Cape Peninsula
University of Technology

VEGETATIVE GROWTH AND ALKALOID CONCENTRATION OF *SCELETIUM TORTUOSUM* (L.) N.E. Br. IN RESPONSE TO DIFFERENT SOILLESS GROWING MEDIA AND FERTIGATION REGIMES IN HYDROPONICS

by

RICHARD JAMES FABER

Thesis submitted in fulfilment of the requirements for the degree

Master of Horticultural Science

in the Faculty of Applied Sciences

at the Cape Peninsula University of Technology

Supervisor: Prof CP Laubscher
Co-supervisor: Prof JC Coetzee

Bellville Campus
October 2019

DECLARATION

I, Richard James Faber, 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**

ABSTRACT

The purpose for this study was to investigate whether *Sceletium tortuosum* was suitable for cultivation in hydroponics and to determine whether different soilless media and fertigation regimes would have an effect on the vegetative growth and alkaloid concentration of the plant. The experiment was conducted over a period of 6 weeks. Three hundred plants were cultivated from one initial mother plant obtained from Verve Dynamics (Pty) Ltd, Somerset West. Twenty treatments were evaluated with 15 sample replicates. Treatments were made up of 4 different soilless growing media, namely: pure silica sand (SS), 50% silica sand with 50% coco-peat (SC), 50% silica sand with 50% vermiculite (SV), and 50% silica sand with 50% perlite (SP). These growing media were tested in conjunction with 5 different fertigation regimes (FR), plants treated with fertigation regime 1 (FR1) received aqueous nutrient solution once every week, fertigation regime 2 (FR2) received aqueous nutrient solution once every second week, fertigation regime 3 (FR3) received aqueous nutrient solution once every third week, fertigation regime 4 (FR4) received aqueous nutrient solution once every fourth week and fertigation regime 5 (FR5) received aqueous nutrient solution once every fifth week respectively.

Chapter 2 reviewed the importance of *S. tortuosum* and its viability as a Traditional African Medicinal Plant. It was found that *S. tortuosum* has clear pharmaceutical and economical importance and is one of the only known plants to contain the alkaloids mesembrenone and mesembrine which can be utilized for the promotion of health and treating a variety of psychological disorders such as anxiety and depression.

In chapter 3 it was seen that the various treatments had significant effects in terms of plant root growth, shoot growth and dry weight. Treatment SCFR3 showed the highest individual mean value for root growth, while the average from treatments SVFR1-5 displayed the highest average value. The lowest individual value for root growth was observed in treatment SPFR5. Overall treatments with fertigation regime FR3 had better root growth, while fertigation regimes FR5 showed sub-optimal root growth. For shoot growth the highest individual mean value was found in treatment SVFR1, while the highest average value was observed in treatments SCFR1-5.

In chapter 4 treatments also had a significant effect on alkaloid concentrations. It was observed that shoot extracts contained a higher concentration of total alkaloids than root extracts, however root extracts had an overall higher amount of delta 7

mesembrenone, and mesembrenone in terms of area %, while shoots had higher amounts of mesembrine. Further the mesembrine standard as mentioned in 4.4.5, shoots clearly have an overall higher concentration of mesembrine than roots. These results suggest that roots of *S. tortuosum* should be harvested for the purpose of extracting delta 7 mesembrenone and mesembrenone molecules, while the shoots should be harvested for extracting mesembrine.

Chapter 5 further investigated the interaction between the vegetative growth and alkaloid concentration of *S. tortuosum.* There appears to be a clear trend that displays higher concentrations of mesembrine where shoot growth was more optimal, however more optimal growth did not display a higher concentration of total alkaloids. In terms of root growth and total alkaloid concentration, it did not appear that more optimal growth induced higher concentrations of total root alkaloids, meaning reasonable stress on plant root and shoot growth could possibly promote higher concentrations of total alkaloids. It is also clear that overall roots contain more delta 7 mesembrenone and mesembrenone than shoots, suggesting roots should be harvest for extracting these molecules specifically. In most cases high results of delta 7 mesembrenone in roots also had similar amounts of mesembrenone, however certain treatments resulted in higher concentrations of the former and the latter, therefore their similar molecular structure does not always permit similar manifestation in the plant material.

Overall this study has found that *S. tortuosum* is suitable for cultivation in hydroponics, and that soilless media, fertigation regimes as well as soilless media in conjunction with fertigation regimes affected the vegetative growth and alkaloid concentration of *S. tortuosum.* This research has shown that some soilless media and fertigation regime treatments had more desirable results in terms of vegetative growth and/or alkaloid concentration of the plant.

ACKNOWLEDGEMENTS

I wish to thank:

- My parents, Richard James Faber and Anna Elizabeth Susanna Faber, who provided me with support emotionally and financially.
- My supervisor, Prof Charles Laubscher for his guidance, research and technical expertise leading to the successful completion of this thesis.
- My co-supervisor, Prof Johannes Coetzee for his guidance and research expertise leading to the successful completion of this thesis.
- Cape Peninsula University of Technology for providing the necessary infrastructure and funding to conduct the research for this thesis.
- Mr. Fanie Rautenbach for his knowledge, guidance and assistance in the antioxidant laboratory of the Cape Peninsula University of Technology which was essential for analysing plant extracts.
- Mr. Richard Davies, Managing Director of Verve Dynamics (Pty) Ltd who supplied the initial *Sceletium tortuosum* mother plant for this study and gave special permission for the use of their mesembrine standard.
- The financial assistance of the National Research Foundation towards this research is acknowledged. Opinions expressed in this thesis and the conclusions arrived at, are those of the author and are not necessarily to be attributed to the National Research Foundation.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

CHAPTER ONE:

PROBLEM STATEMENT, AIMS, HYPOTHESIS AND OBJECTIVES

PROBLEM STATEMENT, AIMS, HYPOTHESIS AND OBJECTIVES

1.1 Problem statement

Gradual depletion of natural resources such as water and inorganic nutrients, as well as the harvesting of plant material from the wild has led to a drastic need for more sustainable, affordable and effective cultivation methods of medicinal and food crops. Conventional agricultural systems have proven to be obsolete in terms of conserving natural resources and the ecology.

The majority of African populations rely on Traditional African Medicinal Plants (TAMP) for health care. Some medicinal plants are cultivated while a large amount are harvested from the wild. Harvesting of plant material from the wild has led to some species reaching threatened status. Human populations are increasing and therefore the amount of plant material required is also increasing, placing further pressure on wild plant populations. Minimal research has been done on indigenous African medicinal plants for determining optimal cultivation methods in hydroponics.

Traditional healers and experts argue that cultivated plants differ in medicinal quality from plants harvested in the wild. The quality of medicinal plants is mainly determined by the concentration of secondary metabolites present in the plant material. Factors that could influence the quality of medicinal plants range from harvesting method and time, environmental conditions, the amount of resources available such as water and nutrients and the genetic compositions of various chemotypes. Investigating the effect of different fertigation regimes and soilless growing media on the vegetative growth and alkaloid concentration of *Sceletium tortuosum* will assist in establishing an optimal growth protocol for cultivating high quality medicinal plant material of this species in hydroponics.

1.2 Aims

This study aims at cultivating *S. tortuosum* in hydroponics using four different soilless growing media and five different fertigation regimes in order to determine which treatment is optimal for cultivation of the plant in hydroponics in terms of vegetative growth and alkaloid concentration.

1.3 Hypothesis

Different soilless growth media and fertigation regimes will have varying effects on the vegetative growth and alkaloid concentration of *S. tortuosum.*

1.4 Objectives of the research

1.4.1 Main Objective

To investigate the vegetative growth and alkaloid concentration of *S. tortuosum* when treated with different growth media and fertigation regimes.

