cultivation of *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt (Zingiberaceae).
The main objectives of this chapter were (i) to evaluate the water holding capacity of different substrate combinations using coconut fiber as the main component and recommend substrate combination that will minimize water usage while optimizing plant growth and (ii) evaluate the effect of the substrate blends and watering regimes on

growth parameters of *S. aethiopicus*. The research rationalization, materials and methods, results and discussions are presented.

Chapter Three: Effect of different substrate combinations and watering regimes on nutrient uptake, anti-*Fusarium oxysporum* (Ascomycota: Hypocreales) activity and secondary metabolite profile of rhizomes and foliage extracts of hydroponically-cultivated *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt (Zingiberaceae).
The objective of this chapter was to evaluate the effect of substrate combinations and watering regimes on macro- and micronutrient uptake, anti-*F. oxysporum* activity and secondary metabolite profile of *S. aethiopicus*. The research rationalization, materials and methods, results and discussions are presented.

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

1.3. Background to the research problem

The basic needs for human survival are food, cloth and shelter however, the need for medicine to prevent diseases and illness and to lead a healthy life is paramount importance (Mitra' & Mukherjee, 2005). Traditional medicines, particularly herbal medicines have been increasingly used worldwide during the last two decades (WHO, 2003). According to recent estimates by the World Health Organisation, more than 3.5 billion people in the developing world rely on plants as components of their primary health care (Balick & Cock, 1996; Bodeker et al., 1997). Medicinal and aromatic plants occupy a priming economic position because of the continuous and increased demand of their products at local, national and international markets (Kumari & Prasad, 2013). Medicinal and herbal plants are largely used for their contents of bioactive compounds and are increasingly cultivated on a commercial scale to satisfy the large demand for natural remedies (Maggini et al., 2012).

South Africa is home to over 30,000 species of higher plants and 3000 of these species have been found to be used in traditional medicine across the country (Van Wyk et al., 1997) and about 350 species are the most commonly used and traded medicinal plants (SANBI, 2006). In South Africa, the national value of trade in medicinal plants alone (approximately 20 000 tonnes), is estimated at R270 million annually (Dold & Cocks, 2002).

Increasing human activities is one of the major factors that have led to a number of plant species to have become extinct or under pressure. Habitats throughout the subcontinent are transformed to meet the needs of people such as food, water, disease management, climate regulation etc. Studies have shown that 62 southern African plant taxa are believed to be extinct, 277 endangered, 445 vulnerable and 1446 rare (Hilton-Taylor, 1997; Botha et al., 2004). For example in Africa, *Warburgia salutaris* and *S. aethiopicus* are among the most highly valued species that are in high demand but very scarce supply (Bodeker et al., 1997; Kuipers, 1997). South Africa has the richest plant biodiversity in the world, many of which are medicinally useful (Afolayan & Adebola, 2004). The rich resources in plant biodiversity are decreasing at an alarming rate as a result of over- exploitation. It is estimated that over half a million people are directly involved in medicinal plant trade in the country. With the current rate of harvesting, the plant supplies will, in time decline and many of the species will eventually become extinct (Afolayan & Adebola, 2004). Today, many medicinal plants face extinction or severe genetic loss (Akerele et al., 1991), but cultivation of medicinal plants to provide an additional or alternative stock of plants is very limited (Geldenhuys & Mitchell, 2006 In: Diederichs, 2006). The documentation of commercial cultivation of South African medicinal plants is recommended due to rapid loss and high demand of natural habitat. Liu et al. (2007) stated that cultivation can be an effective way to decrease the pressure on utilization of wild medicinal plants for commercial trade or household consumption.

Hydroponic farming is becoming a favourite method to produce crops in South Africa, and one major advantage is that plants and flowers are available all year round. South Africa has enormous areas of arid land ranging from semi-desert to desert as well

as places with only limited ground water. Farmers in most cases have no alternative but to resort to high density crops where the minimum amount of water is used (van Zyl, 2012). Production of medicinal herb and root crops in controlled environments provides opportunities for improving the quality, purity, consistency, bioactivity, and biomass production of the raw material (Hayden, 2006). Producers of medicinal plants are also attracted to hydroponic systems for cultivation because recent studies proved that growing in soilless cultures in controlled environments results in higher concentrations of active principles in plants compared to traditional soil cultivation (Giurgiu et al., 2014). It is therefore hypothesized that hydroponic technology can influence growth and production of secondary metabolites in medicinal plants.

1.4. Statement of the research problem

One of the bioactive compounds from *S. aethiopicus* (Zingiberaceae) is being developed in South African traditional medicine for the management of asthma and allergies (Fouche et al., 2013). Scientific research has confirmed the medicinal properties of this plant, but unfortunately it has been overharvested and is nearly gone into extinction in most of Africa (Anonymous, 2014). Street and Prinsloo (2012) stated that the increasing demand for this plant has led into serious overharvesting from the wild and presents an opportunity for potential profitable cultivation. Public cultivation information for *S. aethiopicus* is poorly developed (Hartzell, 2011). Cultivation of medicinal plants is minimal in South Africa. Consequently, certain popularly traded species including *S. aethiopicus* have become over-exploited and are now rare or extinct in the wild (Diederichs et al., 2002). Some of the obstacles in the cultivation of medicinal plants include lack of seed stock or cutting material and a lack of knowledge on how to cultivate the plants (Jager & van Staden, 2005). Although *S. aethiopicus* is a rare plant in South Africa that has become extinct in some provinces (Hilton-Taylor, 1996), information regarding its cultivation by means of hydroponic technology is lacking. There is also inadequate information about the effect of different substrate combinations and watering regimes on growth and secondary metabolite production of *S. aethiopicus*.

1.5 Literature review

1.5.1 *Siphonochilus aethiopicus*

Siphonochilus aethiopicus (Schweinf.) B.L. Burtt is one of the most popular medicinal plants in South Africa (Williams, 1996). The generic name *Siphonochilus* is derived from the Greek *siphono* meaning tube, *chilus* meaning lip in reference to the shape of the flower, and the specific name *aethiopicus* means from southern Africa (Hankey & Reynolds, 2002). *S. aethiopicus* (Schweinf.) B.L. Burtt, commonly known as wild ginger or Natal ginger, belongs to the monocotyledonous family Zingiberaceae. The Zingiberaceae is a family well known for its spice plants, including real ginger, *Zingiber officinale*; cardamom, *Elletaria cardamomum*; and turmeric, *Curcuma longa* (Light, 2002). Light (2002) also stated that many members of the family are used in traditional medicine around the world and a variety of compounds which demonstrate anti-inflammatory activity have been isolated from a number of species of the Zingiberaceae. The species are usually caulescent aromatic herbs with thickened rhizomes and secretory cells with volatile oils (Hutchings et al., 1996). Over 40 genera and about 1300 species are found in the Zingiberaceae. They occur mainly in the tropics of the Old World (Indomalasia), with some representatives in South and Central America (Smith, 1998).

1.5.2 Geographical distribution of *S. aethiopicus*

Wild ginger is native to southern tropical Africa (South of Malawi to the eastern part of South Africa) in South Africa it is distributed in Mpumalanga and Limpopo (extinct in KwaZulu-Natal) (Department of Agriculture, 2009). Hartzell (2011) reported that *S. aethiopicus* (Schweinf.) B.L. Burtt is widespread in the savanna regions of tropical Africa, from Senegal to Ethiopia, south Zimbabwe, Mozambique, Zambia and South Africa. Its habitat is deciduous woodland, bushland and wooded grassland. (Street & Prinsloo, 2012) (Figure 1.1).

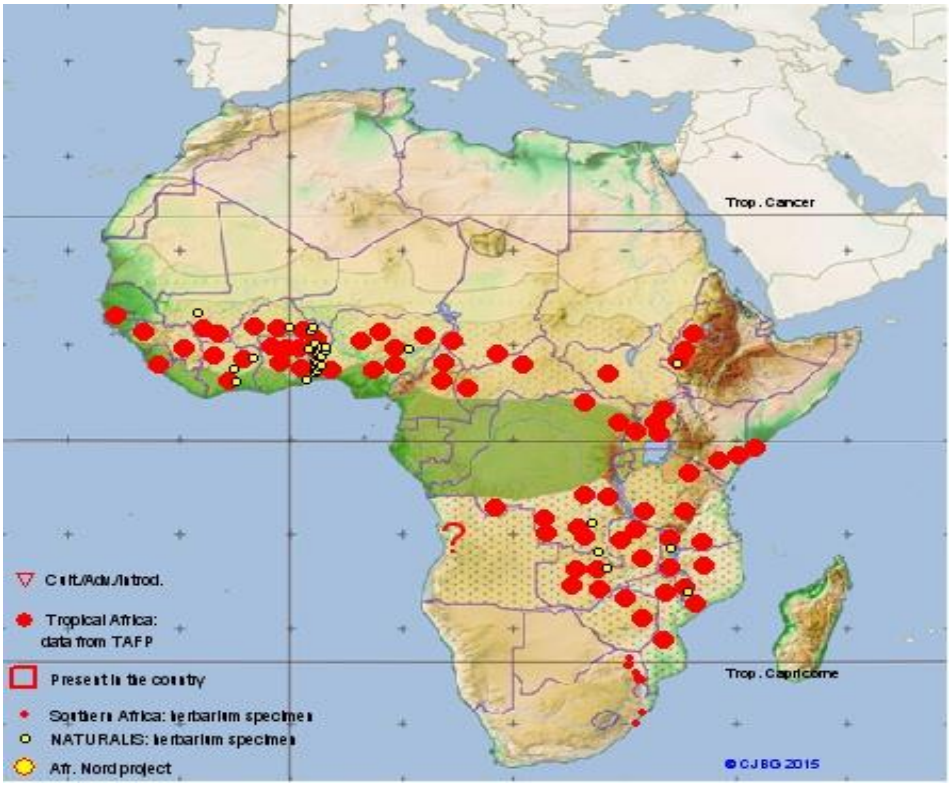

Figure 1.1: Geographical distribution map of *S. aethiopicus* (Adapted from: http://www.ville-ge.ch/musinfo/bd/cjb/africa/details.php?langue=an&id=20110).

1.5.3 Morphological characteristics of *S. aethiopicus*

Wild ginger produces broad grass-like leaves that emerge in spring from the underground rhizome (Manzini, 2005). The leaves are deciduous and sprout annually; they may reach a height of up to 400 mm. The leaves are light green, lanced shaped and borne on the end of stem like leaf bases (Hankey & Reynolds, 2002). The stems grow up to a maximum height of 2 m (Directorate: Plant Production, 2013).

Wild ginger has tremendously attractive flowers, which are borne at ground level and are very short-lived (Manzini, 2005). Reminiscent of orchid flowers, the blooms which are borne from October to February are delicate in texture. They may vary in colour from bright pink to white with a yellow centre and are delicately fragranced

(Hankey & Reynolds, 2002) (Figure 1.2A). Flowers often appear before the leaves in spring, perhaps to allow them to be more visible to pollinators. About 15 flowers are produced per plant over the flowering season, each lasting a single day (Nichols, 1989). Small, berry-like fruits ripen below or above the ground. The plants are characterised by a small cone-shaped rhizomes, which have a distinctive pungent smell (Viljoen et al., 2002) (Figure 1.2B). Up to twenty swollen tubers may be attached to the rhizome, each connected by succulent roots (van Wyk & Gericke, 2000). It is a forest floor plant with aromatic rhizomatous roots (Hankey & Reynolds, 2002).

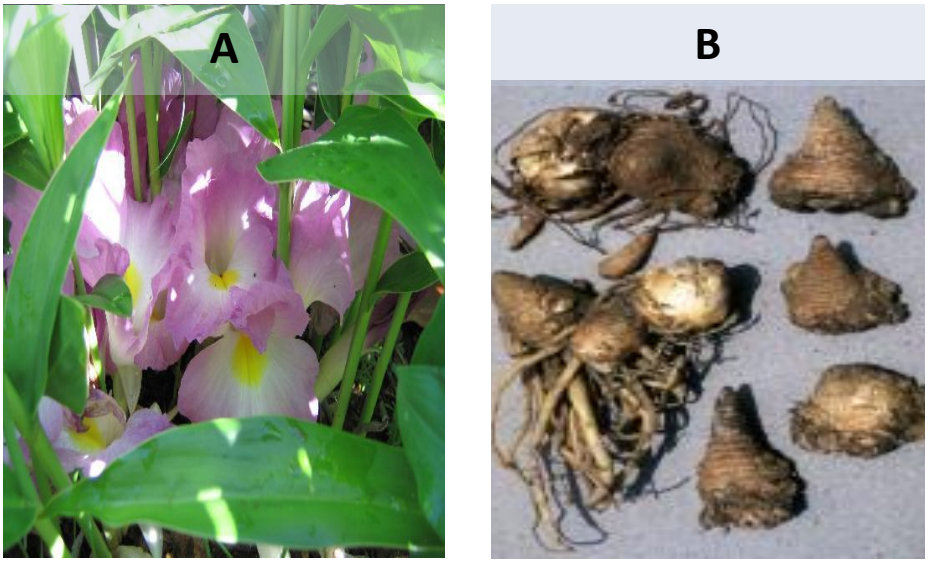

Figure 1.2: (A) *S. aethiopicus* bright pink flowers to white with a yellow centre (Adapted from: http://pacificbulbsociety.org/pbswiki/index.php/Siphonochilus (B) Cone-shaped rhizomes and the aromatic roots of *S. aethiopicus* (Adapted from: http://www.bigtreehealth.com/product-information-africanginger.php).

1.5.4 Growth requirements of *S. aethiopicus*

The wild ginger is easy to cultivate provided it is given a well-drained, compost rich soil and a warm, but shady position either in a container or in the garden (Nichols, 1989). It prefers high temperatures and grow best in a red, yellowish-brown soil rich in organic matter. The best soil pH for wild ginger is 6.0 to 7.0 (Department of Agriculture,

2009). During the early establishment phase when more roots are required, a slightly above ambient temperature of between 20-22 ºC and air temperature of around 20 ºC should be maintained (Manzini, 2005; Mirza, 1996). Watering should be reduced to a minimum during the winter months while plant is dormant and may be resumed with the onset of spring. During the growing season plants respond very well to high levels of feeding with organic matter (Nichols, 1989). According to Mirza (1996), in the early stages of plant development the requirement of phosphorus is high.

1.5.5 Propagation

For propagation of wild ginger, the rhizomes are divided in winter when the plants are dormant. Plants do not produce much seed and splitting rhizomes is the best available option for plant propagation (Manzini, 2005; Pooley, 1998). According to Nichols (1989), plants can be propagated from seed, which can take up to a year to germinate. Tissue culture technique has been successfully used to propagate *S. aethiopicus* (Hankey & Reynolds, 2002; Diederichs, 2006).

1.5.6 Current status of *S. aethiopicus*

S. aethiopicus is well known for its popularity in traditional medicine and there is a growing concern about its conservation status. It is often quoted as being locally extinct in some parts of South Africa as a result of over-exploitation (van Wyk, 2008). This plant is currently listed as critically endangered on the South African Red Data list of plants (Hankey & Reynolds, 2002; Raimondo, 2011). Hartzell (2011) stated that according to the Swaziland Flora Database, *S. aethiopicus'*s Red Data Book status is EN A1d, formerly listed as rare. Wild ginger was listed as an endangered species in the NATIONAL ENVIRONMENTAL MANAGEMENT; BIODIVERSITY ACT, 2004 (ACT 10 of 2004), one category lower than critically endangered, facing a high risk of extinction in the wild in the near future as opposed to an 'extremely high risk' (Hartzell, 2011). Even though the plant is now so scarce, demand continues unabated and the plants price continues to soar (Diederichs, 2006); as a result it is one of the top five, most frequently used and high valued medicinal plants in Africa (Table 1.1).

Table 1.1: Top five most frequently used and high valued medicinal plants

Species	Distribution	Traditional use	Reference
Agathosma betulina	Western Cape	High blood pressure, UTI infections, arthritis, gout	Anton, 2013
Harpagophytum procumbens	North West, Free State, Northern Cape, Limpopo, Namibia and Botswana	Pain, enhance mobility, relief from musculoskeletal conditions, diabetes, neuralgia, headaches and menstrual problems	Anton, 2013 Smithies, 2006
Hypoxis hemerocallidea	Eastern Cape, KwaZulu-Natal, Mpumalanga, Gauteng and Limpopo	Immune boosting properties, Cancer, tuberculosis, Asthma, HIV Aids	Anton, 2013
Perlargonium sidoides	Eastern Cape, Free State, Lesotho and Gauteng	Bronchitis, sore throat, sinusitis, colds and flu	Anton, 2013 Lawrence, 2001
Siphonochilus aethiopicus	Mpumalanga and Northern Cape	Coughs, colds, asthma, flu, candida and menstrual cramps	Anton, 2013 Hankey & Reynolds, 2002

1.5.7 Some of the medicinal and traditional uses of *S. aethiopicus*

The freshly cut rhizomes and roots of wild ginger are very popular in traditional medicine in southern Africa (van Wyk et al., 1997). The highly aromatic roots have a variety of medicinal and traditional uses and the native South African people have cultivated this plant for many years. The rhizomes and roots are chewed fresh to treat asthma, hysteria, colds, coughs and flu (Hankey & Reynolds, 2002). Wild ginger is also used to treat malaria, oral and vaginal thrush, headache, chest ailments (Department of Agriculture, 2009) and it is chewed by women during menstruation (Nichols, 2005). Elsewhere in the region, rhizomes have been employed in the treatment of rheumatism, toothache, neuralgia and to decongest nasal passages (Crouch et al., 2000 In: Nichols, 2005).

According to Hankey and Reynolds (2002), preparation of this plant is administered to horses as prevention against horse sickness and it is used by Zulu people as a protection against lightning and snakes. It is also used as a mosquito repellent and wound dressing (Lategan, 2008). The Xhosa of Idutywa (Eastern Cape) use the powdered roots of *S. aethiopicus* commonly known as isphephetho to ward off evil spirits. The wild ginger belongs to the same family as the true ginger which is widely used for culinary purposes. It can also be used in food for human consumption (meat stews and salads) and forest revegetation (Department of Agriculture, 2009).

1.5.8 Some of the phytochemicals and antimicrobial activity of *S. aethiopicus*

Phytochemical analysis of *S. aethiopicus* extracts showed some of the potential chemical compounds. Three fatty acids were noted in the parts of the plant these include palmitic acid, oleic acid and linoleic acid. The characterization tests discovered that *S. aethiopicus* organs were important source of saponins. Catechin tannins, mucilage's and leucoanthocyanes were particularly abundant in rhizomes (Noudogbessi et al., 2013). Little is known of the secondary chemistry of this species. A report of the

volatile oil composition of an unidentified *Kaempferia* (now *Siphonochilus*) species appeared in 1915 and ongoing work has identified α-terpineol and a sesquiterpene as constituents of the oil in *S. aethiopicus* rhizomes (Kanfer, 2010). In a study carried out by Igoli and Obanu (2011), terpenes were the major constituents of the volatiles of both fresh and roasted rhizomes of wild ginger.

The members of the family Zingiberaceae antimicrobial activities against a wide range of microorganisms and their antioxidant activities have been documented. Many studies have demonstrated that this family contain bioactive compounds that have excellent antimicrobial activities against a diverse group of pathogens (Voravuthikunchai, 2007; Lindsey et al. 1999; Coopoosamy et al. 2010). Light et al. (2002) found that extracts from the leaves, rhizomes and roots of *S. aethiopicus* showed antibacterial and anti-inflammatory activity. The ethanol and ethyl acetate extracts showed great antibacterial activity at minimal inhibitory concentrations ranging from 0.78 to 3.13 mg/ml against *Bacillus substilis, Staphylococcus aureus*. Similarly, Lindsey et al. (1999) discovered antibacterial and cyclooxygenase activities from the rhizome of *S. aethiopicus* using ethanol extracts. Coopoosamy et al. (2010) also observed that both the rhizomes and leaves of *S. aethiopicus* exhibit antibacterial and antifungal activities. Although the antimicrobial activity in leaves is more limiting, it could assist in reducing the use of the rhizomes for traditional treatments ensuring a more sustainable use of the plant.