1.4.2. Specific objectives

- To assess the effect of different soilless growing media and fertigation regimes on the vegetative growth of *S. tortuosum* in hydroponics.
- To assess the effect of different soilless growing media and fertigation regimes on the total alkaloid concentration of *S. tortuosum* grown in hydroponics.
- To assess the effect of different soilless growing media and fertigation regimes on delta 7 mesembrenone, mesembrenone and mesembrine area % of *S. tortuosum* grown in hydroponics.
- To assess the effect of different soilless growing media and fertigation regimes on the mesembrine concentration of *S. tortuosum* grown in hydroponics.
- To assess the effect of different soilless growing media, fertigation regimes, as well as different soilless media in conjunction with fertigation regimes on the vegetative growth and alkaloid concentration of *S. tortuosum* in order to assist in determining an optimal hydroponic growing protocol for producing high quality medicinal plants.

CHAPTER TWO:

THE IMPORTANCE OF *SCELETIUM TORTUOSUM* AND ITS VIABILITY AS A TRADITIONAL AFRICAN MEDICINAL PLANT: A REVIEW

THE IMPORTANCE OF *SCELETIUM TORTUOSUM* AND ITS VIABILITY AS A TRADITIONAL AFRICAN MEDICINAL PLANT: A REVIEW

RJ Faber, CP Laubscher, JC Coetzee

Department of Horticultural Sciences, Faculty of Applied Sciences, Cape Peninsula University of Technology, PO Box 1906, Bellville, 7535, South Africa.

*Email: laubscherc@cput.ac.za

2.1 Abstract

Sceletium tortuosum (L.) N.E. Br. and *Sceletium expansum* L. Bolus (formerly known as *Mesembryanthemum tortuosum* L. and *Mesembryanthemum expansum* L.) forms part of the succulent group of plants within the Mesembryanthemaceae family. The common names given to *Sceletium* are kanna and kougoed. However, some may argue that the name kougoed refers to the finished traditional preparation made by drying and fermenting the harvested plant material, which increases its stimulating effect.

Sceletium tortuosum is a succulent plant that belongs to the family Mesembryanthemaceae. It is indigenous to South Africa, where it is well known by the indigenous people, especially in Namaqualand where the plant is utilized regularly for its medicinal and psycho-active properties. The main alkaloids responsible for these properties are mesembrine, mesembrenine (mesembrenone), and mesembrenol.

Interest in the knowledge and use of Traditional African Medicinal Plants (TAMP) as well as an ever increasing human population has led to the commercialization of traditional African medicines at a fast rate. The economic value of indigenous medicinal plants in South Africa is approximately US$60 000 000 or R4 000 000 000 annually.

Interest in *S. tortuosum* has been growing for its potential to be an alternative supplement in the promotion of health and treating a variety of psychological and psychiatric disorders such as depression and anxiety.

It was found that *S. tortuosum* has clear pharmaceutical and economical importance and is one of the only known plants to contain the alkaloids mesembrenone and

mesembrine which can be utilized for the promotion of health and/or treating a variety of psychological disorders such as anxiety and depression.

2.2 Introduction

Sceletium tortuosum is a succulent plant that belongs to the family Mesembryanthemaceae. It is indigenous to South Africa, where it is well known by the indigenous people, especially in Namaqualand, where the plant is utilized regularly for its medicinal and psycho-active properties (Smith *et al*., 1996). According to Schultes *et al*. (2001) and Harvey *et al*. (2011) the main substances responsible for these properties are the alkaloids mesembrine, mesembrenine (mesembrenone), and mesembrenol.

Interest in *S. tortuosum* has been growing for its potential to be an alternative supplement in the promotion of health and treating a variety of psychological and psychiatric disorders such as depression and anxiety (Gericke & Viljoen, 2008). Studies on the chemistry and biological activity on Traditional African Medicinal Plants (TAMP) have only recently (1997–2008) been published, despite TAMP having been reported as one of the oldest medicinal systems in various ethnobotanical reports (Hutchings *et al*., 1996; Van Wyk *et al*., 1997).

Soilless culture systems (SCS's) in controlled greenhouse environments have proven to be the most effective strategy for agricultural production by providing flexibility as well as control. Crops can be produced in and out of season, while water and soilless media can easily be monitored for its total nutrient status. For these reasons SCS's within a greenhouse environment provide for high quality products and high yields, even in places where environmental conditions would not usually permit (Agung Putra & Yuliando, 2015).

Relevant natural compounds, mainly secondary metabolite concentration and composition, determine the quality of medicinal plants. Water availability, light intensity and temperature are examples of various environmental conditions which affect the quality and quantity of such secondary metabolites (Kleinwächter *et al*., 2014).

Investigating the effect of different soilless growing media and fertigation regimes on the vegetative growth and alkaloid concentration of *S. tortuosum* will contribute to developing optimal growing protocols for cultivating high quality medicinal plants in hydroponics for the ethno-pharmaceutical industry.

The aim of this review paper was to determine whether *S. tortuosum* had pharmaceutical and economic viability and how the medicinal value of the plant could be affected by applying various treatments when cultivating the plant.

2.3 Mesembryanthemaceae FENZL. A sub family of Aizoaceae

Within the family Aizoaceae Martinov. there are currently four sub-families, namely Sesuvioideae, Aizooideae, Ruschioideae, and Mesembryanthemoideae (Klak *et al*., 2007). Succulent plants within the Aizoaceae family are popularly termed “Mesembs”, and sometimes placed in their own family, the Mesembryanthemaceae (Smith *et al*., 1998). Common terms used to describe this group of succulent plants are vygies, fig-marigolds, flowering-stones, ice plants and also midday flowers, amongst others. These plants fascinate many plant enthusiasts and have become popular collector’s items due to their remarkable variation in leaf architecture, flower colour and form, and fruit structure. Different genera within the family grow in various habitats, and examples can thus be found growing in rocky crevices, silty flats and also in saline wastelands. Mesembs occur mainly in south-western Africa, including Angola, South Africa, Zimbabwe, Botswana and Namibia (Smith *et al*., 1998; Hartmann, 2001).

This family has received a large amount of attention in the present century both in herbaria collections and in the field. There are several reasons why the family is important in the ecosystems where they occur: they stabilise soil, which prevents erosion; various insects are catered for year-round by their blossoms, while some leaves serve as fodder for livestock. Apart from its ecological importance, this group of plants also has ethnobotanical value, and is used in making soap, poultices, preserves and also in some cases can serve as a type of psycho-active stimulant (Smith *et al*., 1998).

2.4 The genus *Sceletium* (L.) N.E. BR.

S. tortuosum (L.) N.E. Br. and *S. expansum* L. Bolus (formerly known as *Mesembryanthemum tortuosum* L. and *Mesembryanthemum expansum* L.) forms part of the succulent group of plants within the Mesembryanthemaceae family. The name *Sceletium* is derived from the Latin word sceletus, or skeleton in English, due to the noticeable leaf veins resembling skeleton-like structures within dried leaves of the plants. *Sceletium* spp. are easily identified by this skeletonised structure of the leaves (Smith *et al*., 1998; Klak *et al*., 2007). The common names given to *Sceletium* are kanna and kougoed. However some may argue that the name kougoed refers to the

finished traditional preparation made by drying and fermenting the harvested plant material, which increases its psychoactive effect (Smith *et al*., 1998; Van Wyk & Wink, 2012).

Strong evidence suggest that the indigenous people of southern Africa used one or both of these *Sceletium* species as a vision-inducing narcotic. However, the hallucinogenic effect of kanna/kougoed could have been confused with other intoxicating plants such as *Cannabis* spp. or *Sclerocarya* spp. as the narcotic use of the plant was never observed directly. Despite this, alkaloids possessing sedative, cocaine-like effects have been found within both of these species of *Sceletium* (Schultes, 1976). Other known species of *Sceletium* include the following: *S. crassicaule* L. Bolus*, S. exalatum* Gerbaulet*, S. expansum* L. Bolus*, S. rigidum* L. Bolus*, S. strictum* L. Bolus*,* and *S. varians* (Haw.) Gerbaulet (Smith *et al*., 1998; Klak *et al*., 2007)*.*

S. tortuosum is now considered a medicinal crop plant and is classified as mind-altering, sedative, euphoric, and not hallucinogenic (Van Wyk & Wink, 2012; Van Wyk & Wink, 2015). The alkaloids responsible for these psychoactive properties are mesembrine and mesembrenone. However, the concentration of alkaloids within individual plants may vary depending on their chemotype. Uses of the plant include the treatment of anxiety, stress, nervous tension, alcohol addiction, colic in infants and also for suppressing hunger and thirst. With clear ethno-pharmaceutical value it is also worthy to mention that the use of *S. tortuosum* develops no physical or psychological dependency (Van Wyk & Wink, 2015).

Figure 2.1

Sceletium plant and its “skeletonised appearance” of the dried leaves

(Gericke & Viljoen, 2008).

Figure 2.2

Sceletium tortuosum plant surrounded by its white flowers (Patnala & Kanfer, 2017).

Figure 2.3

Geographical map indicating the distribution of *Sceletium* in South Africa (Gerike & Viljoen, 2008).

2.5 Relevance of Traditional African Medicinal Plants

Interest in the knowledge and use of Traditional African Medicinal Plants (TAMP) as well as an ever increasing human population has led to the commercialization of traditional African medicines at a fast rate (Van Wyk, 2008). According to Keirungi and Fabricius (2005) the economic value of indigenous medicinal plants in South Africa is approximately US$60 000 000 or R4 000 000 000 annually.

The number of people in South Africa that depend on TAMP to aid their medical needs is estimated at 27 million (Fennell *et al*., 2004). The majority of plants used for traditional medicine are harvested from the wild except for some which are selected and cultivated by traditional healers (Van Andel & Havinga, 2008).

In 1998 it was estimated that 20 000 tonnes of plant material was being traded in South African markets (Mander, 1998). Seven hundred thousand tonnes of plant material have been extracted from the wild for this market which mostly consist of people with disadvantaged socio-economic situations or backgrounds (Makunga *et al*., 2008). According to Mander and Mckenzie in 2005, US$ 50-100 million in the form of approximately 1000 plant species are being exchanged in this informal sector (Makunga *et al*., 2008).

Figure 2.4

A commercial product by Medico Herbs containing dried *S. tortuosum* in capsules (https://medicoherbs.com/products/kanna-capsules-60).

Figure 2.5

A commercial product by Phyto Force containing tinctured *S. tortuousm* (https://www.phyto-force.co.za/product/*Sceletium*/).

2.6 Soilless culture

Soilless culture, also known as hydroponics and/or hydro-culture is the term that is used when methods of growing plants without soil is utilized. Artificial or soilless substrate may or may not be used to provide structural support for the plants depending on the grower and method used (Venter, 2010).

Ecological imbalances such as extreme temperatures, chemical toxicity and oxidative stress are threatening conventional agricultural practices. With an annual rise in population and consumers becoming more aware of the quality, quantity and nutritious value of products consumed, challenges within agricultural systems to keep up with demands and standards are becoming more complex. The need for more efficient and controlled cultivation methods have risen dramatically. Soilless culture systems have been proved to be one of the most efficient and effective cultivation method in the agriculture industry of today (Agung Putra & Yuliando, 2015).

2.7 Secondary metabolites and alkaloids

Plant secondary metabolites are divided into three categories, namely terpenoids, flavonoids, and alkaloids. Consisting of multiple chemical structures and biological activities, secondary metabolites are an extremely wealthy source of compounds and are utilized in pharmaceutical, nutraceutical, cosmetic and fine chemical industries. Examples of familiar natural plant products that are used as drugs and/or dietary supplements are: artemisinin, paclitaxel, ginsenoside, lycopene, and resveratrol (Song *et al*., 2014).

Alkaloids are secondary metabolites that consist of one or several nitrogen (N) atoms in their molecular structure. There are approximately 20 000 alkaloid structures that have been described, and are classified into groups according to their molecular ring (heterocyclic) structure. These groups are indole, isoquinoline, quinolone, tropane, pyrrolizidine and quinolizidine alkaloids. Some alkaloids are neurotoxins and/or mind-altering substances. Most have pharmacological or toxicological relevance, and many isolated alkaloids serve as therapeutic agents in medicine (Wink & van Wyk, 2008).

2.8 Electrical conductivity and nutrients

Serving as indicators for soil fertility, nutrient concentrations within soil have been of interest for decades. Nutrients can be organic or inorganic. Availability, utilization, translocation and absorption of nutrients by crop plants for growth and development are referred to as mineral nutrition. Plants require a variety of nutrients in order to successfully grow and develop to their full potential. The most important mineral nutrients are the macro nutrients, namely nitrogen, phosphorous and potassium, although plants also require micro-nutrients in smaller amounts which can be argued to be equally important (Fageria, 2009).

Plants require nitrogen (N) in the largest quantities compared to other elements. N serves as a constituent for many plant cell components such as, amino acids, proteins and nucleic acids. When there is a lack of N availability to a plant, the plants growth will be inhibited rapidly, followed by the common characteristic symptom, chlorosis in older leaves (Frink *et al.*, 1999; Taiz & Zeiger, 2010).

Phosphorous (P) serves as an integral component of valuable compounds found in plant cells. These include phospholipids as well as sugar-phosphate intermediates of respiration and photosynthesis. Necrotic spots, dark-green colouration of leaves, which could also become malformed, as well as stunted growth are common characteristic symptoms of P deficiency (Marschner & Rengel, 2007; Taiz & Zeiger, 2010).

Various enzymes that are important in respiration and photosynthesis are activated by potassium (K). The osmotic potential of plant cells are also partly regulated by K. Marginal chlorosis of leaves, which further develops into necrosis of leaf tips or margins and in between veins is the most common symptom of K deficiency in plants (Liu & Zhu, 1997; Taiz & Zeiger, 2010).

Cell wall synthesis and mitotic cell division depend on the availability of calcium (Ca) ions. Normal functioning of plant membranes and various plant responses to environmental and hormonal signals require Ca. Necrosis of young meristematic regions where cell division and cell wall formation is most prominent is a characteristic symptom of Ca deficiency (White & Broadley, 2003; Taiz & Zeiger, 2010).

Cystine, cysteine, and methionine are amino acids in which sulphur (S) is found. Sulphur is also a constituent of a number of co-enzymes and vitamins, namely coenzyme A, S-adenosylmethionine, biotin, Vitamin B1 and pantothenic acid, which are all essential for optimal metabolism in plant cells (Scherer, 2001;Taiz & Zeiger, 2010).

Electrical conductivity (EC) is the measurement used to indicate the total concentration of nutrients within an aqueous solution. High EC indicates a high concentration of nutrients within the solution, while a low EC indicates a low concentration of nutrients (Liopa-Tsakalidi *et al*., 2015). When plants are supplied with a high EC nutrient solution, the nutrient concentration within the leaves will not necessarily be higher than in plants supplied with a low EC nutrient solution (Suzuki *et al*., 2015), suggesting that the nutrient uptake in plants is not necessarily based on the amount of nutrients available.

2.9 Water amounts

Production of plants in the modern sense requires advancements in technology that will allow the optimization of cultivating high quality plant material while minimizing the use of natural resources, such as water (Schnitzler *et al*., 2003). This is also true for the growing of medicinal and aromatic plants, as well as plant production in general (Manukyan, 2011).

South African agriculture faces increasing pressure to use water more efficiently, as the industry must oblige to demonstrate efficient and effective water use due to limited valuable natural resources (Olivier & Singels, 2015).

It has been observed that a considerably higher concentration of secondary metabolites are produced in medicinal or spice plants grown under water deficient conditions, compared to identical plants of the same species grown with ample amounts of water (Selmar & Kleinwächter, 2013).

Although changes in the synthesis of desired natural compounds is clear when drought stress is applied to plants, the overall effect of applying drought stress for optimizing specific secondary metabolites in plants remains complex. The amount of water also influences other relevant factors such as plant biomass yield and rate of growth. Depending on the plant and the growers' desired outcome with regards to quality, quantity, and rate of growth, the amount of water applied should be carefully considered as there is no prevalent recommendation that can be made for all plants. By deliberately applying drought-stress without first thoroughly investigating how different plants react to different amounts of water and the method of applying it could yield undesirable results (Kleinwächter & Selmar, 2014).

2.10 References

Agung Putra, P. & Yuliando, H. 2015. Soilless culture system to support water use efficiency and product quality: A Review. *Agriculture and Agricultural Science Procedia*, 3: 283–288.