1.6 Challenges allied with soil cultivation of medicinal plants

Cultivation of some herbs has proved difficult because of low germination rates or specific ecological requirements (Vines, 2004). Plants are susceptible to pathogenic microorganisms mainly bacteria and fungus, which cause dramatic negative effects on cultivated-plant yields leading to loss of all production (Mhadhbi, 2012). Under favorable environmental conditions such as high temperature and humidity, microorganisms can proliferate to an extent that could cause negative effects to plant yields (Mhadhbi, 2012). Secondary metabolite accumulation is similarly affected by water availability,

variations in soil pH and nutrients (McChesney, 1999). Soil-based agriculture is facing some major challenges with the advent of civilization all over the world, such as decrease per capita land availability. Apart from this, due to rapid urbanization and industrialization as well as threats from climate change and its related adverse effect, the soil-based cultivation is going to face further challenging threats (Hussain et al., 2014). At present, most medicinal plants are grown under field conditions but it is difficult to control growing conditions and maintain consistent production yields under such circumstances (Simeunovic, 2002).

Each day, the natural resources like soil and water are becoming scarce and require new ways to rationalize their use. Nowadays, opening new agricultural frontiers is not practicable due to deforestation and concern for the environment. It is becoming increasingly necessary to improve the productivity of different species of plants by breeding and/or by other techniques such as hydroponics that guarantees the preservation of natural resources like water and soil and increases the productivity (Correa et al., 2012).

1.7 Role of biotechnology for protecting medicinal plants

1.7.1 Tissue culture (micropropagation)

Plant tissue culture, also called micropropagation, is a practice used to propagate plants under sterile conditions or in a controlled environment, often to produce clones of a plant (Akin-Idowu et al., 2009). Plant tissue culture technology is being widely used for large scale plant multiplication. Apart from their use as a tool of research, plant tissue culture techniques have in recent years become a major industrial importance in the area of plant propagation, disease elimination, plant improvement and production of secondary metabolites. However, micropropagation technology is expensive as compared to conventional methods of propagation by means of seed, cuttings and grafting etc. (Hussain et al., 2002). Disadvantages of tissue culture are economic (pricey tools, high ostentation) and multiplication of elevated number of genetically alike plants has no logic from defense point of view (Maryam et al., 2014).

Micropropagation by tissue culture has bought *S. aethiopicus* back from the brink of extinction although the wild population are reportedly almost totally depleted (Hankey & Reynolds, 2002). Both Kirstenbosch and Durban Botanic Gardens have developed tissue culture, but this is an expensive way to source plant material. Although tissue culture may be popular with biotechnology funders, it is not really a practical option for most farmers for source material, due to the high costs of purchasing tissue culture plants (Hartzell, 2011).

1.7.2 Hydroponic technology (Soilless Culture)

An alternative to conventional crop production of edible and medicinal species is hydroponic cultivation (Montanari et al., 2008). Growing of plants in water or nutrient solutions, referred to as hydroponics, has been practiced for centuries. The term hydroponics was given by Dr. W.F Gericke in 1936, who did several studies on planting vegetables using water (Abdullah, 2001). Hydroponics is becoming a very interesting alternative compared to traditional farming cultivation on soil. It can be used in regions where there is limited availability of arable land and in regions where there was an excessive use of the soil, causing imbalance of chemical and biological characteristics, and high infestation of plant pathogens, frequent problems in protected cultivation (Correa et al., 2012). The switching over from the soil-based cultivation to hydroponic techniques has led to a decrease in the application of pesticides and other toxic agrochemicals, which are often used in soil-grown crops to disinfect the soil and to control soil-borne pathogens (Savvas, 2003).

Hydroponics is used mainly as a controlled system for the production of out of season crops, for growing crops in areas where the soil is not suitable for cultivation, or where water supply is limited (Anderson et al., 1989). In general, hydroponic plants only use one-tenth of the amount of water used by plants grown in soil because in traditional farming majority of the water supply leaches through the root layer quickly (Ortiz et al., 2009). Wahome et al. (2011) testified that plants grow faster in hydroponics because they get all the nutrients they need in the proper amounts and proportions. In soil, plants

develop a large root system to enable them search for nutrients and water. In hydroponics, nutrients and water are provided directly to the roots. The root environment of hydroponic plants is abundantly provided with water, nutrients and air, with a corresponding increase of the metabolic and absorbing activity of roots, which in its turn, initiates development of plant over ground part with increasing biological and economical productivity in several folds (Mairapetyan, 1999).

The controlled environments can also produce a more consistent herbal raw material by accommodating clonal propagation techniques, allowing multiple harvests of both aerial parts and roots from a single crop, and extending the growing season (Hayden, 2006). Hydroponic technology may be applied to produce high standard plant material all year-round in consideration of the possibility to control growing conditions and to stimulate secondary metabolism by appropriate manipulation of mineral nutrition (Maggini et al., 2012).

According to Manukyan (2011), soilless greenhouse production of medicinal and aromatic plants is a perspective and economical alternative for high productivity, superior quality with ecological sound and ensures that the plants are free from biotic and abiotic contaminations with consistent biochemical profiles. Medicinal plants are inherently variable in content of secondary metabolites. It is desirable to develop systems that can assure production of plants with high levels of medicinally active compounds (Fonseca et al., 2006). The use of controlled environments can overcome cultivation difficulties and could be a means to manipulate phenotypic variation in bioactive compounds and toxins. Controlled growth systems also make it feasible to contemplate manipulation of phenotypic variation in the concentration of medicinally important compounds present at harvest (Canter et al., 2005). Greenhouse production due to a better control of the environment appears to be an ideal system to produce plants with consistently high levels of desired phytochemicals (Fonseca et al., 2006). Al-karaki and Othman (2009) proposed that soilless cultivation is capable of superior yields compared to conventional field production and should serve as a model for cultivation of other medicinal/aromatic plants under soilless conditions.

1.8 Growing substrates and substrate mixes

1.8.1 Growing substrates

Soil is the natural growth media for cultivation of many crops. However, it has created problems such as soil borne diseases, undesirable microbial activities and nematodes, changing acidity levels, salinity, poor drainage, poor nutrient levels and undesirable soil characters (Ananda & Ahundeniya, 2012). Soil has been replacing by many organic and inorganic substrates, since they are disease and pest free inert material capable of holding required sufficient moisture and can be reused year after year (Asaduzzaman et al., 2015). Growing media serves four functions. It provides water to the plant roots, supplies nutrients, allows for gas exchange to and from the roots, and provides physical support as an anchor for the plant (Fonteno, 1996). The application of a soilless culture system using artificial substrates could result in efficient and effective use of water and fertilizers and minimize the use of chemicals for pest and disease control (Hassain et al., 2014). According to Ghehsareh et al. (2012) different substrates have several materials (chemical and physical properties) which could have direct or indirect effects on plant growth and development (Table 1.2). This study focused on four growing substrates (coconut fibre (coir), perlite, vermiculite and bark).

1.8.1.1 Coconut fiber (coir)

Coconut is grown commercially in Sri Lanka, the Philippines, Indonesia, southern India and Latin America. These countries are the main source of coir for use in horticulture. Coconut coir, also known as coco dust, coco peat and coco fiber is derived from the mesocarp tissue, or husk of the coconut (*Cocos mucifera*) fruit. The husk contains 60−70% pith tissue and the remainder is mainly fibre (Raviv et al., 2002). With the addition of water, coir expands to 5 to 9 times its compressed volume. Coir dust has been claimed to enhance rooting due to the presence of root- promoting substances (Raviv et al., 2002). Coir is known for its water retention quality. It holds the water and keeps plant hydrated. This is the reason why coconut coir makes gardening a lot easier with less water (Henrymorduch, 2013). The ease of re-wetting and the quick drainage characteristics of coir mean that it needs to be irrigated less frequently and for shorter

periods. These characteristics lead to reduced leaching and lose of nutrients and lower water use (Wakenbake, 2007).

Coconut coir is an inert medium; this implies that growers have full control over maintaining optimum nutrient levels. It is also better than most other mediums at promoting beneficial bacterial growth and protecting the root zone from heat stress (Withers, 2014). Coir contains natural anti-fungal properties, offering the plant protection from many common root diseases (Mahmood, 2004). It is a highly versatile substrate that can be used in most hydroponic systems and can be mixed with other mediums like clay pebbles (Withers, 2014). Coir can be used in soilless potting mixes or mixed with soil in concentrations of up to 80% of the mixture (Kokemuller, 2014). Many growers find that a 50/50 mix of coir and clay pebbles is an effective formula, providing high moisture retention, optimum nutrient control, and plenty of oxygenation around the root zone for increased uptake and healthier plant development (Withers, 2014).

1.8.1.2 Perlite

Perlite is composed mainly of minerals and is an expanded volcanic glass. The expansion process gives perlite a very porous structure and fantastic aeration properties. It also has a neutral pH and a highly absorbent substance that is great at retaining water and nutrients, without shrinking or getting soggy (Withers, 2014). Perlite holds three to four times its weight of water. It has a pH of 6.0 to 8.0 with no buffering capacity. Unlike vermiculite, it has no cation exchange capacity and contains no mineral nutrients (Hartmann & Kester, 1983). Expanded perlite is very light with a particle and bulk density of 0.9 and 0.1g cm^{-3}, respectively. It is provided in various grades, the most common being 0-2 and 1.5-3.0 mm in diameter (Raviv et al., 2002).

Horticultural perlite has a long and enviable record of performance as a propagating and growing medium throughout the world. It has been successfully used in virtually all horticultural applications including glasshouse growing, landscaping, lawn and stadium turf and in a variety of container applications (Hall, 2014). Nutrient enriched

water is trapped in the tiny irregularities on the surface of perlite particles where it is available for use by the plant roots. Roots in perlite are always well aerated and well watered (Hall, 2014). Perlite is widely preferred as it encourages faster root development, reduces risk of damping off, avoids water logging and provides an optimum balance of air and water (Asaduzzaman et al., 2013). Another benefit to using perlite is its potential to be reused in multiple growing seasons, thereby cutting costs (Hanna, 2010). Perlite is now used extensively for its light weight, physical stability and ability to provide non-capillary pore space in a mix (Ingram et al., 1993). It is usually included in a mix to increase drainage but does not increase the retention of nutrients (Robbins & Evans, 2004).

1.8.1.3 Vermiculite

Vermiculite like perlite is a volcanic rock that is heated at high temperatures and forms expanded glass pebbles (Rebel, 2012). It is a micaceous mineral that expands markedly when heated. Extensive deposits are found in Montana and North Carolina (Hartmann & Kester, 1983). Vermiculite holds more water than perlite and has a natural wicking property that can draw water and nutrients into a passive hydroponic system (Venter, 2010). Its high water absorption capacity and the fact that it can hold nutrients in reserve for later use make it beneficial to many growers (Rapaka, 2013). It is very porous, has a strong capillary action and can hold 3-4 times its weight of water. Vermiculite is neutral, with a pH of 7.0 to 7.5 and low electrical conductivity (EC). Like the raw material, it has a permanent negative charge. Disposal of vermiculite is not hazardous to the environment (Raviv et al., 2002).

1.8.1.4 Bark

The major component of nursery substrates for many years, which has also become important in greenhouse substrates in more recent years, is aged softwood bark. (Fields, 2012). Bark is a by-product of the wood and paper industries. Bark is stripped off the logs in the sawmills by debarking machines and has to be hammer milled to pass an appropriate screen, before being used in growing media, either fresh or after composition (Raviv et al., 2002). Bark has been recognized as a suitable component for container growth media since the 1960’s and in some cases it is a good

single component growth medium (Ingram et al., 1993). Both fresh softwood bark and composted softwood and hardwood bark are among the most popular growing and rooting media. Bark is a light weight material, having a bulk density of 0.1-0.3 g $cm^{-3.}$ The main characteristic of bark is its high air filled porosity. Being a naturally biodegradable material, used bark can be recycled in many ways, including soil application (Raviv et al., 2002). Bark has proven to be a successful component of many peat-based mixes improving the drainage of these mixes largely due to its generally high air filled porosity and low water holding capacity (Alexander, 2014). Pine bark is the least expensive media (Hanna, 2009). Snyder (1993 and 1994), found that yields from plants grown in pine bark were either superior or did not differ compared to other growing media, like perlite and rock wool.

Table 1.2: Chemical and physical properties of substrates used in this study. Adapted from Wilkinson et al., 2014 and Douglass et al., 2009.

Substrate	**Bulk density**	**Porosity (Water)**	**Porosity (Air)**	**Ph**	**Cation Exchange Capacity**
Vermiculite	Very low	Very high	High	3-6	High
Perlite	Very low	High	High	6-8	Very low
Coconut fiber (Coir)	Low	High	High	6-7	Low
Bark	Low	Low	Very high	3-6	High

1.8.2 Substrate mixes

Fifteen years ago most nurseries obtained container media components and blended them according to their specifications. During the past ten years, there has been a strong trend among nursery owners to purchase pre blended potting mixes from specialty firms (Ingram et al., 1993). Most soilless media are usually blends of two or more components and the components and their ratios affect both the physical and chemical properties of the media (Fonteno, 1996). Raviv and Lieth (2008) stated that the components of soilless growing media and potting mixes used in horticulture are

primarily selected based on their physical and chemical characteristics and in particular, their superior ability to provide simultaneously sufficient level of oxygen and water to the roots. Nursery producers create their own substrates by mixing two or more components (Gabriel et al., 2009).

When various components are mixed together, a homogenous mixture must be obtained. This includes fertilizer amendments as well as growth medium components (Ingram et al., 1993). Gardening with soilless potting mix does not include the use of soil. Instead, plants are grown in a variety of organic and inorganic materials. Mediums are mixed together rather than used alone, as each usually provides its own function (Phipps, 2014). Individual components of mixed substrate are often chosen considering their properties so that they complement each other and the resultant medium possesses most of the desirable attributes for good plant growth and production (Raviv & Lieth, 2008). Soilless mixes are frequently used for container gardening, wick systems and non-recovery drip systems. The best soilless mixes for semi-hydroponics should have great wicking action and retain water and air well.

1.8.3 Importance of substrate (growing media) water holding capacity

Good quality water is becoming scarcer and more expensive (Reddy, 2012). Globally, water is one of the main limiting factors in agricultural production. However, a large amount of water is consumed while producing plant mass (Graber et al., 2010). Greenhouse growers are looking for ways to reduce their water bills. Growing media is the most important factor in how successful a grower is when watering. A proper growing media helps growers save water. One way to save water is to make the growing media capture more of the water supplied (Reddy, 2012). Robbins and Evans (2004) indicated that the media should be well drained and yet retain sufficient water to reduce the frequency of watering. Water efficiency of containerized crops produced in soilless substrates has become an important topic in horticultural research (Nemali & van Iersel, 2008). The choice of the growing media can be enhanced by using detailed

study of the physical and hydraulic characteristics of the growing media (Raviv et al. 2001). The water holding capacity of any medium is a very important characteristic. Organic soilless mixes that hold generous amount of water are less subject to leaching losses of nutrients. (Mahmood, 2005). The water holding capacity of a medium is the volume of water that is retained by a medium after irrigation and drainage (Ingram et al., 1993). Irrigation frequency is an important consideration when it comes to selecting the ideal substrate; it depends on the water holding characteristics of the substrates (Buechel, 2015). Minimizing the water discharge in closed, recirculated growing systems increases the water use efficiency considerably (Graber et al., 2010). Managing water resources is one of the most important challenges in production (van Iersel et al., 2010). Therefore, knowledge about substrates, substrate mixes and their water holding capacity is important.

1.9 Use of improved irrigation technology (watering regimes) to enhance water use efficiency

Water scarcity is regarded as the main limiting factor affecting worldwide crop production (Nuruddin et al., 2003). South Africa is a semi-arid, water stressed country. It is estimated that by 2025 based on current usage trends, water demand will exceed availability of economically usable fresh water resources. Agriculture irrigation represents approximately 60% of the total water requirement (Anonymous, unpublished) (Figure 1.3). Reducing agricultural water use while maintaining or improving economic productivity of the agricultural sector is a major challenge in arid and semi-arid regions (Al-Karaki & Al-Hashimi, 2011). Irrigation technologies are very important for cultivation of commercial plants because the relative amount of water available to agriculture is declining worldwide, due to the rapid population growth and the greater incidence of drought in recent years as caused by climate change and different human activities (Wahid et al. 2011).

To solve the water crisis problem it is vital to establish the optimum water quantities to be supplied, to obtain tremendous yields of high quality. This will help growers in reducing extra expenditure on water and recording good profits

(Sonnenberg, 2012). Water management plan for each crop is a crucial factor for maintaining a regular consistent supply of soil water. Water scarcity is a growing concern for sustainable agriculture worldwide. Therefore, many nations have attempted to reform water management systems by improving irrigation systems (Mohamed, 2014). Different irrigation regimes and their effect on water relations have been studied in many tree crops, and other perennial and annual plants (Massenti, 2013). In recent years the effective role of water supply on the growth and production of several medicinal plants was observed by many researchers (Mohamed et al. 2014). Irrigation may also affect the volatile oil composition. According to Hassan and Ali (2014) the volatile oil of various medicinal and aromatic plants was affected as a result of applying different irrigation treatments. Limited water supply is an important factor affecting growth and metabolic activities of plant species (Hassan & Ali, 2014).

Comparing to sprinkler irrigation, drip irrigation system can reduce water use by 50 percent (Mohamed, 2014). An understanding of water use in relation to plant growth is of importance for sustainable agriculture (Harb et al., 2010).

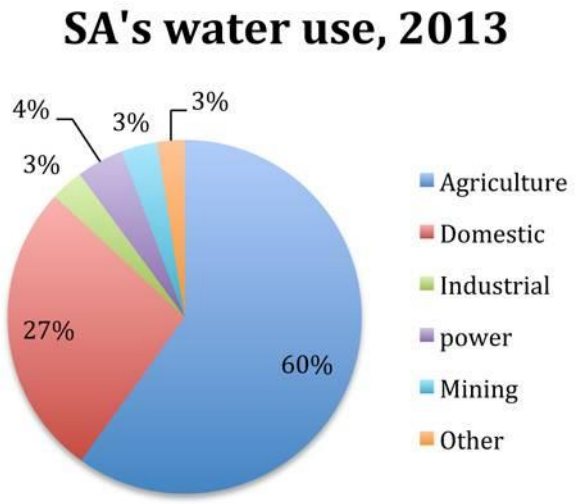

Figure 1.3: Major water use sectors

(Adapted from: http://www.dailymaverick.co.za/article/2015-11-06-no-drop-to-waste-tackling-south-africas-water-crisis/#.VxmXxdQrLrc.)

1.10 *Fusarium oxysporum* (Hypocreales)

The genus *Fusarium* collectively represents the most important group of fungal plant pathogens, causing various diseases on nearly every economically important plant species. *Fusarium oxysporum* is a broad host range pathogen that has caused some of the world's most drastic and economically devastating plant disease epidemics. Recently *F. oxysporum* has also emerged as a model for soil-borne fungal diseases with Arabidopsis and tomato (*Lycopersicom esculentum*) as hosts (Broad Institute, 2010). Collectively, these *F. oxysporum* strains infect and kill a large number of host these include many commercially harvested crops such as species in the Solenaceae family (tomatoes, peppers, potatoes and eggplant), watermelon, lettuce, legumes, beets, basil, strawberries, chrysanthemum, sugarcane, bananas, and multiple other species. *F. oxysporum* spores survive dormant in soil up to 30 years. They are easily spread via water, machinery and seeds, and can colonize in the rhizomes or vegetative cuttings of infected plants, showing no symptoms until transmitted to other individuals. All these are qualities that make this fungus an important and potentially devastating agricultural pest (Gonsalves & Ferreira 1993; Miller et al. 1996; New York Botanical Garden 2003; Wikipedia 2014a, b). *F. oxysporum* causes vascular wilt in a wide variety of crops (Roncero et al., 2003).

Healthy plants can become infected by *F. oxysporum* if the soil in which they are growing is contaminated with the fungus. The fungus can invade a plant either with its sporangial germ tube or mycelium by invading the plant's roots. The roots can be infected directly through the root tips, wounds in the roots, or at the formation point of lateral roots (Agrios, 1988). *F. oxysporum* is primarily spread over short distances by irrigation water and contaminated farm equipment. The fungus can also be spread over long distances either in infected transplants or in soil. Although the fungus can sometimes infect the fruit and contaminate its seed, the spread of the fungus by way of the seed is very rare (Agrios, 1988). It is also possible that the spores are spread by wind (Gonsalves & Ferreira, 1993). The primary solution to control such diseases is through the development of disease resistant plant cultivars (Broad Institute, 2010) and

through chemical soil fumigation (Fravel et al., 2003). Besides their economic importance, species of *Fusarium* also serve as key model organisms for biological and evolutionary research (Broad Institute, 2010).

1.11 Hypothesis

a) Different substrate combinations will influence growth and secondary metabolite production of *S. aethiopicus* depending on water retention of the substrate mix.
b) Cultivation of *S. aethiopicus* under different watering regimes in addition to saving water will increase the concentration of secondary metabolites.
c) Growth parameters per plant will vary with each combination of substrates.
d) LC-MS analysis will indicate different results on the secondary metabolites concentrations of the aerial parts and rhizomes of *S. aethiopicus.*
e) *S. aethiopicus* can be cultivated hydroponically.

1.12 The overall aim of the study

The predominant aim of the study was to determine the effect of watering regimes and combination of coconut fiber with other commonly used hydroponic substrates on plant growth, production of antimicrobial activity and secondary metabolites of *S. aethiopicus*. Furthermore, evaluate water holding capacity of the combined substrates to enable the identification of the best blend for cultivation of this species through semi hydroponics.

1.13 Specific objectives of the research

a) To evaluate water holding capacity of different combinations of substrates so as to identify the best blend that can retain more water.
b) To evaluate the effect of combined substrates and watering regimes with respect to plant growth parameters.

c) To evaluate the effects of different substrate combinations and watering regimes on anti-fungal activities of the plant extracts, nutrient uptake and production of active secondary metabolites.
d) Assess the total activity of *S. aethiopicus* against *F. oxysporum.*

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CHAPTER TWO

Optimizing water holding capacity using inert substrate mixes under different watering regimes for hydroponic cultivation of *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt (Zingiberaceae).

2.1 Introduction

Medicinal plants are an important aspect of the daily lives of many people in rural areas and an essential part of the South African cultural heritage (Van Wyk et al., 2009). The current demand for numerous plant species including *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt used for indigenous medicines exceeds supply and are in danger of extinction (Mander, 1998; Manzini, 2005). *S. aethiopicus* commonly known as wild ginger is one of the most popular (Manzini, 2005) and important medicinal plant species used to treat a variety of ailments (Smith, 1998; van Wyk & Gericke, 2000; Hankey & Reynolds, 2002; Diederichs, 2006; Directorate: Plant Production, 2013). It is a slow growing plant with limited distribution and in high demand (Speirs, 2014). The increasing demand presents an opportunity for potentially profitable cultivation (Street & Prinsloo, 2012). However, lack of appropriate cultivation skills of many medicinal plant species including *S. aethiopicus* has led to scarcity of these plants (Anon, 1998 cited by Manzini, 2005). *S. aethiopicus* has a high water requirement and warrants the adoption of innovative cultivation strategies amidst acute and persistent water scarcity in southern Africa (Department of Agriculture, 2009).

As global soil fertility is on the decline, the use of hydroponic systems in plant research communities is becoming more widespread (Lefever, 2013). Jensen (1997) defines hydroponics as a technology for growing plants in nutrient solutions (water and fertilizers) with or without the use of an artificial medium to provide mechanical support. Soilless greenhouse cultivation of medicinal plants has many valuable advantages such as high productivity and superior quality, year round production, production of drugs with minimum herbicides and pesticides residues, clean cultivation, and minimized use of water (Mairapetyan, 1999; Dorais et al., 2001; Manukyan et al., 2004; Al-Karaki & Othman, 2009). A common practice inside these protective structures is the use of non-

mineral growing media or substrates (Rodriguez et al., 2006). Soilless growing media are composed of different organic and inorganic components such as peat, perlite, vermiculite, coir, rockwool, and bark blended together to create a growing environment with good aeration, nutrient supply and plant available water (Albaho et al., 2009; Matt, 2015). Three functions of growing media are to support plant in soil, to hold and provide water and nutrient element and to enable plant roots to get sufficient amount of oxygen (Ingram et al., 2003). These non-soil materials can be manipulated or processed in different ratios and combinations to provide the superior physical and chemical environment for optimal plant growth (Fields, 2012; Kukal et al., 2012). According to Aklibasinda et al. (2011) water retained in media is necessary for plant growth and other physiological processes. Water and oxygen availability are determined by the water retention characteristics of the growth medium and the irrigation regime used (Heiskanen, 1995). Appropriate water holding capacity avoids water wastage, nutrient leaching and runoff (Krucker, 2003). The substrate should possess the correct physical and chemical characteristics and should drain freely while retaining the correct amount of moisture (Nektarios et al., 2011). Substrates such as coconut fiber (coir), has the unique property of retaining water for longer duration of time; this property may facilitate the continuous and prolonged availability of water for the plants (Paramanandham et al., 2014). Coir has high water holding capacity and has been conventionally used to improve physical and chemical properties of soils (Arenas et al., 2002; El-Hamed et al., 2011). Physical properties of substrates are predominantly important as it relates to water retention, water availability, and aeration in order to provide most favorable growing conditions while conserving water (Deepagonda et. al*.,* 2013).

At present, approximately 7% of the world's population lives in areas where water is scarce (Wallace, 2000). Council for Scientific and Industrial Research (CSIR) researchers warned that continued population and economic growth, combined with climate change, could result in serious water shortages in some parts of the country by 2025 (Sainfo, 2015). Faced by growing water demands and increased climate variability, recent years have seen much attention on drought scenarios and drought adaptation measures (Ward, 2014). Consequently, the search for technologies and

measures to save or conserve water in irrigated agriculture has increased (Kulkarni, 2011). Blokker et al. (2012) identified the following as key water saving methods: drip irrigation, irrigation scheduling, salt tolerance, drought resistance, crop variety and soil structure. The development of improved technologies such as drip irrigation systems that can control the amount and frequency of water applications is a very powerful tool, increasing productivity and lessening adverse water quality impacts (Evans & Sadler, 2008; Khan, 2014). Drip irrigation can conserve water; growers typically convert to drip irrigation not only to conserve water but to secure higher economic return from irrigation through increased yields, reduced costs of water, and reduced variable production costs (Ward, 2014).

Therefore, the objectives of the present study were; (i) to evaluate the water holding capacity of different substrate combinations using coconut fiber as the main component and recommend substrate combination that will minimize water usage while optimizing plant growth and (ii) evaluate the effect of the substrate blends and watering regimes on growth parameters of *S. aethiopicus*.

2.2 Materials and Methods

2.2.1 Plant material

S. aethiopicus rhizomes were obtained from a commercial nursery; Afro Indigenous at Centurion, Gauteng, South Africa. The rhizomes were dipped in Captab (4 g in 1 L) to prevent development of fungi; and were imbedded in 49 plastic pots containing a medium mix of (2 parts pine bark, 1 part perlite and 1 part vermiculite). The pots were placed in a controlled tunnel with the following growing conditions; temperature; day (17 °C-21 °C) and evening (15 °C-18 °C). These pots were hand irrigated with sterile distilled water as needed until crop emergence which was noted. Six weeks old healthy and vigorous seedlings with two to three leaves were each transplanted into separate pots (12.5 cm) filled with substrate combinations according to treatments, inside the controlled tunnel.

2.2.2 Substrates preparation

Coconut fiber (coir) was used as the main component for the preparation of media in different proportions and combinations. The combinations used in this study composed of coir, vermiculite, perlite obtained from The Cape Agricultural Suppliers, Cape Town and bark from the Department of Horticulture, Cape Peninsula University of Technology. The unexpanded coir blocks were then fully expanded by soaking in water and the fibre was sun dried to reduce wetness. The treatments consisted of four different combinations of organic and inorganic substrates in different proportions (Table 2.1).

Table 2.1: Composition of different substrate blends used in the study

Treatment	Composition
T1	Coir + vermiculite + perlite + bark (1:1:1:1)
T2	Coir + bark (1:1)
T3	Coir + perlite (1:1)
T4	Coir + vermiculite (1:1)

The quantity of substrate blend in each pot was approximately 200 g. Each combination was thoroughly mixed in a bucket before filling into pots.

2.2.3 Experimental design

The experiment was conducted in a tunnel within the nursery of the Department of Horticultural Sciences, Cape Peninsula University of Technology, Bellville campus; Cape Town, South Africa, 33° 55’ 48.8” S, 18° 38’ 32.7’’ E. The structure was made of galvanized steel frame with transparent polycarbonate. Steel tables (2.5 × 1 m) were used as a flat surface for white plastic gutters (1.36 m long). Eight plastic gutters were placed on two steel tables; each steel table had four gutters which were held in place by cable ties. Plastic gutters and cable ties were supplied by Builders Warehouse, Cape Town. Each gutter had eight plastic pots (12.5 cm) containing different substrate combinations as treatments. Pots were lined at the bottom with shade cloth to prevent substrates leaving through the drainage holes. Beneath the steel table there were four

fish tanks (60 L) each containing one submersible water pump, which recirculates water through a 20 ml black plastic pipe to one gutter only. All gutters were wrapped with black plastic polyethylene sheets to avoid algae build-up. Nutrient solution was supplied to the plants by spaghetti tubing with drippers (one dripper/plant) at a rate of 2 L/h controlled by a timer. Plants were fertigated with Nutrifeed fertilizer supplied by Starke Ayres, Cape Town containing the following ingredients: 65 g/kg N, 27 g/kg P, 130 g/kg K, 70 mg/kg Ca, 20 mg/kg Cu, 1500 mg/kg Fe, 10 mg/kg Mo, 22 mg/kg Mg, 240 mg/kg Mn, 75 mg/kg S, 240 mg/kg B and mg/kg Zn. Fertilizer group 1 Reg No: K2025 (Act 36/ 1947). Nutrient solutions were prepared by dissolving 60 g of fertilizer in 60 L reservoir with tap water. The pH and electrical conductivity (EC) were maintained at 6.4 and 1.8, respectively. The nutrient solutions were refreshed every 2 weeks to minimize build- up of salts in the substrates. As the water drained out of the pots it drained back into the reservoirs and was reused. The experiment was arranged in a randomized block design (Figure 2.1) and was allowed to run for 9 weeks. The tunnel had the following experimental conditions; average temperature: morning (18-33 °C), day (26-49 °C) and evening (19-34 °C), relative humidity; morning (67-92%), day (80-94%) and evening (81-92%), and average light intensity; morning (3917 Klux), day (19270 Klux) and evening (2685 Klux).

Figure 2.1: (A) The experimental arrangement (setup). (B) Table represents watering regimes (3-days and 5-days interval), three gutters with different treatments and one gutter without plants used to determine water holding capacity.

2.2.4 Watering regimes

Two watering regimes were used in this study to evaluate their effect on plant growth. Seedlings in four different treatments were grown under two watering regimes: watering twice in three days and watering twice in five days. One set of 24 plants planted in four treatments (each treatment replicated three times) received 3-days interval watering and another set received 5-days interval watering. All the 24 plants were allocated to each of the watering regimes giving a total population of 48 plants for the experiment. In both watering regimes, treatments were irrigated for 15 minutes twice a day; at 10:00 h and 18:00 h. The volume of nutrient applied was 1380 mL (1.38 L) per pot per watering regime. All plants within each watering regime received the same cultivation procedure viz; application of fertilizer, experimental conditions, and treatments.

2.2.5 Determination of water holding capacity (WHC)

Three separate pots for each treatment filled with different substrate combinations but without plants were used to determine WHC (Figure 2.1B). Water was added to each combination for 15 minutes. After irrigation, water was left to spread and soak in the substrates and allowed to drain for 30 minutes. Following this, single samples (50 g) taken from each treatment were placed in containers and weighed then transferred to labelled brown paper bags. The paper bags were then placed in a 105 ºC oven for 48 hours and were allowed to cool down at room temperature, then placed back to containers and reweighed to determine dry mass of the substrate. The test was conducted with three separate samples (replicates) for each treatment. After the tests, substrates were removed and substituted with new substrate combinations for the next test. WHC of different substrate combinations was measured according to the methods described by Reddy (2002).

2.2.6 Plant growth parameters

Observations on the following parameters were recorded: Plant emergence (number of days for the rhizomes to germinate), stem length (cm), number of leaves were counted on each plant, newest leaf was measured for leaf length (cm), number of rhizome eyes, new shoots were counted per plant, plant total weight (g), newly

developed rhizome length (cm); old rhizome length (cm), dry weight of aerial parts (g), fresh weight of aerial parts (g), new shoots total weight (g), root length (cm), root weight (g) and number of roots. Plant growth parameters were evaluated once every week. Three replicates were used per treatment in each watering regime.

2.2.7 Calculating quantity of irrigation water used

Total amount of water applied to each watering regime during the experimental period was calculated: The following two steps were used:

(i) Total amount of water applied per plant throughout the study was calculated: the number of water applications in each watering regime were counted and multiplied by the amount of water applied per plant.

(ii) Calculate the total amount of water applied per watering regime throughout the study: total amount of water applied per plant (step one) multiplied by number of plants per watering regime.

2.2.8 Statistical analysis

The experimental data collected were analyzed using one-way analysis of variance (ANOVA) and Tukey HSD test was used to separate the means at a level of significance, $P < 0.05$. These computations were performed using STATISTICA software (StatSoft, 2013). Sigma Plot 10.0 package was used for plotting graphs.

2.3 Results

2.3.1 Water Holding Capacity

Results pertaining to water holding capacity (WHC) of four different substrate combinations are shown in Figure 2.2. There were significant differences ($P < 0.05$) detected in WHC among treatments. The WHC of treatments ranged from 174% to 365%. Maximum (average) WHC (337.86%) was found in T3 (50% coconut fiber + 50% perlite) followed by T4 (277.66%) (50% coconut fiber + 50% vermiculite) and T1 (216.33%) (25% coconut fiber + 25% vermiculite + 25% perlite + 25% bark) respectively, while minimum WHC (201.06%) was observed in T2 (50% coconut fiber +

50% bark). T3 was significantly different (df = 3, 56; P < 0.05) from the other three (3) treatments and there was no significant difference (P > 0.05) between T1 and T2 when WHC means were separated using Tukey HSD test.

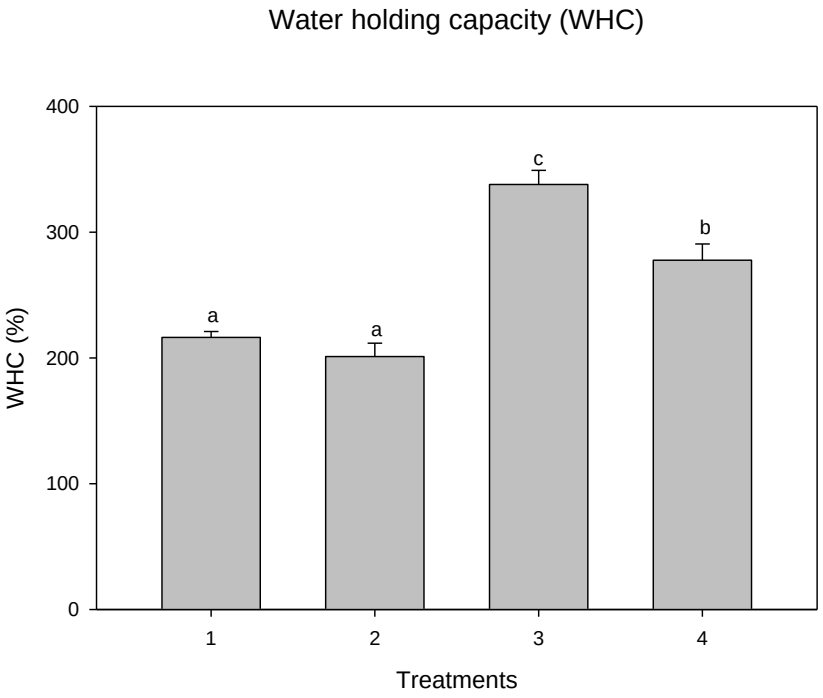

Figure 2.2: Water holding capacity of four different substrate combinations (see Table 2.1 for details on treatments). Vertical columns are means and the bars on each column are ± standard errors of mean. Means followed by the same lower case letters on top of the bars means not significant different (P > 0.05) following comparison using Tukey Test.

2.3.2 Plant emergence

S. aethiopicus seedlings emerged very late, emergence began 70 days after planting. The plants produced flowers in November before seedlings emerged. The flowers appeared directly from the rhizome, independently of the leaves (Figure 2.3A, B & C) and each flower lasted for a day to three days. About 4–8 flowers were produced per plant. Plants emerged after flowering while some developed during flowering.

Figure 2.3: *S. aethiopicus* flowers appearing directly from the rhizome, independently of the leaves.

2.3.3 Growth parameters

2.3.3.1 Number of leaves

There was no marked difference in number of leaves for all treatments in both 3-days (df = 3, 20; F = 0.952; P = 0.434) and 5-days (df = 3, 20; F = 1.935; P = 0.156) watering intervals following ANOVA analysis (Table 2.2a). However, at nine weeks after planting under 3-days interval watering; the highest number of leaves was observed in T3 (8 ± 0.447) compared to the other treatments followed by T4 (7.83 ± 0.307) and T1 (7.5 ± 0.223), respectively. On the contrary, T2 produced the least numbers (7.33 ± 0.21) of leaves. Surprisingly, in 5-days interval watering T2 (6.83 ± 0.307) gave the highest number of leaves compared to T1, T3 and T4 whereas; T1 produced the least number (5.83 ± 0.166) of leaves. Plants watered every 3 days had highest mean values than those watered every 5 days.

2.3.3.2 Stem length

At nine weeks post treatment, no significant difference (df = 3, 20, P = 0.550) in terms of stem length of *S. aethiopicus* was found among treatments in 3-days interval watering; the highest stem length (27.08 ± 4.424 cm) was obtained in T3 while T4

recorded the shortest stem length (21.73 ± 2.407 cm) (Table 2.2a). When the means of stem length were compared in 5-days interval watering regime, there was statistical difference among treatments (df = 3, 20, P = 0.02) with T2 producing the highest stem length (23.25 ± 1.07 cm) which was significantly different (P < 0.05) compared to the other treatments; (T3 [18.5 ± 1.39 cm], T1 [18.36 ± 0.96 cm] and T4 [17.25 ± 1.865 cm] respectively). The data showed that the highest mean values were obtained in 3-days interval watering when means were separated using Tukey HSD test.