Fageria, N.K. 2009. *The use of nutrients in crop plants*. *1st ed*. Boca Raton: CRC Press.

Fennell, C.W., Light, M.E., Sparg, S.G., Stafford, G.I. & Van Staden, J. 2004. Assessing African medicinal plants for efficacy and safety: Agricultural and storage practices. *Journal of Ethnopharmacology*, 95(2–3): 113–121.

Frink, C.R., P.E. Waggoner & J.H. Ausubel, 1999. Nitrogen fertilizer: retrospect and prospect. *Proceedings of the National Academy of Sciences*, 96: 1175-1180.

Gericke, N. & Viljoen. M. 2008. *Sceletium*—A review update. *Journal of Ethnopharmacology*, 119(3): 653–663.

Hartmann, H.E.K. 2001. *Illustrated Handbook of Succulent Plants: Aizoaceae F–Z.* Berlin: Springer.

Harvey, A.L., Young, L.C., Viljoen, A.M. & Gericke, N.P. 2011. Pharmacological actions of the South African medicinal and functional food plant *Sceletium tortuosum* and its principal alkaloids. *Journal of Ethnopharmacology*, 137(3): 1124–1129.

Hutchings, A., Scott, A.H., Lewis, G., & Cunningham, A. 1996. *Zulu Medicinal Plants*. *1st ed*. Pietermaritzburg: Natal University Press.

Keirungi, J. & Fabricius, C. 2005. Selecting medicinal plants for cultivation at Nqabara on the Eastern Cape Wild Coast, South Africa. *South African Journal of Science*, 101(11–12): 497–501.

Klak, C., Bruyns, P. V. & Hedderson, T. J. 2007. A phylogeny and new classification for Mesembryanthemoideae (Aizoaceae). *Taxon*, 56: 737–756.

Kleinwächter, M., Paulsen, J., Bloem, E., Schnug, E. & Selmar, D. 2014. Moderate drought and signal transducer induced biosynthesis of relevant secondary metabolites in thyme (*Thymus vulgaris*), greater celandine (*Chelidonium majus*) and parsley (*Petroselinum crispum*). *Industrial Crops & Products*, 64: 158–166.

Kleinwächter, M. & Selmar, D. 2014. Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. *Industrial Crops & Products*, 42: 558–566.

Liu, J. & Zhu, J.K. 1997. An Arabidopsis mutant that requires increased calcium for potassium nutrition and salt tolerance. *Proceedings of the National Academy of Sciences*, 94 (26): 14960-14964.

Liopa-Tsakalidi, A., Barouchas, P. & Salahas, G. 2015. Response of zucchini to the electrical conductivity of the nutrient solution in hydroponic cultivation. *Agriculture and Agricultural Science Procedia*, 4: 459–462.

Makunga, N.P., Philander, L.E. & Smith, M. 2008. Current perspectives on an emerging formal natural products sector in South Africa. *Journal of Ethnopharmacology*, 119(3): 365–375.

Mander, M. 1998. Marketing of indigenous medicinal plants in South Africa - a case study in Kwazulu-Natal. *FAO - Food and Agriculture Organization of the United Nations*: 1–158.

Manukyan, A. 2011. Effect of growing factors on productivity and quality of lemon catmint, lemon balm and sage under soilless greenhouse production: 1. drought stress. *Medicinal and Aromatic Plant Science and Biotechnology*, 5(2): 119–125.

Marschner, P. & Rengel, Z., 2007. *Nutrient Cycling in Terrestrial Ecosystems.* Berlin: Springer.

Olivier, F.C. & Singels, A. 2015. Increasing water use efficiency of irrigated sugarcane production in South Africa through better agronomic practices. *Field Crops Research*, 176: 87–98.

Patnala, S. & Kanfer, I., 2017. *Sceletium* plant species – Alkaloidal components, chemistry and ethnopharmacology. *Journal of Ethnopharmacology* 121(1), pp. 86-91.
Scherer, H.W. 2001. Sulphur in crop production - Invited paper. *European Journal of Agronomy*, 14(2): 81–111.

Schultes, R.E. 1976. *Hallucinogenic Plants*. New York: Golden Press.

Schultes, R.E., Hofmann, A., & Rätsch, C. 2001. *Plants of the Gods: Their sacred, healing, and hallucinogenic Powers*. 2nd ed. Lucerne: EMB-service for Publishers.

Schnitzler, W.H., Trüggelmann, L., Toth, A., Woitke, M., Tüzel, Y., Tüzel, H., Hanafi, A., Jaquet, F., Le Grusse, P., Junge, H., Giuffrida, F., Leonardi, C., Wadid Awad, M.M., El-Behairy, Fort, F., Codron, J.M. & Qaryouti, M.M. 2003. Efficient water use for high quality vegetables through the environmentally sound hydroponic production 'Ecoponics'. *Acta Horticulturae*, 609: 493–495.

Selmar, D. & Kleinwächter, M. 2013. Stress enhances the synthesis of secondary plant products: The impact of stress-related over-reduction on the accumulation of natural products. *Plant and Cell Physiology*, 54(6): 817–826.

Smith, G.F., Chesselet, P., van Jaarsveld, E.J., Hartmann, H., Hammer, S., van Wyk, B.-E., Burgoyne, P., Klak, C. & Kurzweil, H. 1998. *Mesembs of the World*. *1st ed*. Pretoria: Briza Publications.

Smith, M.T., Crouch, N.R., Gericke, N. & Hirst, M. 1996. Psychoactive constituents of the genus *Sceletium* N.E.Br. and other Mesembryanthemaceae: A review. *Journal of Ethnopharmacology*, 50(3): 119–130.

Song, M.C., Kim, E.J., Kim, E., Rathwell, K., Nam, S. & Yoon, Y.J. 2014. Microbial biosynthesis of medicinally important plant secondary metabolites. Natural Product Reports, 1–13.

Suzuki, M., Umeda, H., Matsuo, S., Kawasaki, Y., Ahn, D., Hamamoto, H. & Iwasaki, Y. 2015. Effects of relative humidity and nutrient supply on growth and nutrient uptake in greenhouse tomato production. *Scientia Horticulturae*, 187: 44–49.

Taiz, L. & Zeiger, E. 2010. *Plant Physiology*. *5th ed*. Sunderland: Sinauer Associates.

Van Andel, T. & Havinga, R. 2008. Sustainability aspects of commercial medicinal plant harvesting in Suriname. *Forest Ecology and Management*, 256(8): 1540–1545.

Van Wyk, B.E. 2008. A broad review of commercially important southern African medicinal plants. *Journal of Ethnopharmacology*, 119(3): 342–355.

Van Wyk, B.E, Van Oudtshoorn, B., & Gericke, N. 1997. *Medicinal Plants of South Africa*. Pretoria: Briza Publications.

Van Wyk, B. & Wink, M. 2012. *Medicinal Plants of the World*. 5th ed. Pretoria: Briza.

Van Wyk, B.E. & Wink, M. 2015. *Phytomedicines, Herbal Drugs and Plant Poisons*. *1st ed*. Pretoria: Briza Publications.

Venter, G. 2010. *Successful hydroponics, a comprehensive practical guide to scientifically based hydroponic crop production*. Pretoria: Xlibris Corporation.

Wink, M. & van Wyk, B.E. 2008. *Mind-altering & Poisonous Plants of the World*. *1st ed*. Pretoria: Briza Publications.

White, P.J. & Broadley, M.R. 2003. Calcium in plants. *Annals of Botany*, 92: 487-511.

CHAPTER THREE:

VEGETATIVE GROWTH OF *S. TORTUOSUM* IN RESPONSE TO DIFFERENT SOILLESS GROWING MEDIA AND FERTIGATION REGIMES IN HYDROPONICS

VEGETATIVE GROWTH OF *S. TORTUOSUM* IN RESPONSE TO DIFFERENT SOILLESS GROWING MEDIA AND FERTIGATION REGIMES IN HYDROPONICS

RJ Faber, CP Laubscher*, JC Coetzee

Department of Horticultural Sciences, Faculty of Applied Sciences, Cape Peninsula University of Technology, PO Box 1906, Bellville, 7535, South Africa.