2.3.3.3 Leaf length

There was no significant difference in leaf length of *S. aethiopicus* among treatments in both 3-days (df = 3, 20; F = 0.488; P = 0.694) and 5-days interval (df = 3, 20; F = 1.665; P = 0.206) (Table 2.2a). It was however observed that in 3-days interval watering the highest leaf length was obtained in T2 (18 ± 4.244 cm) followed by T1 (16.33 ± 2.859 cm) and T3 (15.91 ± 2.841 cm) respectively, while the lowest leaf length (12.83 ± 1.943 cm) was obtained in T4. Conversely, T2 plants in 5-days interval watering recorded the highest leaf length (22.916 ± 1.445 cm) followed by T3 (20.68 ± 1.216 cm) and T1 (19.13 ± 2.357 cm), respectively. On the other hand plants grown in T4 (18.08 ± 1.164 cm) had the lowest leaf length. Broadly, plants watered every 5-days recorded the highest leaf length.

2.3.3.4 Number of rhizome eyes

At nine weeks post treatment, in both 3 and 5-days intervals no significant difference in number of eyes was found in all treatments (Table 2.2a). In 3-days interval watering (df = 3, 20; P = 0.836), T4 recorded the highest number of eyes (6.166 ± 0.79) followed by T2 (5.33 ± 0.557) and T3 (5.33 ± 1.308), whereas, T1 (5.166 ± 0.477) recorded the least number of eyes. Whilst in 5-days interval watering (df = 3, 20; P = 0.910), T1 (4.6 ± 0.802) and T2 (4.6 ± 0.846) recorded highest number of eyes compared to the other treatments (T3 [4.5 ± 0.619] and T4 [4 ± 0.365] respectively). Generally, the highest mean numbers of rhizome eyes were obtained in 3-days interval when means were separated using Tukey HSD test.

2.3.3.5 New shoots

Treatments in 3-days interval watering had a significant effect (df = 3, 20; P = 0.018) on new shoots of *S. aethiopicus* at nine weeks post treatment. When the means of new shoots were compared (Table 2.2a), T4 produced the highest number of shoots (4.16 ± 0.6), which was significantly different (P < 0.05) compared to the other treatments T1 (2.166 ± 0.307) and T2 (2.166 ± 0.542) with the exception of T3 (2.83 ± 0.307) which was not significantly different to T2. Whereas, watering every 5 days had no significant difference (df = 3, 20; P = 0.08) in new shoots between treatments, the highest number of shoots was obtained in T3 (2.33 ± 0.421) and T4 (2.33 ± 0.494) unlike plants in T1 which yielded the lowest number of shoots (1 ± 0.365). The data showed that the maximum mean values were recorded in 3-days interval watering.

2.3.3.6 Plant total weight

There was no marked difference in plant total weight of *S. aethiopicus* among treatments in both 3-days (df = 3, 20; F = 3.347; P = 0.039) and 5-days (df = 3, 20; F = 1.829; P = 0.174) watering intervals (Table 2.2a). Nonetheless, it was observed that in 3-days interval the highest total weight was obtained in T4 (181.63 ± 18.89 g) followed by T3 (124.28 ± 2.859 g) and T2 (117.75 ± 13.939 g) respectively. While the lowest total weight (116.316 ± 8.286 g) was obtained in T1. Similarly, in 5-days interval the highest plant total weight was recorded in T4 (86.66 ± 17.498 g) when compared to T3, T2 and T1 where, T1 (54.33 ± 4.153 g) obtained the least total weight. Broadly, the highest mean values were recorded in 3-days interval watering.

2.3.3.7 Rhizome length (fresh)

The different treatments did not significantly affect the length of *S. aethiopicus* fresh rhizomes in both watering regimes. At nine weeks post treatment (Table 2.2a), in 3-days interval watering; T2 produced the highest rhizome length (1.56 ± 0.335 cm), although it was not statistically different (P > 0.05) compared to the other treatments (T3 [1.53 ± 0.275 cm], T4 [1.51 ± 0.207 cm], and T1 [1.5 ± 0.322 cm] respectively). Also the results showed that in 5-days interval watering (df = 3, 20; P = 0.244); T4 (1.78 ± 0.153 cm) obtained longer rhizome lengths than plants in T2 (1.71 ± 0.13 cm), T3 (1.68 ± 0.11

cm) and T1 (1.4 ± 0.148 cm) respectively (Table 2.2a). In addition, the best result for rhizome length was recorded in plants watered every 5-days interval.

2.3.3.8 Dry weight (aerial parts)

Treatments in both 3-days (df = 3, 20; P = 0.193) and 5-days (df = 3, 20; P = 0.528) watering intervals did not significantly influence dry weight (aerial parts) of *S. aethiopicus* at nine weeks post treatment (Table 2.2a). In 3-days interval, T4 recorded the highest dry weight (11.66 ± 1.20 g) while the minimum dry weight was found in T1 (8.8 ± 0.814 g). On the contrary; in 5-days interval, the maximum dry weight (6.4 ± 0.556 g) was observed in T2 followed by T3 (6.183 ± 0.61 g) and T4 (6.166 ± 0.79 g) respectively, while the minimum dry weight of the aerial parts (5.23 ± 0.317 g) was found in T1. The results also showed that the highest mean values were recorded in plants that received water every 3 days.

2.3.3.9 New shoots total weight

At nine weeks post treatment, there was no significant difference in new shoots total weight of *S. aethiopicus* among the treatments in both 3-days (df = 3, 20; F = 3.271; P = 0.042) and 5-days (df = 3, 20; F = 0.053; P = 0.983) watering intervals following ANOVA analysis (Table 2.2a). However, it was observed that in 3-days interval the highest total weight was obtained in T4 (36.28 ± 4.33 g) followed by T2 (21 ± 5.66 g) and T3 (18.23 ± 5.107 g) respectively. While the lowest total weight (15.196 ± 5.128 g) was obtained in T1. Furthermore, in 5-days interval the highest new shoots total weight was obtained in T2 (8.08 ± 2.648 g) when compared to T1, T3 and T4 where, T1 (6.616 ± 4.367 g) obtained the least total weight. Generally, the highest mean values were recorded in 3-days interval watering.

2.3.3.10 Fresh weight (aerial parts)

There was no significant increase in aerial parts total weight for all treatments in both 3-days (df = 3, 20; F = 0.143; P = 0.932) and 5-days (df = 3, 20; F = 1.795; P = 0.180) watering intervals following ANOVA analysis (Table 2.2b). However, at nine weeks after planting in 3-days interval, the highest total weight was observed in T4

(56.78 ± 5.795) followed by T1 (55.2 ± 3.41 g) and T3 (55.2 ± 4.287 g) respectively. The lowest total weight (52.63 ± 4.349 g) was recorded in T2. Furthermore, in 5-days interval T2 (42.78 ± 2.039 g) gave the highest aerial parts total weight compared to T1, T3 and T4 where, T1 produced least (32.45 ± 4.55 g) total weight. Also the results showed that the highest mean values were recorded in 3-days interval watering.

2.3.3.11 Rhizome length (old)

There was no marked difference in rhizome length of *S. aethiopicus* among treatments in both 3-days (df = 3, 20; P = 0.799) and 5-days (df = 3, 20; P = 0.977) watering intervals following ANOVA analysis (Table 2.2b). It was however observed that in 3-days interval the highest rhizome length was obtained in T1 (4.2 ± 0.299 cm) followed by T4 (4.2 ± 0.196 cm) and T3 (4.05 ± 0.19 cm) respectively. The lowest rhizome length (3.86 ± 0.387 cm) was obtained in T2. Conversely, in 5-days interval the highest rhizome length was shown by T2 (3.65 ± 0.218 cm) followed by T4 (3.55 ± 0.218 cm) and T3 (3.53 ± 0.47 cm) respectively, while T1 had the lowest (3.46 ± 0.14 cm) rhizome length. It was however observed that plants grown in 3-days interval watering recorded the highest rhizome length.

2.3.3.12 Rhizome weight

At nine weeks post treatment, there was no significant difference in rhizome weight of *S. aethiopicus* among the treatments in both 3-days (df = 3, 20; F = 2.109; P = 0.131) and 5-days (df = 3, 20; F = 0.348; P = 0.79) watering intervals (Table 2.2b). However, it was observed that in 3-days interval the highest rhizome weight was obtained in T4 (92.3 ± 8.426 g) followed by T3 (70.3 ± 4.32 g) and T1 (67.9 ± 8.586 g) respectively. While the lowest rhizome weight (66.48 ± 10.77 g) was obtained in T2. Furthermore, in 5-days interval watering the highest rhizome weight was obtained in T3 (57.08 ± 10.907 g) when compared to T1, T2 and T4 where, T1 (45.06 ± 3.419 g) obtained the least rhizome weight. Generally, the highest mean values were recorded in 3-days interval watering.

2.3.3.13 Root length

The different treatments did not significantly affect the root length of *S. aethiopicus* in both watering regimes. At nine weeks post treatment (Table 2.2b), in 3-days interval watering (df = 3, 20; P = 0.601), T1 produced the highest root length (11.16 ± 0.843 cm) and was not statistically different (P > 0.05) compared to the other treatments (T2 [10.75 ± 2.13 cm], T3 [9.91 ± 1.66 cm], and T4 [8.33 ± 1.364 cm] respectively). Similarly, in 5-days interval watering (df = 3, 20; P = 0.887); T1 (7.58 ± 1.28 cm) produced longest root length than plants in T3 (7.33 ± 1.12 cm), T2 (6.916 ± 1.502 cm) and T4 (6.23 ± 1.207 cm) respectively. In addition, the best result for root length was recorded in 3-days interval.

2.3.3.14 Root weight

There was no significant difference in root weight for all treatments in both 3-days (df = 3, 20; F = 2.399; P = 0.098) and 5-days (df = 3, 20; F = 0.327; P = 0.805) watering intervals following ANOVA analysis (Table 2.2b). Nonetheless, at nine weeks after planting in 3-days interval, the highest root weight was observed in T4 (56.65 ± 5.848 g) followed by T3 (48.316 ± 5.176 g) and T1 (42.63 ± 5.447 g) respectively. The lowest root weight (41.68 ± 4.79 g) was recorded in T2. Furthermore, in 5-days interval T3 (32.76 ± 3.67 g) gave the highest root weight compared to T1, T2 and T4 whereas; T1 produced least (27.13 ± 3.05 g) root weight. Generally, the results showed that the highest mean values were recorded in 3-days interval watering.

2.3.3.15 Number of roots

Treatments watered every 3 days had a significant effect (df = 3, 20; P = 0.008) on number of roots of *S. aethiopicus* at nine weeks post treatment (Table 2.2b). When the means were compared, T4 produced the highest number of roots (75.83 ± 4.35), which was significantly difference (P < 0.05) from other treatments (T1 [53.16 ± 5.306], T2 [51.5 ± 5.136] and T3 [55.33 ± 5.226]). Whereas, in 5-days interval watering no significant difference (df = 3, 20; P = 0.553) in number of roots was found between treatments; the highest number of roots was obtained in T4 (46 ± 6.537) followed by T3 (45.83 ± 4.377) and T2 (40.66 ± 4.23) respectively, while T1 obtained the lowest

number of roots (37.83 ± 3.229). The data showed that the maximum mean values were recorded in 3-days interval watering.

Table 2.2a: Effect of substrate combinations and watering regimes on growth parameters of *S. aethiopicus* at 9 weeks after planting.

Parameters	Treatment	3-days interval (watering regime)	5-days interval (watering regime)
Number of leaves	T1	7.5 ± 0.223 a	5.83 ± 0.166 a
	T2	7.33 ± 0.21 a	6.83 ± 0.307 a
	T3	8 ± 0.447 a	6.33 ± 0.210 a
	T4	7.83 ± 0.307 a	6.33 ± 0.421 a
Stem length	T1	22.25 ± 2.622 a	18.36 ± 0.966 ab
	T2	27.08 ± 4.424 a	23.25 ± 1.07 b
	T3	25.41 ± 2.014 a	18.5 ± 1.39 ab
	T4	21.73 ± 2.407 a	17.25 ± 1.865 a
Leaf length	T1	16.33 ± 2.859 a	19.13 ± 2.357 a
	T2	18 ± 4.244 a	22.916 ± 1.445 a
	T3	15.916 ± 2.841 a	20.68 ± 1.216 a
	T4	12.83 ± 1.943 a	18.08 ± 1.164 a
Number of eyes	T1	5.166 ± 0.477 a	4.6 ± 0.802 a
	T2	5.33 ± 0.557 a	4.6 ± 0.846 a
	T3	5.33 ± 1.308 a	4.5 ± 0.619 a
	T4	6.166 ± 0.792 a	4 ± 0.365 a
New shoots	T1	2.166 ± 0.307 a	1 ± 0.365 a
	T2	2.166 ± 0.542 a	2.16 ±0.307 a
	T3	2.83 ± 0.307 ab	2.33 ± 0.421 a
	T4	4.16 ±0.6 b	2.33 ± 0.494 a
Plant total weight	T1	116.316 ± 8.286 a	54.33 ± 4.153 a
	T2	117.75 ± 13.939 a	76.9 ± 1.587 a
	T3	124.28 ± 23.445 a	75.86 ± 8.977 a
	T4	181.63 ± 18.892 a	86.66 ± 17.498 a
Rhizome length (fresh)	T1	1.5 ± 0.322 a	1.4 ± 0.148 a
	T2	1.56 ± 0.335 a	1.71 ± 0.132 a
	T3	1.53 ± 0.275 a	1.68 ± 0.113 a
	T4	1.51 ± 0.207 a	1.78 ± 0.153 a
Dry weight (Aerial parts)	T1	8.8 ± 0.814 a	5.23 ± 0.317 a
	T2	8.9 ± 0.987 a	6.4 ± 0.556 a
	T3	9.28 ± 1.07 a	6.183 ± 0.61 a
	T4	11.66 ± 1.202 a	6.166 ± 0.79 a
New shoots (total weight)	T1	15.916 ± 5.128 a	6.616 ± 4.367 a
	T2	21 ± 5.66 a	8.083 ± 2.648 a
	T3	18.23 ± 5.107 a	7.433 ± 2.168 a
	T4	36.28 ± 4.33 a	8.066 ± 2.27 a

Means followed by same lowercase letters in the same column are not significantly different ($P > 0.05$) following comparison using Tukey test. Grey and white colors are used to differentiate columns with growth parameters.

Table 2.2b: Effect of substrate combinations and watering regimes on growth parameters of *S. aethiopicus* at 9 weeks after planting.

Parameters	Treatments	3-days interval (watering regime)	5-days interval (watering regime)
Aerial parts (total weight)	T1	55.21 ± 3.41 a	32.45 ± 4.55 a
	T2	52.63 ± 4.349 a	42.78 ± 2.039 a
	T3	55.2 ± 4.287 a	41.06 ± 2.629 a
	T4	56.78 ± 5.795 a	38.9 ± 3.686 a
Rhizome length (old)	T1	4.2 ± 0.299 a	3.466 ± 0.14 a
	T2	3.866 ± 0.387 a	3.65 ± 0.218 a
	T3	4.05 ± 0.192 a	3.53 ± 0.47 a
	T4	4.2 ± 0.196 a	3.55 ± 0.218 a
Rhizome weight	T1	67.9 ± 8.586 a	45.06 ± 3.419 a
	T2	66.48 ± 10.77 a	47.76 ± 5.928 a
	T3	70.3 ± 4.32 a	57.08 ± 10.907 a
	T4	92.3 ± 8.426 a	48.95 ± 4.91 a
Root length	T1	11.16 ± 0.843 a	7.583 ± 1.28 a
	T2	10.75 ±2.132 a	6.916 ±1.502 a
	T3	9.91 ± 1.66 a	7.33 ± 1.122 a
	T4	8.33 ± 1.364 a	6.23 ± 1.207 a
Root weight	T1	42.63 ± 5.447 a	27.13 ± 3.05 a
	T2	41.68 ± 4.794 a	29.05 ± 2.45 a
	T3	48.316 ± 5.176 a	32.76 ± 3.672 a
	T4	59.65 ± 5.848 a	32.08 ± 7.479 a
Number of roots	T1	53.16 ± 5.306 a	37.83 ± 3.229 a
	T2	51.5 ± 5.136 a	40.66 ± 4.232 a
	T3	55.33 ± 5.226 a	45.83 ± 4.377 a
	T4	75.83 ± 4.354 b	46 ± 6.537 a

Means followed by same lowercase letters in the same column are not significantly different ($P > 0.05$) following comparison using Tukey test. Grey and white colours are used to differentiate columns with growth parameters.

2.3.4 Quantity of irrigation water used

During the 9-week study, plants were watered with 1380 mL (1.38 L) of water per pot in each watering regime. The results indicated that, plants in 3-days interval received 21 watering applications (number of irrigations) while 5-days interval received 12 applications throughout the study (Table 2.3a). Furthermore, in 3-days interval total amount of water applied per plant was 28.98 L while in 5-days interval each plant received 16.56 L of water through the experimental period. Generally, 3-days interval received the highest application of water 695.52 L whereas; 5-days interval total amount of water applied throughout the study was 397.44 L (Table 2.3b). The 5-days interval reduced water application by 55% when compared to 3-days interval watering.

Table 2.3a: Total amount of irrigation water applied per plant during the experimental period.

	Watering regimes	
	3-days interval	5-days interval
Water applications (number of irrigations)	21	12
Water applied per day/plant	1.38 L	1.38 L
Total amount of water per plant	**28.98 L**	**16.56 L**

Table 2.3b: Total amount of irrigation water applied per watering regime during the experimental period.

	Watering regimes	
	3-days interval	5-days interval
Total amount of water per plant	28.98 L	16.56 L
Number of plants per watering regime	24	24
Total amount of water per watering regime	**695.52 L**	**397.44 L**

2.4 Discussion

2.4.1 Plant emergence

It was observed that *S. aethiopicus* plants emerged very late; similarly, Wilson & Ovid (1993) discovered that the germination of ginger began 56 days after planting. Rhizomes were planted in August instead of September as recommended and winter lasted longer than expected. In general, emergence of the plant was prolonged due to low temperature which ranged between 17 ºC–21 ºC during the day. These results further strengthened the argument that *S. aethiopicus* prefers warm temperatures (Manzini, 2005; Department of Agriculture, 2009). Results concerning flowering are in agreement with previously published findings by Nichols (1989); Wilson and Ovid (1993) and Edwards et al. (2004), that wild ginger produce flowers during November before seedlings appear and also confirmed that flowers are short-lived lasting only about a day or two. Emerging plants only continue to grow once flowering is completed.

2.4.2 Water holding capacity

In this study, all the substrate combinations had high water holding capacity (WHC) ranging from 174% to 365%; although some were statistically different, they were close to each other. All substrates appeared to have satisfactory WHC. Kukal et al. (2012) showed that coir mixes exhibited significantly higher water holding capacity; the WHC was 6.1 times higher ranging between 283–256%. The high WHC of coir based substrates has also been reported by Evans and Stamps (1996), Prasad (1997), Dombrowsky (2012) and Joshua and Vincent (2015). According to Hernandez-Apaolaza et al. (2005) and Kukal et al. (2012) when coir is used as the main component in substrate combinations, it has a potential to increase WHC (Treder, 2008). The combination of coir and perlite (T3) showed the highest results while coir and bark (T2) combination had the lowest WHC, these findings are in agreement with findings of Torres-Quezada (2012), which showed that pine bark potting mix had lower water retention capacity compared to other mixes due to its lower water-filled pore percentage and reduced capillarity. Differences in water retention capability can be explained by the variation in particle sizes between the substrates. Kukal et al. (2012) who studied water retention characteristics of growing media highlighted that the differences in WHC

among the media could be due to diversity in total porosity and pore size distribution. The high WHC of the different substrate combinations indicate that plants grown in coir amended mixes could lessen the frequency of watering, which characterizes coir as a positive property to save water. Tramp et al. (2009) highlighted that high WHC allows the plants growing in the media to withstand longer periods between watering events and reduces the effect of drought.

2.4.3 Growth parameters

From the results, the number of leaves, leaf length, number of rhizome eyes, plant total weight, rhizome length (fresh), dry weight (aerial parts), new shoots total weight, aerial parts fresh weight, rhizome length (old), rhizome weight, root length and root weight were not statistically different among treatments and watering regimes. Similarly, Shinohara et al. (1999) found no differences in growth parameters between plants grown in coconut fiber substrates. Researchers have observed equal or improved plant growth in substrates with coconut fiber (Evans & Stamps, 1996; Stamps & Evans, 1997). Perhaps the inclusion of coconut fiber in all treatments could have masked the effects of the other substrates. The use of different substrate combinations had little effect on plant growth, indicating that *S. aethiopicus* can be grown in a variety of substrates.

The number of roots and new shoots showed significant differences in 3-days interval watering among treatments; coir and vermiculite combination (T4) produced the best results when compared to other treatments. These results are in accordance with Colombo et al. (2016) who observed that a mix of coir and vermiculite produced the best results and differed statistically from other mixes for container grown plants. These findings are also in agreement with findings of Tramp et al. (2009), which showed that mixes containing vermiculite had higher water holding capacity and resulted in the greatest growth. The good results obtained with the combination are probably related to physical characteristics of coir and vermiculite, in particular, to its higher water holding capacity. The WHC tests conducted in this study indicated that coir and vermiculite combination had higher WHC (277.66%) compared to other combinations used.

According to Mabengwa (2013), plants grow well in media that holds water as evenly as possible and also provide sufficient nutrient holding capacity. On the contrary, stem length displayed significant differences in 5-days interval watering among treatments; plants in T2 (coir and bark) were significantly higher than other treatments. These observations are in line with the findings of Franco et al. (2007) who reported that coconut fiber mixed in equal parts with pine bark was the most efficient substrate when different growth parameters were evaluated. Similarly, Poulter and Aleksandra (2010) detected that pine bark and coir mix produced more stems per plant and had higher production rate.

The highest mean values due to watering regimes were recorded with plants that received water every third day (3-days interval watering). In line with these results, Isah et al. (2013) established that *Acacia senegal* performed better when watered once in three days. Similarly, Sale (2015) also observed that plants watered once in three days yielded the highest number of leaves. According to Abdelaziz (2010) high irrigation frequency may improve crop performance due to higher availability of nutrients. On contrary, watering every 5 days had the lowest mean values; studies have shown that the longest intervals of 5 days had the lowest biomass value and limits production (Ismail & Ozawa, 2009; Emmanuel, 2014). Although it is clear from the results obtained in this study, that different watering regimes have affected the growth of *S. aethiopicus* differently, it also shows that the plant could have the ability to tolerate different watering regimes. There were no major differences between the watering regimes, whereas, the most effective application was 3-days interval on growth of the plant. The results show that with regard to water shortage in the country, it seems watering interval of 5-days not only save water but also result in an acceptable economic production. 5-days interval has a potential to save water without any significant negative impact on plant growth.

According to Scott-Shaw (1999) *S. aethiopicus* produces 4-8 leaves; our results showed that the healthy plant produces as many as 7-12 leaves. These findings are in harmony with those documented by Azarmi et al. (2012) who established that

hydroponically grown medicinal plants had high productivity. The findings of this study demonstrated that hydroponic cultivation is an important means for successful cultivation of *S. aethiopicus* and would help to stimulate delivery of the plant to the market. Mairapetyan (1999) and Manukyan et al. (2004) stated that greenhouse production of valuable herbs and aromatic plants in soilless cultivation is an attractive and economical alternative for high productivity and superior quality. Under hydroponic conditions *S. aethiopicus* grow and develop more intensively and in three months they surpass the development of soil grown plants. A study undertaken by Manzini (2005) indicated that majority of traditional healers estimated that the growing period of wild ginger ranged from five to eight months while others believed that it took nine to twelve months to grow the plant. Furthermore, the plants were cultivated outside their restricted areas (natural conditions); this conforms to Melody (2010) and Gromicko (2016) who reported that hydroponic plants can grow out of their natural areas.

2.4.4 Quantity of irrigation water used

Hydroponic systems use significantly less water than conventional ones, especially when water is reused and recirculated through the system; this result in lower water bill (Gromicko, 2016). Water applied to 5-days interval was lower than 3-days interval nonetheless there were no differences in the responses of plants to reduced water application. These findings are in agreement with those of Zayton et al. (2014), who said that the lower the irrigation frequency (five days), the smaller the total amount of water applied. According to The Gardener (2012), an average person with a home garden can use 150 to 200 liters of water per day to water the garden however, in this study about 33 liters of water per day was applied. Hydroponic production of *S. aethiopicus* used up to 6 times less water than home garden (open field) production.

In conclusion, both watering regimes produced plants of greater vigour and therefore the choice of 5-days interval for these substrate combinations would be recommended to decrease water use and increase production cost. The potential of the plant to survive under less frequent irrigation can assist in water saving strategies. It is also concluded that application of coir reduces the negative effects of water shortage while optimizing plant growth and has greater longevity which could lead to additional

cost efficiencies. Long irrigation intervals and substrate combinations have a potential to economize water consumption.

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Chapter Three

Effect of different substrate combinations and watering regimes on nutrient uptake, anti-*Fusarium oxysporum* (Hypocreales) activity and secondary metabolite profile of rhizomes and foliage extracts of hydroponically cultivated *Siphonochilus aethiopicus* (Schweinf.) B.L. Burtt (Zingiberaceae)

3.1 Introduction

Studies on plant secondary metabolites have been increasing over the last 50 years (Bourgaud et al., 2001). These molecules are known to play a major role in the adaptation of plants to their environment, but also represent an important source of active pharmaceuticals (Ramachandra Rao & Ravishankar, 2002; Pandey et al., 2015; Yadav & Yadav, 2016). Due to their large biological activities, plant secondary metabolites have been used for centuries in traditional medicine. Today, they correspond to valuable compounds such as pharmaceutics, cosmetics, fine chemicals, or more recently nutraceutics (Bourgaud et al., 2001). Plants are tremendous source for the discovery of new products of medicinal value for drug development. At present, numerous distinct chemicals derived from plants are important drugs used in one or more countries in the world (Vanisree et al., 2003). Within these groups of plant secondary metabolites, the three largest molecule families are: phenolics, terpenes and steroids, and alkaloids. A good example of a widespread metabolite family is given by phenolics because these molecules are antecedents to lignin synthesis and they are commonly found in all higher plants (Bourgaud et al., 2001; Nguyen et al., 2013). For many of the medicinal plants of current interest, a primary focus of research to date has been in the areas of phytochemistry, pharmacognosy and horticulture. In the area of phytochemistry, medicinal plants have been characterized for their possible bioactive compounds, which have been separated and subjected to detailed structural analysis. Horticultural research on medicinal plants has focused on developing the capacity for optimal growth in cultivation (Briskin, 2000).

The production of plant secondary metabolites has, for a long time been achieved through the field cultivation of medicinal plants. However, it happens that some plants

do not withstand large field cultures due to pathogen sensitiveness (Bourgaud et al., 2001). According to Kirakosyan and Kaufman (2002) contemporary, the bulk of the market products such as crude extracts from higher plants are obtained from the wild, providing an opportunity for development of production strategies that are environmentally sustainable and economically viable. Wild plants are exposed to a myriad of environmental factors viz. temperature, humidity, the supply of water, nutrients, which vary in space and time and thus result to variation in secondary metabolite production (Ramakrishna & Ravishankar, 2011; Anderson et al., 2014; Liu et al., 2015). This has led scientists, researchers and farmers consider other cultivation and propagation methods such as hydroponics and tissue culture respectively as alternative ways to produce the corresponding secondary metabolites (Bourgaud et al., 2001). Greenhouse production facilities can be maintained throughout the year, leading to increased concentration of biologically active phytochemicals and the medicinal capacity of the plant tissues (Murch et al., 2002). Controlled growth systems make it feasible to contemplate manipulation of phenotypic variation in the concentration of medicinally important compounds present at harvest (Pedneault et al., 2002; Canter et al., 2005). Researchers such as Marapetyan (1984), Gontier et al. (2002), Manukyan (2005) to mention the few have employed hydroponic cultivation for growth of aromatic and medicinal plants (Dimaki, 2007). These studies showed that hydroponic systems could be a promising way for production of aromatic and medicinal plants and could also meet the increasing demand of the pharmaceutical industry for natural medicinal plant material. Hydroponics may provide a suitable growing system for high quality biomass production while allowing the regulation of secondary metabolism by managing the nutrient solution (Bolonhezi et al., 2010). Also, adoption of hydroponics can increase water-use efficiency therefore reducing wastage (Putra & Yuliando, 2015).

Environmental conditions could be modified in greenhouse production to maximize the accumulation of key compounds. Subjecting plants to controlled stress in particular can result in changes in levels of secondary metabolites production, some of which they may be of medicinal interest (Tuomi et al., 1984). Selmar (2008) and Manukyan (2011) indicated that the formation of bioactive constituents of plants depend on the actual

environment and on growing conditions. A successful and effective application of deliberate and manipulated drought stress for quality improvement, such as applying special watering regimes in combination with efficient soil draining is an encouraging new tool for the production of spice and pharmaceutical relevant plants (Selmar, 2008). Limited water supply has a generally negative effect on plant growth and development. However, there are reports on the positive effect of limited water supply on the biosynthesis of secondary metabolites (Singh-Sangwan et al., 2001). It seems necessary to do research related to the relationship between medicinal plants and water deficit for the increasing need of medicinal plants (Jaleel et al. 2007).

Siphonochilus aethiopicus is a deciduous plant which bears cone-shaped rhizomes; the medicinal value of the plant is associated with these rhizomes (Golding, 2003; FAO, 2008). According to Fouche et al. (2011) *S. aethiopicus* has anti-inflammatory properties supporting anecdotal accounts of its effectiveness against asthma, sinusitis, colds and flu. Knowles (2005) also affirmed that the essential oil of the roots and rhizomes are virtually identical in composition and are generally used for their decongestant properties, provide some rational for the use of wild ginger in the traditional treatment of flu and coughs. It contains a volatile oil with the antiseptic alpha-terpineol and other monoterpenoids. The main compound is a highly characteristic sesquiterpenoid (FAO, 2008). Plant extracts mainly added to herbal preparation for their interesting use, are associated with several antimicrobial properties including anti-inflammatory and antioxidant properties. Herbal extracts have also shown to exhibit antifungal properties (Lall & Kishore, 2014). Antifungal and antibacterial activities of *S. aethiopicus* have been confirmed by several researchers (Coopposamy et al., 2010; Igoli et al., 2012; Lategan et al., 2009) using extracts from the rhizomes of the plant but little attention has been given to antifungal activities of the leaves. Due to its popular use amongst traditional healers and the method of harvesting (removal of the entire rhizome) the plants have become extinct in many parts of its natural habitat (Viljoen et al., 2008).

The increasing interest of the pharmaceutical industry in exploiting natural products has initiated scientific studies on the so-called aromatic and medicinal plants. However, information regarding their cultivation is scarce and as bioactive phytochemicals are highly at risk to environmental regulation and optimization; and full control of growth conditions is vital for consistent and high quality medicinal product (Dimaki, 2007). Thus, *S. aethiopicus* was cultivated by means of hydroponics under various watering regimes and substrates combinations. The objective of this chapter was to evaluate the effect of substrate combinations and watering regimes on macro- and micronutrient uptake, anti-*F. oxysporum* activity and secondary metabolite profile of *S. aethiopicus*.

3.2 Methods and materials

3.2.1 Plant material

S. aethiopicus rhizomes were obtained from a commercial nursery Afro Indigenous at Centurion, Gauteng, South Africa. The rhizomes were originally grown in a tissue culture lab as they have literally disappeared from the wild in South Africa. The rhizomes were dipped in Captab (4 g in 1 L) to prevent development of fungi and were imbedded in 49 pots containing a medium mix of (2 parts pine bark, 1 part perlite and 1 part vermiculite). The pots were placed in a controlled tunnel with the following growing conditions; temperature; day (17 °C-21 °C) and evening (15 °C-18 °C). Pots were hand irrigated with sterile distilled water as needed until crop emergence which was noted. Six weeks old healthy and vigorous seedlings with one to three leaves were each transplanted into separate pots (12.5 cm) filled with substrate combinations according to treatments inside the controlled tunnel.

3.2.2 Substrates preparation

Coconut fiber (coir) was used as the main component for the preparation of media in different proportions and combinations. The combinations used in this study composed of coir, vermiculite, perlite obtained from The Cape Agricultural Suppliers, Cape Town and bark from the Department of Horticulture, Cape Peninsula University of Technology. The unexpanded coir blocks were then fully expanded by soaking in water

and the fibre was sun dried to reduce wetness. The treatments consisted of four different combinations of organic and inorganic substrates in different proportions.

Table 3.1: Composition of different substrate blends used in the study

Treatment	Composition
T1	Coir + vermiculite + perlite + bark (1:1:1:1)
T2	Coir + bark (1:1)
T3	Coir + perlite (1:1)
T4	Coir + vermiculite (1:1)

The quantity of substrate blend in each pot was approximately 200 g. Each combination was thoroughly mixed in a bucket before filling into pots.

3.2.3 Experimental design

The experiment was conducted in a tunnel within the nursery of the Department of Horticultural Sciences, Cape Peninsula University of Technology, Bellville campus; Cape Town, South Africa, 33° 55’ 48.8” S, 18° 38’ 32.7’’ E. The structure was made of galvanized steel frame with transparent polycarbonate. Steel tables (2.5 × 1 m) were used as a flat surface for white plastic gutters (1.36 m long). Eight plastic gutters were placed on two steel tables; each steel table had four gutters which were held in place by cable ties. Plastic gutters and cable ties were supplied by Builders Warehouse, Cape Town). Each gutter had eight plastic pots (12.5 cm) containing different substrate combinations as treatments. Pots were lined at the bottom with shade cloth to prevent substrates leaving through the drainage holes. Beneath the steel table there were four fish tanks (60 L) each containing one submersible water pump, which recirculates water through a 20 ml black plastic pipe to one gutter only. All gutters were wrapped with black plastic polyethylene sheets to avoid algae build-up.

Nutrient solution was supplied to the plants by spaghetti tubing with drippers (1 dripper/plant) at a rate of 2 L/h controlled by a timer. Plants were fertigated with Nutrifeed fertilizer supplied by Starke Ayres, Cape Town containing the following ingredients: 65 g/kg N, 27 g/kg P, 130 g/kg K, 70 mg/kg Ca, 20 mg/kg Cu, 1500 mg/kg Fe, 10 mg/kg Mo, 22 mg/kg Mg, 240 mg/kg Mn, 75 mg/kg S, 240 mg/kg B and mg/kg Zn. Fertilizer group 1 Reg No: K2025 (Act 36/ 1947). Nutrient solutions were prepared by dissolving 60 g of fertilizer in 60 L reservoir with tap water. The pH and electrical conductivity (EC) were maintained at 6.4 and 1.8, respectively. The nutrient solutions were refreshed every 2 weeks to minimize build-up of salts in the substrates. As the water drained out of the pots it drained back into the reservoirs and was reused. The experiment was arranged in a randomized block design and was allowed to run for 9 weeks. The tunnel had the following experimental conditions; average temperature; morning (18–33 °C), day (26–49 °C) and evening (19–34 °C), relative humidity; morning (67–92%), day (80–94%) and evening (81–92%), and average light intensity; morning (3917 Klux), day (19270 Klux) and evening (2685 Klux).

3.2.4 Watering regime

Two watering regimes were used in this study to evaluate their effect on plant growth. Seedlings in four different treatments were grown under two watering regimes: watering twice in three days and watering twice in five days. One set of 24 plants planted in four treatments (each treatment replicated three times) received 3-days interval watering and another set received 5-days interval watering. All the 24 plants were allocated to each of the watering regimes giving a total population of 48 plants for the experiment. In both watering regimes, treatments were irrigated for 15 minutes twice a day; at 10:00 h and 18:00 h. The volume of nutrient applied was 1380 mL (1.38 L) per pot per watering regime. All plants within each watering regime received the same cultivation procedure viz; application of fertilizer, experimental conditions, and treatments.

3.2.5 Tissue analysis

Leaf samples were analysed for macro- and microelements by a commercial laboratory Bemlab (Pty) Ltd in Somerset West, South Africa. Leaves were washed with Teepol solution, rinsed with de-ionised water and dried at 70°C overnight in an oven. The dried leaves were then milled and ashed at 480°C shaken up in a 50:50 HCl (50%) solution for extraction through filter paper (Campell & Plank, 1998; Miller, 1998). The Potassium (K), Phosphorus (P), Calcium (Ca), Magnesium (Mg), Sodium (Na), Manganese (Mn), Iron (Fe), Copper (Cu), Zinc (Z) and Boron (B) content of the extracts were analysed using Ash method. Total Nitrogen (N) content of the leaves was determined through total combustion in a Leco N-analyser. The amounts of N, P, K, Ca and Mg were converted from percentage (%) to mg/kg; 10 000 was used as the conversion factor.

3.2.6 Extraction of plant material

Fresh rhizomes and aerial parts were harvested at 9 weeks post-treatment and air-dried at 28 ± 2 ºC. Dried plant material was cut into smaller pieces and ground using a Jankel and Kunkel Model A 10 mill into fine powder. Powdered plant material (3 g) was extracted with 60 ml of acetone in glass beaker and the supernatant filtered through Whatman No.1 filter paper. Acetone is a useful extractant because it is less toxic, highly volatile and dissolves a wide range of hydrophilic and lipophilic compounds (Eloff, 1998). The extracted material was left to dry over night at room temperature (22 ± 2 ºC) and the dried acetone extracts were weighed.

3.2.7 Antimicrobial activities of extracts

The microdilution method described by Nchu et al. (2010) was employed with minor modifications in determination of the minimum inhibitory concentration (MIC) for the extracts. *S. aethiopicus* extracts were diluted into acetone to obtain a starting concentration of 6 mg/ml. The starting concentration was diluted two fold in each successive serial dilution. The *Fusarium oxysporum* f sp.glycines strain (UPFC no. 21) was obtained through the courtesy of the Phytomedicine Programme, University of Pretoria. The fungus strain was originally isolated by C. Cronje from roots of a maize

plant Delmas, Gauteng. *F. oxysporum* was sub-cultured from stock agar plates and grown into Nutrient Broth (Merck, South Africa) for four hours. The fungal culture (100 ml) was added to each well of the 96-well microplates (10^5 cells/ ml). Amphotericin b (160 µg/mL) was prepared as stock solution in acetone and served as a positive control and acetone was used as a negative control. Forty micro litre (40 µl) of 0.2 mg/ml of *p*-iodonitrotetrazolium chloride (INT) (Sigma) dissolved in sterile distilled water was added to each microplate well, sealed in a plastic bag and incubated at 37 ºC and 100% RH. The MIC values were recorded after 12 and 18 hours. The antifungal bioassay (MIC) consisted of three replicates per treatment and per watering regime.

3.2.8 Total activity (TA)

The total activity in ml/g indicates the volume to which the extract derived from one (1) gram of plant material can be diluted and still inhibits the growth of the tested microorganism. The total activity of the acetone extracts of *S. aethiopicus* was calculated using the following equation: Total activity = total mass (yield) in mg extracted from 1 g of dried plant material divided by the MIC value in mg/ml (Eloff, 2000; Dzoyem et al. 2014). The higher the total activity, the more effective is the plant.