*Email: laubscherc@cput.ac.za

3.1 Abstract

Sceletium tortuosum (L.) N.E. Br is a succulent medicinal crop plant in the family Mesembryanthemaceae, it was formerly known as *Mesembryanthemum tortuosum*. The plant is indigenous to South Africa where is it well known and utilized for its medicinal and psycho-active properties. Interest in *S. tortuosum* has been growing as mesembrine-type alkaloids found in the plant have tremendous potential for promoting health and treating a variety of psychological and psychiatric disorders. The purpose for this study was to investigate whether *S. tortuosum* would be suitable for hydroponic cultivation, as well as how the plant would respond to different soilless media and fertigation regimes in terms of vegetative growth. Three hundred plants were cultivated from one initial mother plant obtained from Verve Dynamics (Pty) Ltd. Twenty treatments were evaluated with 15 sample replicates. Treatments were made up of 4 different soilless growing media, namely: pure silica sand (SS), 50% silica sand with 50% coco-peat (SC), 50% silica sand with 50% vermiculite (SV), and 50% silica sand with 50% perlite (SP). These growing media were tested in conjunction with 5 different fertigation regimes (FR), plants treated with fertigation regime 1 (FR1) received aqueous nutrient solution once every week, fertigation regime 2 (FR2) received aqueous nutrient solution once every second week, fertigation regime 3 (FR3) received aqueous nutrient solution once every third week, fertigation regime 4 (FR4) received aqueous nutrient solution once every fourth week and fertigation regime 5 (FR5) received aqueous nutrient solution once every fifth week respectively. Results from this experiment showed that *S. tortuosum* is suitable for hydroponic cultivation and that different soilless growing media and fertigation regimes as well as soilless media in conjunction with fertigation regimes had varying effects on the plants vegetative growth. The most significant root growth was found in treatment SCFR3, while the most significant shoot growth and dry weight was found in SVFR1.

Key words: dry weight, fertigation regimes, hydroponics, roots, shoots, soilless media, vegetative growth.

Abbreviations: fertigation regime (FR), silica sand (SS), silica sand and coco-peat (SC), silica sand and vermiculite (SV), silica sand and perlite (SP).

3.2 Introduction

3.2.1 *Sceletium tortuosum* (L.) N.E. Br

S. tortuosum is a succulent plant that belongs to the family Mesembryanthemaceae. It is indigenous to South Africa, where it is well known by the indigenous people, especially in Namaqualand, where the plant is utilized regularly for its medicinal and psycho-active properties (Smith *et al*., 1996). According to Schultes *et al*. (2001) and Harvey *et al*. (2011) the main alkaloids responsible for these properties are mesembrine, mesembrenine (mesembrenone), and mesembrenol.

Interest in *S. tortuosum* has been growing for its potential to be an alternative supplement in the promotion of health and treating a variety of psychological and psychiatric disorders such as depression and anxiety (Gericke & Viljoen, 2008).

S. tortuosum is considered a medicinal crop plant and is classified as mind-altering, sedative, euphoric, and not hallucinogenic (Van Wyk & Wink, 2012; Van Wyk & Wink, 2015). The alkaloids responsible for these psychoactive properties are mesembrine and mesembrenone. However, the concentration of alkaloids within individual plants may vary depending on their chemotype. Uses of the plant include the treatment of anxiety, stress, nervous tension, alcohol addiction, colic in infants and also for suppressing hunger and thirst. With clear ethno-pharmaceutical value it is also worthy to mention that the use of *S. tortuosum* develops no physical or psychological dependency (Van Wyk & Wink, 2015).

3.2.2 Vegetative plant growth

Vegetative growth occurs once an embryo has broken its dormancy and starts to mobilize stored reserves. Germination is achieved in response to various factors, such as moisture levels, extended cold or heat periods, as well as light availability. Different species will require different conditions to break dormancy, i.e. germinate. Root and shoot apical meristems initially develop from stored reserves in cotyledons or endosperm. After further development the seedling becomes capable of photosynthesis, allowing for further vegetative growth to take place once reserves have been depleted (Taiz & Zeiger, 2010).

Plant growth occurs when aerial and root organs are able to acquire all essential constituents required for healthy development. When growing plants in soil, the rate of growth and therefore the rate of mineral nutrients absorbed by their roots is naturally governed by soil fertility. To a certain extent, soil fertility is maintained by decomposing organic matter which returns nutrient elements to the soil, also known as the nutrient cycle. In reverse, plants that are cultivated for harvest, are removed from the soil and as a result absorbed nutrient elements in the plant material do not return, thus nutrient elements need to be monitored and amended as required to ensure healthy plant growth (Le Bot *et al*., 1998; Greenwood, 1990).

3.2.3 Soilless growing media and fertigation regimes

Any method of growing plants without the use of soil is known as soilless plant cultivation (Savvas *et al*., 2013). Soilless plant cultivation can produce higher yields and allow for more frequent harvests, making it more cost-effective in comparison to soil-based cultivation (Grafiadellis *et al.*, 2000; Nejad & Ismaili, 2014).

One of the main obstacles for healthy plant growth in containerised gardens is limited root space. This presents two challenges, firstly the shallow layer of growing medium in a container can become fully saturated rather quickly, and secondly water storage capacity is restricted, and therefore would need more frequent irrigation to prevent roots from completely drying out, as opposed to plants cultivated in a normal open ground soil profile (Bunt, 1988).

The physical structure of an optimal growing medium should be capable of storing an appropriate amount of water and air between irrigation regimes. An incorrect balance of water and air could result in root asphyxia or drought stress (Fonteno, 1993; Caron & Nkongolo, 1999). The key driver for the development of soilless growing media is the inability of soil to provide such a balance in minimal volumes. Formulation and modification of soilless media has allowed for the guarded control of water, air and nutrients to root systems while also excluding soil borne pathogens (Raviv *et al*., 2002).

Pure silica sand is inert, contains no nutrients or organic matter, therefore sterilization is not as essential compared to soil, and this together with unlimited supply, relatively inexpensive cost, and reusability makes it an ideal soilless medium for hydroponic plant cultivation (Reversat *et al*., 1999). Coco-peat has high water retaining and air-porosity properties, it is also easy to handle, recyclable and economical. Perlite is light-weight, readily available and chemically stable. It is effective for increasing air

porosity or drainage within growing media, although it is expensive and does not hold an adequate amount of water. Vermiculite is also lightweight and has excellent buffering qualities; however, it is also expensive and can easily be over watered. In soilless culture systems the challenge lies in finding the optimal medium-nutrient combination for different plant species in order to achieve optimal vegetative growth (Brooking, 1976; Jensen, 2017).

The aim of this study was to determine how different soilless media in conjunction with different fertigation regimes would affect the vegetative growth of *S. tortuosum* in order to assist in establishing an optimal protocol for cultivating the plant in hydroponics.

3.3 Materials and methods

3.3.1 Greenhouse Experiment

This investigation was conducted over a period of 7 weeks in the research greenhouse facility at the Cape Peninsula University of Technology, Bellville, Cape Town, South Africa; GPS co- ordinates - 33º 55'45.53S, 18º 38' 31. 16E. The nature of the structure and the technology installed ensured control of the environment within the greenhouse.

3.3.2 Plant Preparation

One *S. tortuosum* mother plant was obtained from Verve Dynamics, Somerset West. An additional ten mother plants were propagated and cultivated from this plant. Once these ten plants were large enough, ± 600 2.5-3 cm stem cuttings were propagated from the mother plants to ensure a supply of more than 300 rooted cuttings, which was the number of plant material required for the experiment.

Cuttings were treated with Dynaroot™ No 1 (Active ingredient: 0.1% I.B.A.) rooting hormone and planted into 200 and 128 cell styrofoam trays containing a 50/50 mixture of silica sand, and coco-peat as rooting medium. The trays were placed in an environmentally controlled propagation greenhouse at the Cape Peninsula University of Technology, Bellville Campus. The first sign of roots was observed after 2 weeks, and roots had fully developed after 2 months. After rooting, trays were moved to the research greenhouse for acclimatization. The cuttings were irrigated with tap water by hand. The most vigorous and uniform rooted cuttings in the batch was selected for the experiment.