3.2.9 Preliminary analysis

Antifungal activity of the rhizome that was originally grown in tissue culture was analysed as described in section 3.2.6; 3.2.7 and 3.2.8 to compare the values with hydroponically grown rhizomes. Suppliers were not able to obtain plant material from the wild and pointed out that *S. aethiopicus* has literally vanished from the wild in South Africa.

3.2.10 Liquid Chromatography-Mass Spectrometry (LC-MS)

Due to limited plant material (aerial parts and rhizomes) of *S. aethiopicus*, only treatments with the highest antifungal activity were subjected to extraction with acetone (analytical grade) for each watering regime. Three replicates were used for each treatment. In each case powdered plant material (3 g) was suspended in acetone (60 ml) followed by stirring for 18 hours. Each mixture was then filtered using Whatman

No.1 filter paper and the supernatant was evaporated to dryness. Methanol (2 ml) was added to the supplied samples, it was sonicated for 10 min and centrifuged for 5 min at 3000 G and transferred to vials. A Waters Synapt G2 quadrupole time-of-flight mass spectrometer was used for LC-MS analysis. It was fitted with a Waters Ultra pressure liquid chromatograph and photo diode array detection. LC separation was attained on a Waters BEH C18, 2.1x100 mm column with1.7 um particles. A gradient was applied using 0.1% formic acid (solvent A) and acetonitrile containing 0.1% formic acid (solvent B). The gradient started at 100% solvent A for 1 minute and changed to 28% B over 22 minutes in a linear way. It then went to 40% B over 50 seconds and a wash step of 1.5 minutes at 100% B, followed by re-equilibration to initial conditions for 4 minutes. The flow rate was 0.3 ml/min and the column was kept at 55 ºC. The injection volume was 2 µL. Data was acquired in MS^E mode which consisted of a low collision energy scan (6V) from *m/z* 150 to 1500 and a high collision energy scan from *m/z* 40 to 1500. The high collision energy scan was done using a collision energy ramp of 30-60V. The photo diode array detector was set to scan from 220-600 nm. The mass spectrometer was optimized for best sensitivity, a cone voltage of 15 V, desolvation gas was nitrogen at 650 L/hr and desolvation temperature 275 ºC. The instrument was operated with an electrospray ionization probe in the negative mode. The negative mode was chosen for the purpose of targeting the phenolic class of compounds, because they are amenable to this method of analysis and they occur broadly in the Zingiberaceae family. Sodium formate was used for calibration and leucine encephalin was infused in the background as lock mass for accurate mass determinations. The entire flow from the LC was directed into the mass spectrometer. LC-MS chromatograms obtained for the crude extracts were analysed by Masslynx to get spectra of various peaks. The initial approach was to extract ion chromatograms of molecular masses corresponding to compounds which had previously been reported for the family Zingiberaceae. Only major peaks in each chromatogram were analysed for determination of molecular masses and fragmentation data for targeted compounds were compared to known data. Retention time peaks for crude extracts were compared to the peak obtained with the standards (protocatechuic acid, caffeic acid, quercetin, p-hydroxybenzoic acid, rutin, kaempferol, epicatechin, naringenin and hesperetin). From the chromatograms, the

observed area under each peak was used to visually estimate the quantity of each of the targeted compounds present in the eluted crude plant extract at a fixed scale.

3.2.11 Statistical analysis

The experimental data collected were analyzed using one-way analysis of variance (ANOVA) and Tukey HSD test was used to separate the means at a level of significance, $P < 0.05$. These computations were performed using STATISTICA software (StatSoft, 2013). Sigma Plot 10.0 package was used for plotting graphs.

3.3 Results

3.3.1 Tissue analysis

3.3.1.1 Macronutrients

In 3-days interval watering, the levels of N, Ca, Mg and Na did not differ significantly between treatments (df = 3, 8; $P \geq 0.05$) (Table 3.2). However, there were significant differences ($P < 0.05$) on the uptake of P and K. The uptake of P increased significantly in aerial parts grown in T3 (6266.6 ± 88.19 mg/kg), while K (63000 ± 763.76 mg/kg) levels were higher in T2. On the contrary, in 5-days interval watering; levels of P, K, Ca and Na did not show significant differences in aerial parts of *S. aethiopicus* in all treatments (Table 3.2). Nevertheless, the levels of N and Mg varied significantly ($P < 0.05$) in different treatments; N uptake (33400 ± 360.55 mg/kg) best result was observed in T4 while Mg (3233.3 ± 66.66 mg/kg) highest uptake was found in T1. The uptake of these macronutrients displayed higher values in watering regime with increased water supply. The best results for each were observed in treatments involved in 3-days interval watering.

3.3.1.2 Micronutrients

As shown in Table (3.3), uptake of Mn, Zn and NO_3^- in 3-days interval watering did not show significant difference ($P > 0.05$) in aerial parts of *S. aethiopicus* between treatments. However, uptake of Fe, Cu, B, and NH_4^- varied significantly ($P < 0.05$) in different treatments. The best Fe uptake (85.3 ± 2.18 mg/kg) was recorded in T1; Cu

(3.3 ± 0.33 mg/kg) and NH_4^- (4646.6 ± 140.1 mg/kg) highest uptake was recorded in T4 while B (73.33 ± 5.89 mg/kg) levels were higher in T3. On the other hand, in 5-days interval watering different treatments did not significantly affect the uptake of Mn, Fe, Cu, Zn, NO_3^- and NH_4^- (Table 3.3). Whereas, only B (82.33 ± 6 mg/kg) levels recorded significant increase in aerial parts when grown in T3. Additionally, micronutrients displayed higher values in watering regime with increased water supply. The best results for each were observed in treatments involved in 3-days interval watering except for Mn and B which showed best results in 5-days interval watering.

Table 3.2: Effect of different substrate combinations and watering regimes on macronutrient uptake of *S. aethiopicus* aerial parts.

		Watering regimes	
Nutrient (mg/kg)	**Treatment**	**3-days interval**	**5-days interval**
N	T1	30600 ± 1686.2 a	29466 ± 751.29 a
	T2	29866.6 ± 1102 a	30533 ± 218.58 ab
	T3	30400 ± 901.85 a	29466 ± 1201.8 a
	T4	33533.3 ± 788.1 a	33400 ± 360.55 b
P	T1	6200 ± 57.73 a	5666.6 ± 240.37 a
	T2	6033.3 ± 88.19 ab	5800 ± 351.18 a
	T3	6266.6 ± 88.19 a	5966.6 ± 284.8 a
	T4	5333.3 ± 284.8 b	5266.6 ± 375.65 a
K	T1	61300 ± 1096.96 ab	57333.3 ± 1328.3 a
	T2	63000 ± 763.76 b	63866.6 ± 1922.9 a
	T3	60366.6 ± 328.29 ab	59466.6 ± 1003.8 a
	T4	59033.3 ± 866.66 a	58633.3 ± 2251.9 a
Ca	T1	6033.3 ± 463.08 a	5233.3 ± 33.33 a
	T2	6166.6 ± 145.29 a	5466.6 ± 133.33 a
	T3	6533.3 ± 185.59 a	5133.3 ± 33.33 a
	T4	6433.3 ± 296.27 a	5533.3 ± 366.66 a
Mg	T1	3166.6 ± 120.18 a	3233.3 ± 66.66 b
	T2	2933.3 ± 33.33 a	2800 ± 0 a
	T3	3000 ± 152.75 a	2866.6 ± 88.19 a
	T4	2933.3 ± 88.19 a	2933.3 ± 88.19 ab
Na	T1	239.66 ± 47.69 a	313 ± 22.47 a
	T2	249.66 ± 35.07 a	365.6 ± 28.75 a
	T3	382.66 ± 86.56 a	337 ± 18.5 a
	T4	255.33 ± 29.62 a	286.6 ± 15.05 a

Means followed by same lowercase letters in the same column are not significantly different ($P < 0.05$) following comparison using Tukey test. Grey and white colours are used to differentiate columns with macronutrients.

Table 3.3: Effect of different substrate combinations and watering regimes on micronutrient uptake of *S. aethiopicus* aerial parts.

		Watering regimes	
Nutrient (mg/kg)	**Treatment**	**3-days interval**	**5-days interval**
Mn	T1	206 ± 19.08 a	234.6 ± 36.19 a
	T2	158.66 ± 7.12 a	240 ± 25.02 a
	T3	161.33 ± 37.22 a	235.3 ± 39.26 a
	T4	220 ± 26.62 a	240.3 ± 49.8 a
Fe	T1	85.3 ± 2.18 b	69 ± 3.21 a
	T2	66 ± 5.68 a	54 ± 3.05 a
	T3	61.3 ± 3.33 a	65.3 ± 6.56 a
	T4	66.3 ± 4.66 a	67.6 ± 3.92 a
Cu	T1	3 ± 0 ab	3.33 ± 0.33 a
	T2	2.6 ± 0.33 ab	3 ± 0 a
	T3	2 ± 0 a	3 ± 0 a
	T4	3.3 ± 0.33 b	3.33 ± 0.33 a
Zn	T1	38.66 ± 1.45 a	33.66 ± 2.18 a
	T2	38.33 ± 2.02 a	37.33 ± 3.92 a
	T3	34.33 ± 1.66 a	33.66 ± 1.33 a
	T4	36.33 ± 1.45 a	31 ± 1.15 a
B	T1	62 ± 2.88 ab	60 ± 4.04 ab
	T2	50.33 ± 2.03 a	55.66 ± 4.05 a
	T3	73.33 ± 5.89 b	82.33 ± 6 b
	T4	66.66 ± 4.63 ab	80.33 ± 7.53 ab
NO_3^-	T1	2679.3 ± 167.61 a	1972.6 ± 183.6 a
	T2	1983.3 ± 265.83 a	1957 ± 124.85 a
	T3	1681.3 ± 192.24 a	1754.6 ± 59.52 a
	T4	2214.6 ± 443.72 a	1497.3 ± 208.22 a
NH_4^-	T1	3888 ± 158.75 a	3549.6 ± 267.12 a
	T2	4511.6 ± 234.9 ab	3927.6 ± 281.34 a
	T3	4251 ± 78.07 ab	3451.6 ± 228.91 a
	T4	4646.6 ± 140.1 b	3751 ± 125.48 a

Means followed by same lowercase letters in the same column are not significantly different ($P > 0.05$) following comparison using Tukey test. Grey and white colours are used to differentiate columns with micronutrients.

3.3.2 Yield, Minimum Inhibitory Concentration (MIC) and Total Activity (TA)

3.3.2.1 Yield

The yield following acetone extraction of aerial parts of *S. aethiopicus* was not statistically different ($P > 0.05$) among treatments and watering regimes. However, it was observed that in 3-days interval (Table 3.4) the highest mean value was obtained in T3 (179 ± 10.17 mg) followed by T1 (162 ± 19.42 mg), T2 (119 ± 61.27 mg) and T4 (104.66 ± 47.47 mg), respectively. Similarly, in 5-days interval watering T3 gave the highest values compared to other treatments (T1, T4 and T2 respectively). On the other hand, there was also no marked difference ($P > 0.05$) in yield of *S. aethiopicus* rhizomes among treatments and watering regimes (Table 3.5). It was however observed that in 3-days interval watering the highest yield was obtained in T2 (166 ± 33.3 mg) followed by T1 (133 ± 33.3 mg) while T3 and T4 had the same and lowest mean values (100 ± 0 mg). Conversely, in 5-days interval the highest yield was observed in T1 and T2 (133 ± 33.3 mg) followed by T3 and T4 (100 ± 0 mg). Additionally, yield of the tissue culture grown rhizome (Table 3.6) (122 ± 0 mg) was not significantly different compared to hydroponically cultivated rhizomes.

3.3.2.2 Minimum inhibitory concentration

The MIC values of acetone extracts of *S. aethiopicus* aerial parts and rhizomes were not statistically different ($P > 0.05$) among treatments and watering regimes.

Aerial parts: it was observed that in 3-days interval watering ($df = 3,8$; $F = 1.8$; $P > 0.05$) (Table 3.4), the MIC value of acetone extracts of the aerial parts grown in T3 and T4 was 0.5 ± 0.125 mg/ml at 12 hours post treatment and T3 remained unchanged at 18 hours post treatment. This MIC value was lower compared to those obtained with acetone extracts of plants that were grown in T4, T2 and T1 at 18 h post treatment. Acetone extracts of aerial parts exposed to T2 and T1 had the same MIC values; 0.625 ± 0.125 mg/ml and 0.75 ± 0 mg/ml at 12 and 18 h post treatment respectively and exhibited the weakest anti-fungal activities. Similarly, in 5-days interval watering ($F = 1.2$; $P > 0.05$) (Table 3.4); the MIC value of acetone extracts of aerial parts grown in T3

was 0.3125 ± 0.062 mg/ml at 12 h post treatment and changed at 18 h post treatment to 0.375 ± 0 mg/ml. T3 MIC values at 12 and 18 h post treatment were lower compared to those obtained with acetone extracts of plants grown in T4, T2 and T1. Acetone extracts of plants that were grown in T4 recorded; 0.375 ± 0 mg/ml and 0.5 ± 0.125 mg/ml in the anti-*F. oxysporum* bioassay at 12 h and 18 h post-treatment respectively. Acetone extracts of plants that were grown in T2 and T1 displayed the weakest and equal MIC values; 0.5 ± 0.125 mg/ml and 0.625 ± 0.125 mg/ml at 12 and 18 h respectively.

Rhizomes: in 3-days interval watering (df = 3, 8; F = 0.88; $P > 0.05$) (Table 3.5), the MIC value of acetone extracts of rhizomes that were grown in T3 was 0.078 ± 0.015 mg/ml at 12 h post treatment and changed at 18 h post treatment to 0.25 ± 0.06 mg/ml. T3 MIC values at 12 and 18 h post treatment were lower compared to those obtained with acetone extracts of plants grown in T4, T2 and T1. Acetone extracts of plants that were grown in T2 and T4 recorded equal MIC values; 0.125 ± 0.03 mg/ml and 0.3125 ± 0.06 mg/ml in the anti-*F. oxysporum* bioassay at 12 h and 18 h post-treatment respectively. Acetone extracts of plants grown in T1 displayed the weakest MIC values; 0.156 ± 0.03 mg/ml and 0.375 ± 0 mg/ml at 12 and 18 h respectively. Also the results showed that in 5-days interval watering (F = 1.8; $P > 0.05$) (Table 3.5), the MIC value of acetone extracts of rhizomes that were grown in T3 was 0.06 ± 0.015 mg/ml while T2 and T1 MIC value obtained was 0.125 ± 0.03 mg/ml at 12 h post treatment. The MIC value of acetone extracts of plants grown in T3 and T4 was 0.1875 ± 0 mg/ml at 18 h post treatment. This MIC value was lower compared to those obtained with acetone extracts of plants that were grown in T2 and T1; T2 exhibited the weakest anti-fungal activities at 18 h post treatment. Additionally, the MIC value of the tissue culture grown rhizome (Table 3.6) (0.3125 ± 0.06 mg/ml) at 18 hour was not significantly different compared to hydroponically cultivated rhizomes. The antifungal activities of the extracts were significantly lower than amphotericin b. Generally, the MIC values of aerial parts and rhizomes in 5-days interval were lower compared to 3-days interval watering.

3.3.2.3 Total activity (TA)

Total activity demonstrates the quantity at which the extract may be diluted with a solvent and still inhibit growth of a microorganism. In the present study, at 12 hours and 18 hours there was no significant difference in calculated TA among substrate combinations and watering regimes. However, acetone extract of aerial parts grown in T3 under 3-days interval watering (129.86 ± 20.86 ml/g [12 and 18 hours] recorded the highest values of TA against *F. oxysporum* compared to the other treatments (T1 [90.5 ± 9.95 and 72.13 ± 8.6 ml/g], T4 [91.4 ± 43.79 and 66.96 ± 34.15 ml/g] and T2 [86.64 ± 59.29 and 52.86 ± 27.2 ml/g] at 12 and 18 hours respectively (Table 3.4). Similarly, in 5-days interval (Table 3.4); T3 (203.2 ± 49.99 and 153.16 ± 11.89 ml/g [12 and 18 hours] respectively, recorded the highest values of the calculated TA compared to the other treatments (T1 [130.76 ± 37.78 and 103.66 ± 36 ml/g], T4 [120.3 ± 21.2 and 103.4 ± 32.49 ml/g] and T2 [97.16 ± 27.05 and 73.6 ± 15.7 ml/g] at 12 and 18 hours respectively.

On the contrary, in 3-days interval acetone extract of rhizomes grown in T2 (532.1 ± 177.1 ml/g) recorded the highest value of TA against *F. oxysporum* compared to T3 (472.8 ± 118.2 ml/g), T1 (296 ± 59.1 ml/g) and T4 (295.66 ± 58.9 ml/g) at 12 hours respectively (Table 3.5). On the contrary, at 18 hours T1, T2 and T3 (148.16 ± 29.6 ml/g) recorded the highest values of TA whereas T4 (118.5 ± 29.6 ml/g) obtained the lowest value. Nonetheless, in 5-days interval (Table 3.5) acetone extract of rhizomes grown in T3 (591 ± 118.2 ml/g) recorded the highest value of TA compared to T2 and T4 (413.86 ± 156.2 ml/g) while T1 (354.9 ± 0.33 ml/g) recorded the lowest TA value at 12 hours. At 18 hours, T1, T4 and T3 (177.8 ± 2 ml/g) recorded the highest values of TA against *F. oxysporum* whereas T2 (148.16 ± 29.6 ml/g) obtained the lowest TA value. Generally, the highest TA values of aerial parts and rhizomes were recorded in 5-days interval compared to 3-days interval watering.

Table 3.4: Results on yield, minimum inhibitory concentration and total activities of acetone extracts obtained from aerial parts of hydroponically-cultivated *S. aethiopicus* following exposure to various substrate combinations and watering regimes.

Treatment	Yield ± SE (mg)		MIC ± SE (mg/ml)		Total Activity (ml/g)	
	3-days	**5-days**	**3-days**	**5-days**	**3-days**	**5-days**
T1 (12 H)	162 ± 19.42 A	168 ± 21.83 A	0.625 ± 0.125 A	0.5 ± 0.125 A	90.5 ± 9.95 A	130.76 ± 37.78 A
(18 H)			0.75 ± 0 a	0.625 ± 0.125 a	72.13 ± 8.6 a	103.66 ± 36 a
T2 (12 H)	119 ± 61.27 A	127.3 ± 15.96 A	0.625 ± 0.125 A	0.5 ± 0.125 A	86.64 ± 59.29 A	97.16 ± 27.05 A
(18 H)			0.75 ± 0 a	0.625 ± 0.125 a	52.86 ± 27.2 a	73.6 ± 15.7 a
T3 (12 H)	179 ± 10.17 A	172.3 ± 13.38 A	0.5 ± 0.125 A	0.313 ± 0.06 A	129.86 ± 20.86 A	203.2 ± 49.99 A
(18 H)			0.5 ± 0.125 a	0.375 ± 0 a	129.86 ± 20.86 a	153.16 ± 11.89 a
T4 (12 H)	104 ± 47.47 A	165.3 ± 23.88 A	0.5 ± 0.125 A	0.375 ± 0 A	91.4 ± 43.79 A	120.3 ± 21.2 A
(18 H)			0.625 ± 0.125 a	0.5 ± 0.125 a	66.96 ± 34.15 a	103.4 ± 32.49 a
Amphotericin b						
(12 H)			0.047 ± 0 A			
(18 H)			0.047 ± 0 a			
Negative control						
(12 H)			No effect			
(18 H)			No effect			

- Means followed by the same uppercase letters in the same column illustrate no significant difference ($P > 0.05$) at 12 hours post treatment following comparison using Tukey test
- Means followed by same lowercase letters in the same column illustrate no significant difference at 18 hours post treatment.