3.3.3 Hydroponic experiment

Five identical hydroponic systems were constructed. Each system consisted of one 70 litre capacity low-density polyethylene (LDPE) reservoir; four polyvinyl chloride (PVC) square gutters (130 mm x 70 mm x 2 500 mm) secured onto wire mesh tables (900 mm x 1 250 m x 2 500 m); sixty 12,5 x 12,5 cm plastic pots, a 2 000 ℓ/h submersible water pump with a 2,5 m head capacity, ten-meter x 20 mm LDPE irrigation piping; four reducers (20 mm–15 mm); four 15 mm flow regulators; one air pump, one air stone (15cm) and eight 20 mm bulk head connectors. Reservoirs were kept filled to a level of approximately 50 ℓ with aqueous nutrient solution. Each gutter was sealed with PVC adhesive and fitted with an inlet guiding water towards the gutters and an outlet to return excess water to the reservoir. Each inlet consisted of LDPE irrigation piping converted from 20 mm to 15 mm diameter and each fitted with a 15 mm valve allowing maximum control of aqueous nutrient solution flow into gutters. All gutters were filled with silica sand to a depth of ± 1,5 cm. Fifteen pots (12,5 cm x 12,5 cm) were placed into each gutter on top of the silica sand. As water passes through the gutter the sand becomes fully saturated with aqueous nutrient solution which is then imbibed by soilless growing media in pots via capillary action. Every gutter for each system represented a different soilless substrate in 15 pots, as follows: 100% silica sand (SS); a 50/50 mixture of silica sand and coco-peat (SC); a 50/50 mixture of silica sand and vermiculite (SV) and a 50/50 mixture of silica sand and perlite (SP).

Each separate system was manually controlled to fertigate plants with the same amount of aqueous nutrient solution at different time intervals.

The five systems were given aqueous nutrient solution amounts as follows: Fertigation regime 1 (FR1) was fertigated once a week at ± 350 ml per minute for 1 hour. Fertigation regime 2 (FR2) was fertigated every second week at ± 350 ml per minute for 1 hour. Fertigation regime 3 (FR3) was fertigated once every third week at ± 350 ml per minute for 1 hour. Fertigation regime 4 (FR4) was fertigated once every fourth week at ± 350ml per minute for 1 hour. Fertigation regime 5 (FR5) was fertigated once every fifth week at ± 350 ml per minute for 1 hour. Fifteen plants, one per pot were placed into each soilless substrate of each system, which totals to 60 plants per hydroponic system and 300 plants for the entire experiment. Therefore 15 replicates in a randomized blocked design were tested for each media and fertigation regime combination.

All aqueous nutrient solutions used in the experiment were kept at a pH level of 6.0, and an electrical conductivity (EC) level of 0.5. EC levels of the aqueous nutrient solutions were monitored with a calibrated hand held digital EC meter (Hanna Instruments®™ HI 98312). Water reservoir pH levels were monitored with a calibrated handheld digital pH meter (Eurotech®™ pH 2 pen). For increasing pH potassium hydroxide was used, while phosphoric acid was used for decreasing pH of aqueous nutrient solutions. For decreasing the EC of aqueous nutrient solutions, reverse osmosis water was added into reservoirs, while adding Hoagland Solution to aqueous nutrient solutions in reservoirs increased the EC. Pots were individually numbered and arranged randomly.

All gutters were slightly tilted to allow aqueous nutrient solutions to flow from one end (inlet) to the other (outlet). Each outlet was guided to a separate smaller gutter which then returned the aqueous nutrient solution back to the reservoir, creating a circulating system.

3.3.4 Treatment preparation

Soilless medium treatments were prepared using four different soilless medium combinations, namely: 100% silica sand (SS); a 50/50 mixture of silica sand and coco-peat (SC); a 50/50 mixture of silica sand and vermiculite (SV) and a 50/50 mixture of silica sand and perlite (SP). For treatments SC, SV and SP equal parts silica sand and coco-peat/vermiculite/perlite was mixed together. All silica sand used was thoroughly rinsed with tap water until water poured through the sand ran clear.

Fertigation regimes were achieved by installing identical pumps to all hydroponic systems. The amount of aqueous nutrient solution delivered to each grow bed was controlled by adjusting all output valves until ±350 ml per minute was measured. Measurement was achieved by placing an empty bucket below each output for 1 minute, the liquid captured in the bucket was decanted and measured using a 500 ml Erlenmeyer flask until the amount of aqueous nutrient solution for each output was measured at ±350 ml for 1 minute.

Treatment SSFR3 was selected for the control, as it contained 100% silica sand, which was the base of all soilless mediums used in this experiment as well as a mid-frequent fertigation regime.

3.3.5 Treatment Application

Treatments applied consisted of 4 different soilless growth media and 5 different fertigation regimes in a circulating capillary action hydroponic system. The control consisted of treatment SSFR3 containing 100% silica sand growing media and a mid-frequent fertigation regime. Each treatment was numbered, as follows:

SSFR1 - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once a week.

SCFR1 – 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once a week.

SVFR1 – 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once a week.

SPFR1 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once a week.

SSFR2 - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every second week.

SCFR2 - 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every second week.

SVFR2 - 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every second week.

SPFR2 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every second week.

SSFR3 (control) - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every third week.

SCFR3 - 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every third week.

SVFR3 - 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every third week.

SPFR3 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every third week.

SSFR4 - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fourth week.

SCFR4 - 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fourth week.

SVFR4 - 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fourth week.

SPFR4 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fourth week.

SSFR5 - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fifth week.

SCFR5 - 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fifth week.

SVFR5 - 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fifth week.

SPFR5 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fifth week.

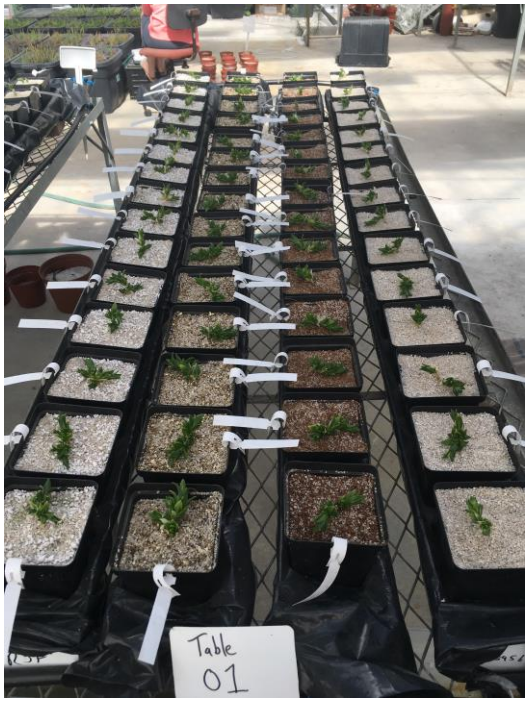

Figure 3.1 – Treatments SSFR1; SCFR1; SVFR1 and SPFR1 in an environmentally controlled greenhouse.

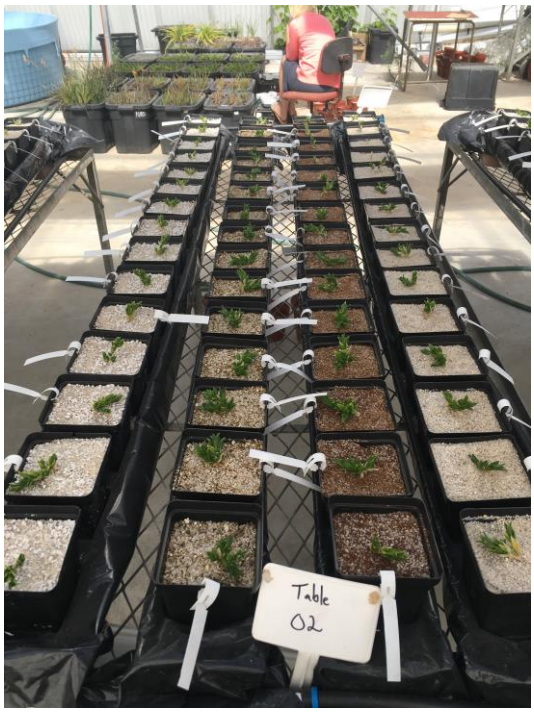

Figure 3.2 – Treatments SSFR2; SCFR2; SVFR2 and SPFR2 in an environmentally controlled greenhouse.