Table 3.5: Results on yield, minimum inhibitory concentration and total activities of acetone extracts obtained from rhizomes of hydroponically- cultivated *S. aethiopicus* following exposure to various substrate combinations and watering regimes.

Treatment	Yield ± SE (mg)		MIC ± SE (mg/ml)		Total Activity (ml/g)	
	3-days	**5-days**	**3-days**	**5-days**	**3-days**	**5-days**
T1 (12 H)	133 ± 33.3 A	133 ± 33.3 A	0.156 ± 0.03 A	0.125 ± 0.03 A	296 ± 59.1 A	354.9 ± 0.3 A
(18 H)			0.313 ± 0.06 a	0.25 ± 0.06 a	148. 16 ± 29.6 a	177.8 ± 2 a
T2 (12 H)	166 ± 33.3 A	133 ± 33.3 A	0.125 ± 0.03 A	0.125 ± 0.03 A	532.1 ± 177.1 A	413.86 ± 156.2 A
(18 H)			0.375 ± 0 a	0.313 ± 0.06 a	148.16 ± 29.6 a	148.16 ± 29.6 a
T3 (12 H)	100 ± 0 A	100 ± 0 A	0.078 ± 0.02 A	0.06 ± 0.02 A	472.8 ± 118.2 A	591 ± 118.2 A
(18 H)			0.25 ± 0.06 a	0.1875 ± 0 a	148.16 ± 29.6 a	177.8 ± 2 a
T4 (12 H)	100 ± 0 A	100 ± 0 A	0.125 ± 0.03 A	0.109 ± 0.04 A	295.66 ± 58.9 A	413.86 ± 156.2 A
(18 H)			0.313 ± 0.06 a	0.1875 ± 0 a	118.5 ± 29.6 a	177.8 ± 2 a
Positive control (Amphotericin b)						
(12 H)			0.047 ± 0 A			
(18 H)			0.047 ± 0 a			
Negative control						
(12 H)			No effect			
(18 H)			No effect			

- Means followed by the same uppercase letters in the same column illustrate no significant difference ($P > 0.05$) at 12 hours post treatment following comparison using Tukey test.
- Means followed by same lowercase letters in the same column illustrate no significant difference at 18 hours post treatment.

Table 3.6: Preliminary results on yield, minimum inhibitory concentration and total activities of acetone extracts obtained from rhizome of tissue culture- cultivated *S. aethiopicus*.

	Yield ± SE (mg)	**MIC ± SE (mg/ml)**	**Total Activity (ml/g)**
12 H	122 ± 0 A	0.078 ± 0.015 A	346.09 ± 86.55
18 H		0.3125 ± 0.06 a	86.76 21.68

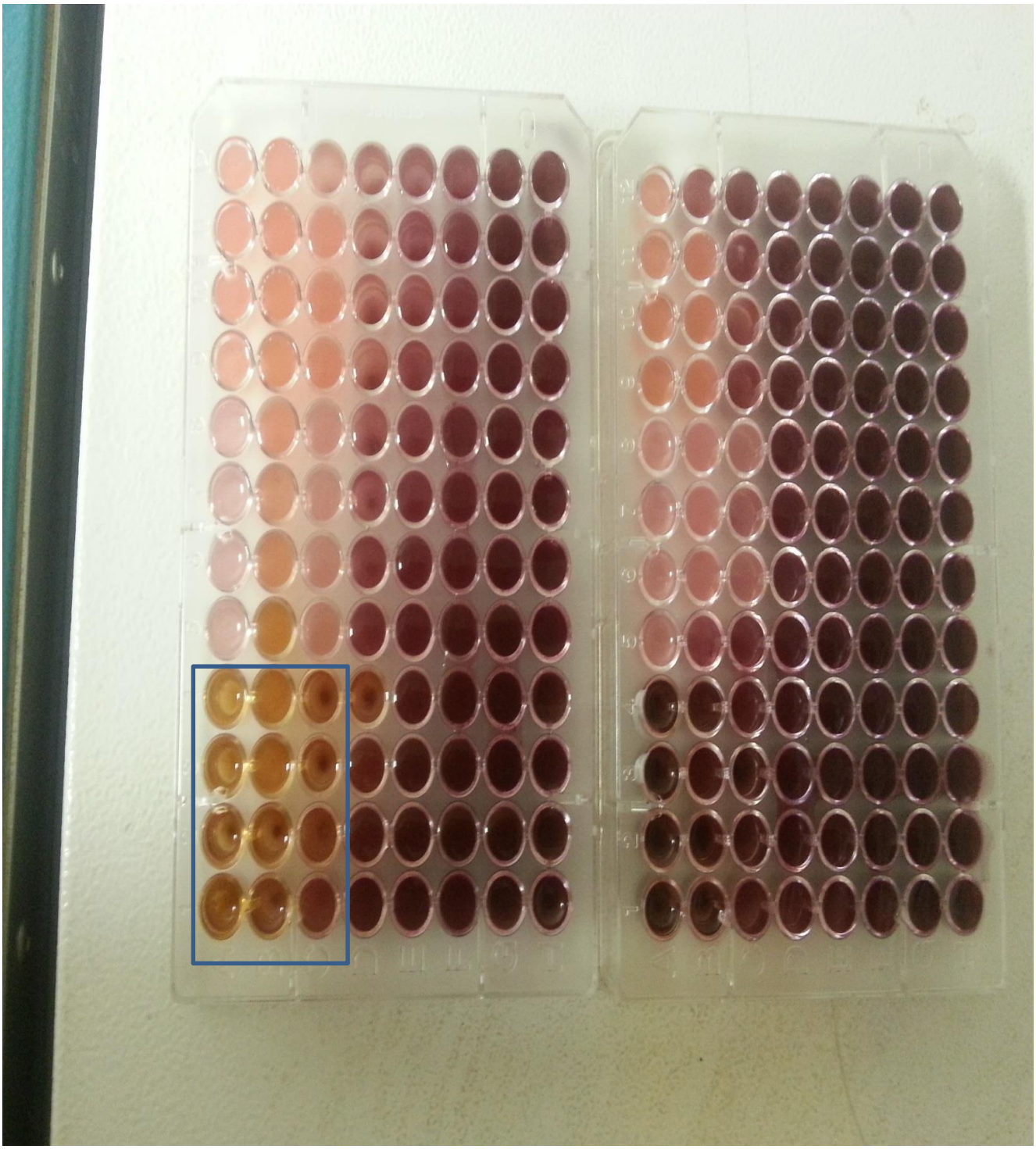


Figure 3.1: Photograph showing anti-*Fusarium* activity of acetone extracts of *S. aethiopicus.*

3.3.3 LC-MS analysis

LC-MS analysis was carried out on the acetone extract of aerial parts and rhizomes of *S. aethiopicus.* Active compounds with their retention time (T_R) and concentrations (ppm) are presented in Table 3.7 (aerial parts) and Table 3.8 (rhizomes). In the present study, a variety of compounds have been detected: protocatechuic acid, caffeic acid, quercetin, p-hydroxybenzoic acid, rutin, kaempferol, epicatechin, naringenin and hesperetin (University of Stellenbosch, Mass Spectrometry Unit); whose structures are presented in Figure 3.4. The results exhibited that the mean concentrations of the isolated compounds were not significantly different ($P > 0.05$) among watering regimes in both aerial parts and rhizome of *S. aethiopicus*. The chromatograms of 3 and 5-days interval watering demonstrated a similar peak profile in both aerial parts and rhizome of the plant. LC-MS chromatograms showing the peak identities of the compounds are depicted in Figure 3.2 and 3.3. However, acetone extracts from the aerial parts showed higher concentration of rutin in 3-days interval [(T_R = 13.3 min) 18.23 ± 5.126 ppm] and 5-days interval watering [(T_R = 13.27 min) 22.88 ± 18.29 ppm]; followed by caffeic acid (found in 5-days interval, only), p-hydroxybenzoic acid, protocatechuic acid, kaempferol, hesperetin and quercetin, respectively. Naringenin had the lowest concentration in 3-days interval [(T_R = 20.75 min) 0.06 ± 0.017 ppm] and 5-days interval watering [(T_R = 20.68 min) 0.09 ± 0.02 ppm] (Table 3.7). On the contrary, acetone extracts from the rhizomes showed higher concentration of p-hydroxybenzoic acid in 3-days interval [(T_R = 6.21 min) 12.03 ± 7.83 ppm] and 5-days interval watering [(T_R = 6.19 min) 19.87 ± 10.05 ppm]; followed by caffeic acid, protocatechuic acid, naringenin and rutin, respectively. Whereas, epicatechin had the lowest concentration in 3-days interval [(T_R = 9.64 min) 0.04 ± 0.02 ppm] and 5-days interval watering [(T_R = 9.63 min) 0.046 ± 0.02 ppm] (Table 3.8). To further interrogate the LC-MS data, the mean concentrations of protocatechuin acid, caffeic acid and rutin were higher in the aerial parts than in rhizomes; while p-hydroxybenzoic acid and naringenin were significantly ($P < 0.05$) higher in the rhizome than in the aerial parts. 5-days interval watering showed the highest concentrations of phenolic compounds than 3-days interval except for protocatechuic acid and kaempferol (aerial parts); and caffeic acid (rhizomes) which were higher in 3-days interval watering. Compounds such as quercetin, kaempferol and

hesperetin were isolated only in aerial parts whereas epicatechin was isolated from the rhizomes, only. Protocatechuin acid, p-hydroxybenzoic acid and epicatechin were isolated from the tissue culture grown rhizome and were higher than hydroponically grown rhizome.

Table 3.7: Effect of watering regimes on phenolic compounds of *S. aethiopicus* aerial parts.

Compounds	Concentration (ppm)			
	T_R	3-days interval	T_R	5-days interval
Protocatechuic acid	4.62	2.02 ± 0.44	4.61	1.59 ± 1.037
Caffeic acid	–	–	8.12	12.33 ± 12.33
Quercetin	18.76	0.23 ± 0.125	18.73	0.34 ± 0.246
p-hydroxybenzoic acid	6.32	6.09 ± 1.62	6.29	6.13 ± 4.45
Rutin	13.3	18.23 ± 5.126	13.27	22.88 ± 18.29
Kaempferol	21.69	0.83 ± 0.236	21.67	0.66 ± 0.16
Naringenin	20.75	0.06 ± 0.017	20.68	0.09 ± 0.02
Hesperetin	18.76	0.326 ± 0.06	18.73	0.33 ± 0.23

- Data presented are in means ± standard error (n = 3)
- (-) represents no phenolic compound isolated, T_R= retention time

Table 3.8: Effect of watering regimes on phenolic compounds of *S. aethiopicus* rhizomes.

Compounds	Concentration (ppm)					
	T_R	3-days interval	T_R	5-days interval	T_R	Tissue culture
Protocatechuic acid	4.62	1.11 ± 0.58	4.63	1.57 ± 0.79	4.59	1.58
Caffeic acid	8.8	4.47 ± 4.47	–	–	–	–
p-hydroxybenzoic acid	6.21	12.03 ± 7.83	6.19	19.87 ± 10.05	6.23	27.16
Rutin	–	–	13.3	0.13 ± 0.078	–	–
Epicatechin	9.64	0.04 ± 0.02	9.63	0.046 ± 0.02	9.63	0.12
Naringenin	20.74	0.19 ± 0.025	20.72	0.25 ± 0.03	20.72	–

- Data presented are in means ± standard error (n = 3)
- (-) represents no phenolic compound isolated, T_R= retention time

Aerial parts

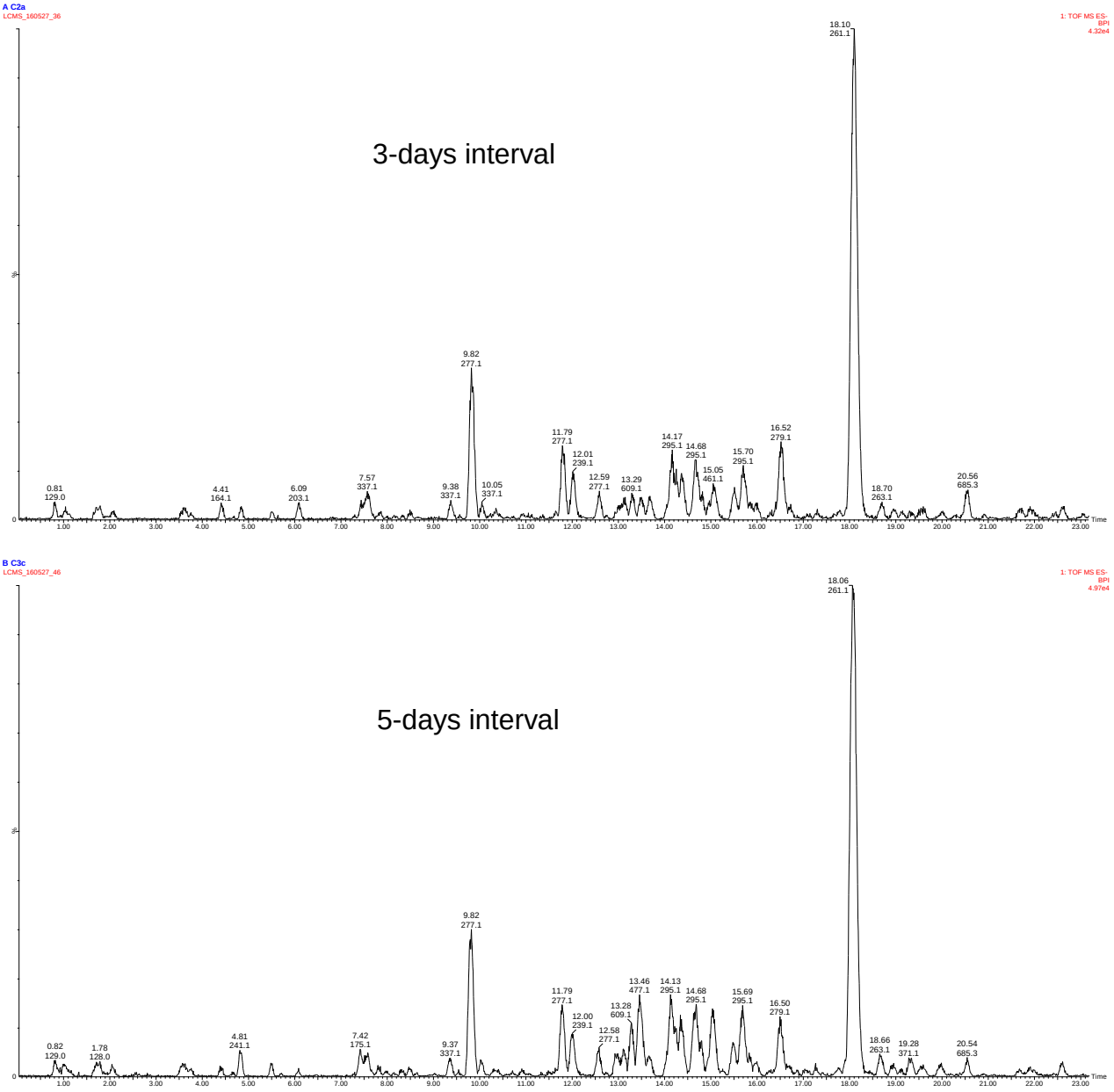


Figure 3.2: LC-MS profiles (TIC = total ion chromatogram) of acetone extract of *S. aethiopicus* aerial parts subjected to different watering regimes (3 and 5-days interval).

Rhizomes

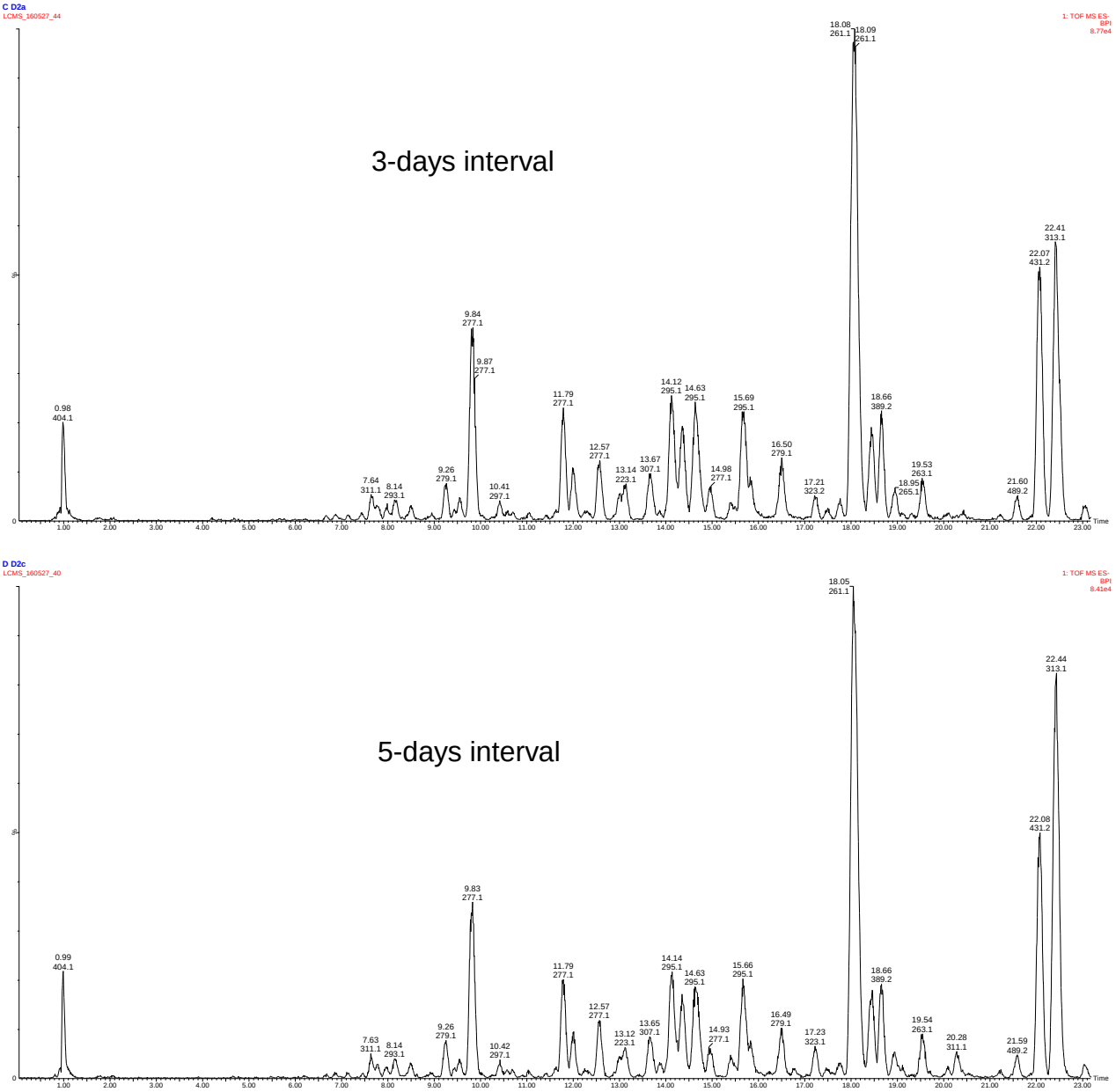
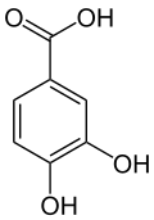
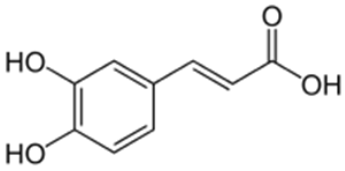


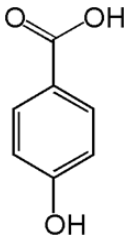
Figure 3.3: LC-MS profiles (TIC = total ion chromatogram) of acetone extract of *S. aethiopicus* rhizomes subjected to different watering regimes (3 and 5-days interval).