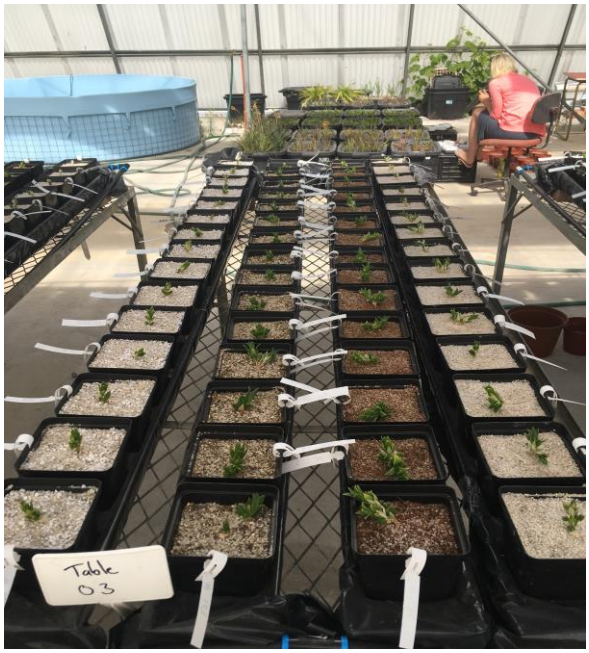

Figure 3.3 – Treatments SSFR3; SCFR3; SVFR3 and SPFR3 in an environmentally controlled greenhouse.

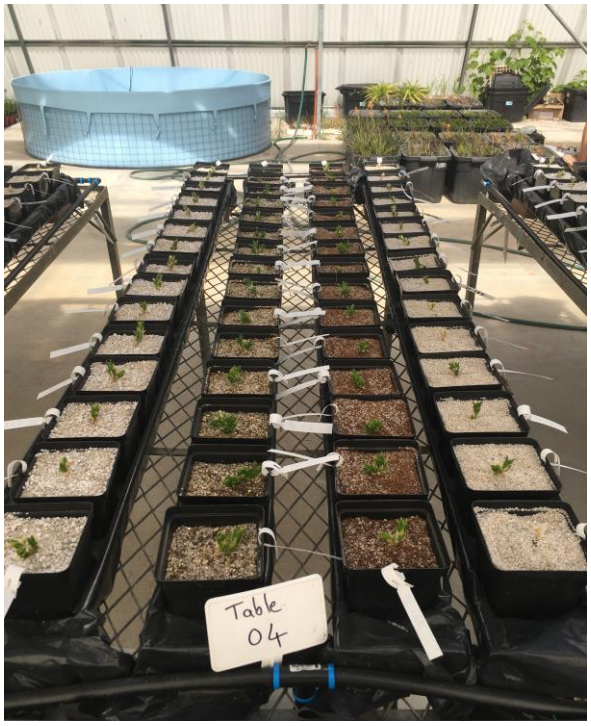

Figure 3.4 – Treatments SSFR4; SCFR4; SVFR4 and SPFR4 in an environmentally controlled greenhouse.

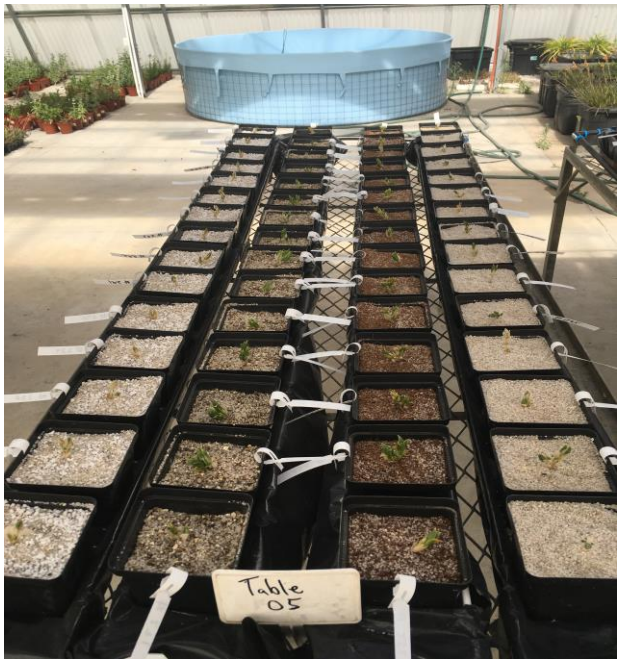

Figure 3.5 – Treatments SSFR5; SCFR5; SVFR5 and SPFR5 in an environmentally controlled greenhouse.

Figure 3.6 – Overview of experimental hydroponics systems displaying various treatments and hydroponic reservoirs.

3.3.6 Data collection

3.3.6.1 Root & shoot growth

Root and shoot lengths were measured prior to transplanting into the hydroponic systems and again at the end of the experiment. Measurements were done in millimetres using a standard ruler and recorded.

3.3.6.2 Dry weight

Plants were placed in brown paper bags post-harvest and dried at 30–31°C in a forced convection oven (Daihan Labtech LDO-150F) until the material was crisp dry. Plants were then weighed using an electronic balance (RADWAG® Model PS 750.R2) with 0.001 g readability and recorded.

Figure 3.7 – *S. tortuosum* plant post-harvest showing healthy root development.

3.3.7 Statistical analysis

All data collected was statistically analysed using two-way analysis of variance (ANOVA). Treatment means were compared using Fischer's Least Significant Difference (L.S.D) at values of $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ (Steel & Torrie, 1980). The software program STATISTICA version 13 was used to perform all calculations on Windows 10.

3.4 Results

3.4.1 Root growth

Soilless media

The effects of soilless media on root growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on root growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on root growth of *S. tortuosum* were found to be statistically significant at the value of $p \leq 0.01$. The highest individual mean value (197,93 mm) for root growth was observed in treatment SCFR3 (see Table 3.1 and Figure 3.8).

Table 3.1

The root growth of *Sceletium tortuosum* in response to four different soilless media and five fertigation regimes in hydroponics.

Treatment		Root Growth (mm)	
Soilless Medium	**Fertigation Regime**	**Mean**	**Standard Error + Mean Group**
SS	FR1	41,07	±6,82i
SS	FR2	62,27	±13,17ghi
SS	FR3	77,20	±13,64fgh
SS	FR4	53,80	±13,36hi
SS	FR5	58,13	±13,15hi
SC	FR1	114,20	±14,96def
SC	FR2	109,40	±16,26def
SC	FR3	197,93	±25,26a
SC	FR4	150,67	±22,54bcd
SC	FR5	67,47	±10,80fgh
SV	FR1	91,87	±14,16efg
SV	FR2	135,47	±19,18cde
SV	FR3	186,93	±18,55ab
SV	FR4	171,40	±27,09abc
SV	FR5	54,53	±20,36hi
SP	FR1	89,33	±13,12efg
SP	FR2	111,73	±14,58def
SP	FR3	95,20	±13,45efg
SP	FR4	109,67	±22,40def
SP	FR5	33,00	±10,22i

Two-way ANOVA F-Statistics

Soilless Medium	20,0***
Fertigation Regime	15,5***
Soilless Medium*Fertigation Regime	2,5**

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤0.01 (**), P ≤0.001 (***) as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4 = fertigation regime 4; FR5= fertigation regime 5.