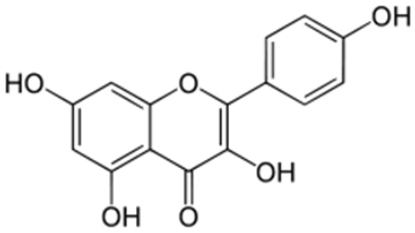
Protocatechuic acid



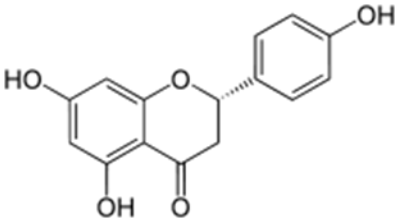
Caffeic acid



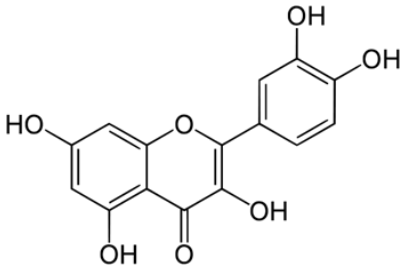
P-hydroxybenzoic acid



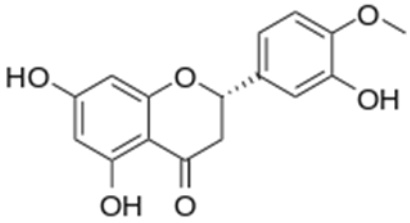
Kaempferol



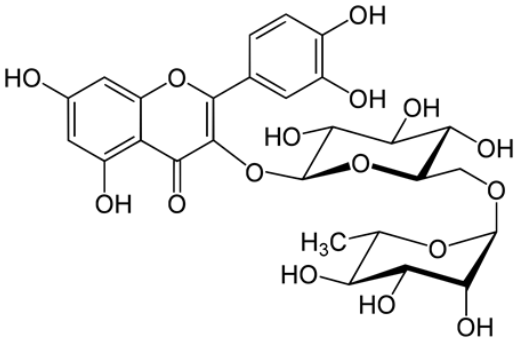
Naringenin



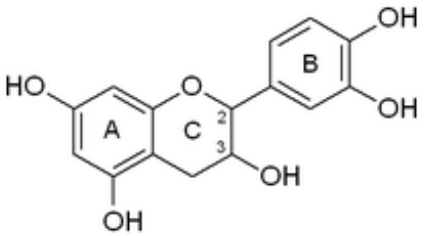
Quercetin



Hesperetin



Rutin



Epicatechin

Figure 3.4: Chemical structures of the nine phenolic compounds isolated from *S. aethiopicus.* Adapted from: https://www.google.co.za/search?q=structures+of+phenolic+compounds

3.4 Discussion

3.4.1 Tissue analysis

In controlled hydroponic systems, nutrient availability is one of the fundamental growth factors that may be influenced by the amount of water supplied to the plants. The quantity of water that plants receive has a significant influence on the amount of nutrients it will contain (Sonnenberg, 2012). In this study, substrate combinations did not have any significant effect on the uptake of the following macro- and micronutrients (Ca, Na, Mn, Zn and NO_3^-) irrespective of the watering regime. Due to difficulty of finding previously published data for leaf nutrient levels to compare with the results of this study, the results were compared to those reported by Alifar and Ghehsareh (2010) which showed that organic and inorganic substrates had no significant difference on concentration of macro and micro elements in the leaf of cucumber. Significant differences were observed in P, K, N, Mg, Fe, Cu, B and NH_4^- (Table 3.2 and 3.3). The best results for each nutrient varied between treatments. Substrate combinations had different effects on different nutrient concentrations. These findings may be explained by the difference in chemical and physical properties of substrates and their interaction with nutrient solution composition and plant nutrient uptake (Abdelaziz, 2010). According to Cuervo et al. (2012) in systems based on organic substrates it is difficult to track the variation of microelements concentration (Cu, Fe, B, Zn and Mn) due to changes in physical, chemical and microbiological properties of the substrates.

From the above results it could be noticed that the uptake of most macro- and micronutrients (Table 3.2 and 3.3) was highly enhanced by exposing plants to higher application of water (3-days interval watering) in comparison with 5-days interval; except for Na, Mn, B and Cu were highly enhanced by 5-days interval. These findings are concurred with findings of Singh and Singh (2004) and Sonnenberg (2012) who discovered that nutrient availability increased by exposing plants to higher doses of water. Sonnenberg (2012) emphasized that an increased nutrient uptake of macro- and micronutrients exposed to higher water quantities, was a result of higher moisture availability and improved transpiration. Plants which received the longest interval of 5

days had the lowest results; this agreed with the findings of Singh and Singh (2004); Hussein and El-Dewiny (2011) that total nutrient content of all the nutrient elements decreased with increasing water stress. Drying of the soil and decrease in irrigation level can reduce the availability, uptake and transport of nutrients (Menzel et al., 1986; Singh & Singh, 2009). Decreasing water availability under drought generally results in reduced total nutrient uptake and frequently in reduced concentration of mineral nutrients in plants (Garg, 2003). Despite significant differences in levels of macro- and micronutrients in aerial parts between watering regimes and substrate combinations no symptoms of deficiency were observed in plants.

3.4.2 Antimicrobial activity and total activity

Results obtained in this study indicated that all the evaluated substrate combinations and watering regimes showed no significant difference on aerial parts (Table 3.4) and rhizome (Table 3.5) extracts of *S. aethiopicus* against *F. oxysporum*. However, acetone extracts of *S. aethiopicus* plants that were grown in T3 (coir and perlite) was the most bioactive against *F. oxysporum* compared to plants grown in T1, T2 and T4. Similarly, Evans (2008) observed that a medium containing 20 percent perlite and 80 percent coconut coir was the most disease suppressive. Generally, acetone extracts of aerial parts and rhizomes of *S. aethiopicus* were found to be bioactive against *F. oxysporum* in the antifungal assay. Nevertheless, there was no statistical difference observed in the anti-*F. oxysporum* activity between coir substrates; this may be due to the presence of phenolic compounds in coir dust. Phenolics in coir may have inhibited disease-causing pathogens (Evans & Stamps, 1996). Phenolic compounds have anti-microbial properties (Zafar et al. 2014) and these compounds play key roles in protecting plants from microorganisms, herbivores (Reidah, 2013). Furthermore, no statistical difference in the antifungal activities of *S. aethiopicus* was observed among watering regimes. The mean values of MIC due to watering regimes showed that increasing period of irrigation (from 3-days interval to 5-days interval) for both aerial parts and rhizomes of *S. aethiopicus* exhibited the highest antifungal inhibitory effects against *F. oxysporum*. Similar results were recorded by Said-Al Ahl et al. (2009). Tissue nutrient content analysis discussed in 3.3.1 showed that manganese, boron, copper and sodium were highly enhanced by 5-days watering interval. There is

much scientific support that micronutrients such as Cu, B and Mn can reduce the severity of plant diseases by increasing disease tolerance and the resistance of plants to pathogens (Parker, 2011). Mn and Cu play a key role in synthesis of phenolic compounds and therefore act as an essential component of plant resistance to a wide range of fungal and bacterial pathogens (Cakmak, unpublished; Spectrum Analytic, Inc). Also, B and Mn have been beneficial in the control of *Fusarium* spp. infections (Walker & Foster, 1946; Dordas, 2008). Spectrum Analytic (unpublished) reported that shortage of the key nutrients such as Mn, Cu, Zn and B reduce the amount of the plants natural antifungal compound at the site of the infection.

In previous studies, *S. aethiopicus* was shown to have antimicrobial activity against the following pathogens; *Aspergillus flavus, Aspergillus glaucus, Candida albicans, Candida tropicalis, Trichophyton mentagrophytes, Trichophyton rubrum, Botrytis cinerea* (Knowles, 2005; Coopoosamy et al., 2010; Fielding et al., 2015). The MIC values obtained in the current study are relatively higher for the rhizomes ranged from 0.078-0.3125 mg/ml but very low in the leaves (0.375-0.75 mg/ml). Correspondingly, Coopoosamy et al. (2010) indicated that an antibacterial activity in the leaf of *S. aethiopicus* was lesser than that of the rhizomes. This can be explained by the fact that the leaf is predominantly involved as a production center, which in turn through transport mechanisms send the formulated products to the rhizomes (Coopoosamy et al., 2010). It is worth mentioning that there was no significant difference in MIC values of plant material grown in tissue culture (0.078 and 0.3125 mg/ml [12 and 18 hours] (Table 3.6) and the one that was used in this study (hydroponically-grown) (Table 3.5). Similarly, Ishimaru et al. (1992) found that roots of the mother plant cultivated under hydroponic conditions contained almost the same phenolic compounds as those cultivated in tissue culture.

In this study, the extracts of aerial parts grown hydroponically in T3 recorded the highest values of TA against *F. oxysporum* when compared to the other treatments; for

both 3-days (129.86 ml/g [12 and 18 hours]) and 5-days (203.2 and 153.16 ml/g) watering intervals at 12 and 18 hours respectively. T2 recorded the least values of TA in 3 and 5-days watering intervals. On the contrary, the extracts of rhizomes grown in T2 recorded the highest values of TA against *F. oxysporum* when compared to the other treatments in both 3 and 5-days intervals. T4 recorded the least values of TA in 3-days interval while T1 and T2 recorded the least values of TA activities in 5-days watering interval. Matanzima (2014) stated that total activity is a very good criterion for comparing biological activities among plant species due to its formula which takes into account the yield and antimicrobial activities of the extracts.

3.4.3 LC-MS analysis

According to the LC-MS analysis, in the examined *S. aethiopicus* acetone extracts nine compounds were identified which matched previously isolated compounds from the family, Zingiberaceae; quercetin, rutin, kaempferol, naringenin, hesperetin and epicatechin (flavonoids), protocatechuic acid, caffeic acid and p-hydroxybenzoic acid (phenolic acids) (Voravuthikunchai, 2007; Ghasemzadeh et al., 2010; Jing et al., 2010; Singh & Gupta, 2013; Taheri et al., 2014; Yashoda et al., 2014; Ghasemzadeh et al., 2016). The results showed that the mean concentrations of the isolated compounds were not significantly different among watering regimes in both aerial parts and rhizome of *S. aethiopicus* (Table 3.7 & 3.8). However, increasing watering interval from 3 to 5-days resulted in enhancement of the identified phenolic compounds. Water deficit is known to increase the secondary metabolites concentration in plant tissues (Ade-Ademilua & Mbah, 2013). In a recent study conducted by Hassan and Ali (2016), it was found that decreasing the irrigation levels increased total phenolic content in cumic (*Cuminum cyminum*). Aziz et al. (2008) also observed that increasing the period between irrigation gave the highest relative constituents of the most important compounds in *Thymus vulgaris*. These findings somewhat support the conclusion that the phenolic compound increase under water deficit. Furthermore, most phenolic compounds were present in aerial parts extract in higher concentration than in rhizome, with exception of p-hydroxybenzoic acid and naringenin which were higher in rhizome. It is worth mentioning that quercetin, kaempferol and hesperetin were isolated in aerial

parts only. Similar results were also obtained in many other studies. In a study conducted by Chan et al. (2007), it was found that for most ginger species screened; leaf phenolic contents were significantly higher than those of rhizome. Also, Tomovic et al. (2015) observed that flavonoid content of aerial part of *Potentilla reptans* was higher than flavonoid content of rhizome. Jing et al. (2010) and Singh and Gupta (2013) determined that the antioxidant activity and phenolic contents of the leaves and the amount of phenolics and flavonoids were higher than those in rhizomes. These findings are in disagreement with the findings of Ghasemzadeh et al. (2010) and Yashoda et al. (2014), which showed that levels of flavonoids and phenolic acids were greater in rhizomes compared to leaves.

Phenolic compounds have been reported to defend the plant against microorganisms and herbivores (Hada et al., 2001). Puupponen-Pimia et al. (2001) stated that many plant phenols are known to possess antimicrobial properties. In particular, the nine compounds isolated from *S. aethiopicus* (quercetin, rutin, kaempferol, naringenin, hesperetin, epicatechin, protocatechuic acid, caffeic acid and p-hydroxybenzoic acid) have been described to possess antimicrobial activity against several bacterial and fungal species (Cowan, 1999; Cetin-Karaca, 2011; Dogasaki et al., 2002; Rauha et al., 2002; Hayek et al., 2013). Since these compounds are categorized as antimicrobials, the effects of antifungal activity of aerial parts and rhizome of *S. aethiopicus* may be attributed to the content of these compounds. Acetone extracts of *S. aethiopicus* possess antimicrobial activity and this potential may be due to the presence of flavonoids and phenolic acids of the plant. It is worth mentioning that catechin has been previously detected in rhizomes of this species (Noudogbessi et al., 2013) while the above mentioned compounds were isolated from the plant for the first time however, they were previously detected in Zingiberaceae species. Furthermore, rutin was the major flavonoid presented in aerial parts, while p-hydroxybenzoic acid was the major compound in rhizome.

3.5 Conclusion

- In conclusion, the uptake of most macro- and micronutrients was highly enhanced by exposing plants to higher application of water (3-days interval watering).
- Antifungal activities were maintained among *S. aethiopicus* plants grown hydroponically and plants exposed to coir and perlite mix under 5-days interval watering were the most bioactive against *F. oxysporum* and yielded the highest plant biomass. Increasing the period between watering (5-days interval) displayed the highest antifungal inhibitory effects. The total activity demonstrates that there is no difference between plant extracts attained from plants grown in different substrate combinations and watering regimes.

- The results show that 5-days interval watering boosts *S. aethiopicus* microbial activity and the accumulation of the phytochemicals, while saving water. Marked antimicrobial potential of *S. aethiopicus* extracts can be attributed to the presence of flavonoids and phenolic compounds. It is hoped that this information may be used to influence traditional healers to use leaves as a replacement for rhizomes. The potential medicinal uses of *S. aethiopicus* are supported by the presence of the above mentioned phenolics and flavonoids activities.

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CHAPTER FOUR

General discussion, conclusions and recommendations

4.1 General discussion

S. aethiopicus (wild or African ginger) is one of the most important medicinal plant species used in traditional herbal medicine in South Africa and facing an extremely high risk of extinction in the wild. It is normally grown in open fields and has a high water requirement; with the current scarcity of water in South Africa, use of more efficient irrigation systems such as drip irrigation, the adoption of water recycling techniques (hydroponics) and maximizing substrates water holding capacity is essential. Economic yields can be achieved by using soilless culture system while conserving water.

Results from this study have shown that growth parameters were not significantly affected by substrate combinations and watering regimes except for the number of roots, new shoots and stem length. However, plants grown in coir and vermiculite combination at 1:1 ratio showed best results in terms of growth parameters when compared to other treatments. The good results obtained with the combination are possibly due to its water holding capacity (WHC), the WHC was higher compared to other treatments. Irrespective of the substrate combinations, growth was influenced by watering regimes; plants grown in 3-days interval watering had the highest mean values compared to plants grown in 5-days interval. The highest mean values due to watering treatments were recorded with plants that received the highest amount of water even though there was no significant difference among watering regimes. The results show that in water limited environmental regions such as South Africa 5-days interval watering could assist growers with reasonable *S. aethiopicus* yields while saving water. Furthermore, it was found that there was no significant difference on nutrient uptake with respect to substrate combinations, this explains the similar results observed in growth parameters of the plant and implies that plants received adequate nutrients due to use of coir which increased WHC of substrate combinations used in this study. To further interrogate the results, 3-days interval exhibited better nutrient uptake compared to 5-days interval watering, which explains the exceptional increase noticed in plant

growth parameters under 3-days interval watering. Higher application of water improved the uptake of nutrients therefore growth improvement was primarily related to enhancement of nutrient uptake.

Furthermore, acetone extracts of *S. aethiopicus* aerial parts and rhizomes subjected to different substrate combinations and watering regimes were found to be bioactive against *F. oxysporum.* Acetone extracts of *S. aethiopicus* plants that were grown in T3 (coir and perlite) and 5-days interval watering were the most bioactive against *F. oxysporum.* These results may be due to high content of Na, Mn, B and Cu that were detected under 5-days interval and have been reported to reduce the severity of plant diseases and act as essential components of plant resistance to a wide range of fungal and bacterial pathogens. Antifungal activities in the aerial parts of *S. aethiopicus* were less than that of the rhizomes. Additionally, using LC-MS analysis nine compounds were isolated from the acetone extract of *S. aethiopicus* aerial parts and rhizomes: quercetin, rutin, kaempferol, naringenin, hesperetin, epicatechin, protocatechuic acid, caffeic acid and p-hydroxybenzoic acid. Watering interval of 5-days resulted to enhancement of the isolated phenolic compounds. Discernable antimicrobial potential of *S. aethiopicus* extracts was due to the presence of phenolic compounds which have been recorded to possess antimicrobial properties.

It is conspicuous that both the rhizomes and aerial parts exhibit antifungal activities and phenolic compounds, this could promote the use of leaves to ensure more sustainable use of *S. aethiopicus* and reduce the use of the rhizomes. Use of the latter could be one of the reasons for the scarcity of *S. aethiopicus* in the wild.

4.2 Conclusions and recommendations

In conclusion, hydroponic cultivation using different substrate combinations and watering regimes has potential to optimize the quality, antimicrobial activity and phytochemical profile of *S. aethiopicus.* From the economic point of view, it could be concluded that watering every 5-days combined with coir based substrates might give

the chance for increasing water use efficiency (save water), produce satisfactory marketable plants and boost accumulation of antifungal activities and phytochemicals.

Based on the above conclusions the following recommendations are made. Although the study found that coir based substrates under 3 and 5-days watering regimes have excellent performance for *S. aethiopicus* production, there is a need for the study to evaluate the effect of water stress (longer watering intervals) on growth and secondary metabolite production with respect to coir growing media. It is also recommended that this study is extended to a wide range of plant species with even higher or less ratios of coir in the growing substrates.

4.3 Appendix 1: Publication (Chapter 1)

Xego et al., Afr J Tradit Complement Altern Med. (2016) 13(3):169-180

THREATENED MEDICINAL PLANTS OF SOUTH AFRICA: CASE OF THE FAMILY HYACINTHACEAE

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Abstract

Background: Traditional medicine plays a major role in the primary health care of many people living in rural areas. South Africa is a home to over 30,000 species of higher plants and 3,000 of these species have been found to be used in traditional medicine across the country. South African medicinal plants are decreasing at an alarming rate as a result of over exploitation. Today many medicinal plants face extinction but detailed information is lacking. The purpose of this paper was to review current and proposed cultivation strategies that could be used to improve plant conservation statuses, livelihoods of the people involved in medicinal plant industry and sustainability of this industry.

Material and Method: In this review, emphasis was on the members of Hyacinthaceae family and the species *Siphonochilus aethiopicus* (Schweinf) B.L. Burtt (Zingiberaceae), which are some of the most traded and used in traditional herbal medicine. Detailed literature search was conducted on the current strategies that are being used for the cultivation of medicinal and food crops and a conceptual analysis of how technologies used for the cultivation of non-medicinal crops could be adopted for cultivation of medicinal plants in Africa. *Siphonochilus aethiopicus* was used as a case study to demonstrate the potential of using alternative cultivation strategy such as hydroponics in the cultivation of medicinal plants.

Result and Conclusion: The results showed that hydroponics has the potential to improve plant growth and yield of desired plant parts even in areas where these plants do not normally grow under natural conditions. This was the case with *Siphonochilus aethiopicus.* There is potential for growth in the medicinal plant industry if optimum cultivation technologies such as hydroponics are implemented despite the perception that Africans have an ingrained traditional preference of wild harvested plants, on the contrary many Africans have no issues with cultivated medicinal plants.