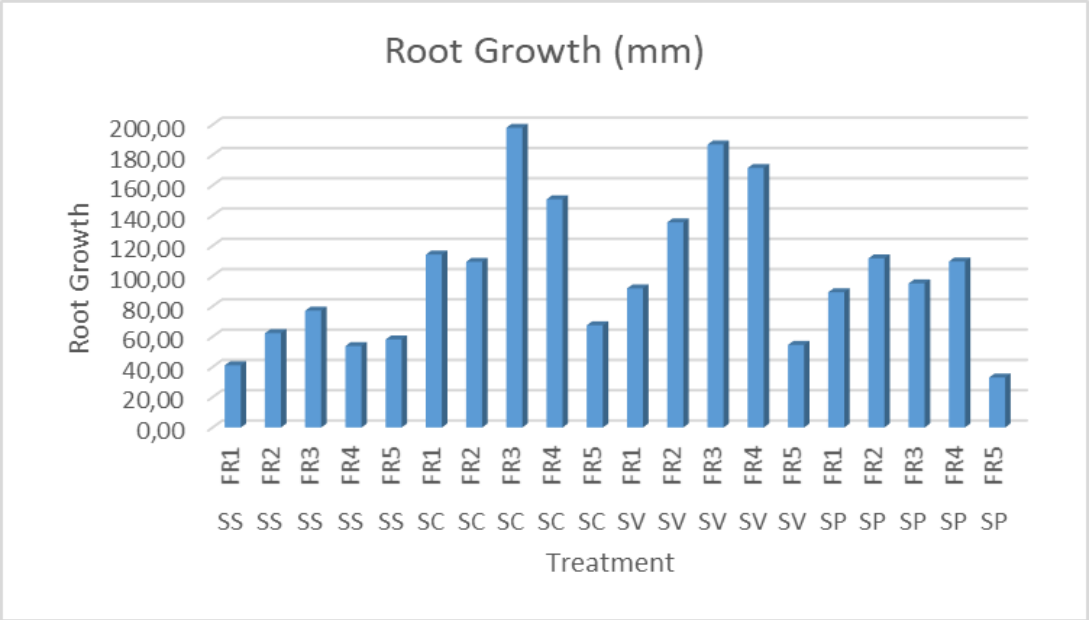

Figure 3.8

Root growth of *S. tortuosum* in response to 4 different soilless media, and 5 different fertigation regimes in hydroponics. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4= fertigation regime 4; FR5= fertigation regime 5. Treatment SSFR3 was selected for the control.

3.4.2 Shoot growth

Soilless media

The effects of soilless media on shoot growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on shoot growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on shoot growth of *S. tortuosum* were found to be not significant. The highest individual mean value (27,07 mm) for shoot growth was observed in treatment SVFR1 (see Table 3.2 and Figure 3.9).

Table 3.2

The shoot growth of *Sceletium tortuosum* in response to four different soilless media and five fertigation regimes in hydroponics.

Treatment		Shoot Growth (mm)	
Soilless Medium	Fertigation Regime	Mean	Standard Error + Mean Group
SS	FR1	15,33	±1,95cde
SS	FR2	10,20	±1,79efg
SS	FR3	8,27	±1,20ghi
SS	FR4	9,40	±6,65fgh
SS	FR5	3,47	±0,83i
SC	FR1	21,13	±2,19abc
SC	FR2	19,67	±3,02bc
SC	FR3	22,87	±2,04ab
SC	FR4	12,80	±0,96def
SC	FR5	10,00	±1,44efg
SV	FR1	27,07	±2,31a
SV	FR2	16,47	±1,60bcd
SV	FR3	16,27	±2,63cde
SV	FR4	12,40	±1,33efg
SV	FR5	9,40	±1,99fgh
SP	FR1	19,27	±2,10bcd
SP	FR2	12,40	±2,09efg
SP	FR3	10,60	±1,49efg
SP	FR4	6,47	±0,97ghi
SP	FR5	4,47	±1,36hi

Two-way ANOVA F-Statistics

Soilless Medium	14,7***
Fertigation Regime	20,1***
Soilless Medium*Fertigation Regime	1,1ns

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤0.001 (***), ns = not significant as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4= fertigation regime 4; FR5= fertigation regime 5.

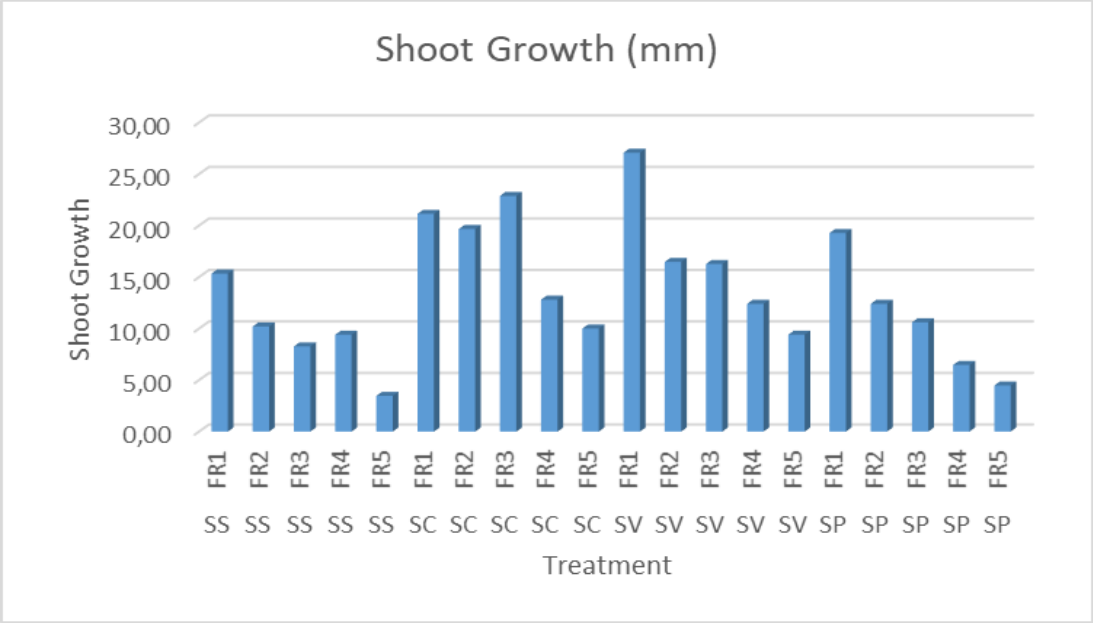

Figure 3.9

Shoot growth of *S. tortuosum* in response to 4 different soilless media, and 5 different fertigation regimes in hydroponics. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4= fertigation regime 4; FR5= fertigation regime 5. Treatment SSFR3 was selected for the control.

3.4.3 Dry weight

Soilless media

The effects of soilless media on the dry weight of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on the dry weight of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on the dry weight of *S. tortuosum* were found to be statistically significant at the value of $p \leq 0.001$. The highest individual mean value (0,97 g) for dry weight was observed in treatment SVFR1 (see Table 3.3 and Figure 3.10).

Table 3.3

The dry weight of *Sceletium tortuosum* in response to four different soilless media and five fertigation regimes in hydroponics.

Treatment		Total Dry Weight (g)	
Soilless Medium	Fertigation Regime	Mean	Standard Error + Mean Group
SS	FR1	0,55	±0,04cde
SS	FR2	0,42	±0,05ghi
SS	FR3	0,36	±0,04hij
SS	FR4	0,18	±0,03kl
SS	FR5	0,15	±0,02l
SC	FR1	0,74	±0,07b
SC	FR2	0,53	±0,06def
SC	FR3	0,64	±0,06bcd
SC	FR4	0,33	±0,03ij
SC	FR5	0,28	±0,03jk
SV	FR1	0,97	±0,06a
SV	FR2	0,75	±0,04b
SV	FR3	0,59	±0,07cde
SV	FR4	0,48	±0,06efg
SV	FR5	0,27	±0,04jkl
SP	FR1	0,67	±0,04bc
SP	FR2	0,43	±0,04fgh
SP	FR3	0,25	±0,03jkl
SP	FR4	0,27	±0,02jkl
SP	FR5	0,17	±0,01kl

Two-way ANOVA F-Statistics

Soilless Medium	42,3***
Fertigation Regime	78,4***
Soilless Medium*Fertigation Regime	2,9***

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤0.001 (***) as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4= fertigation regime 4; FR5= fertigation regime 5.

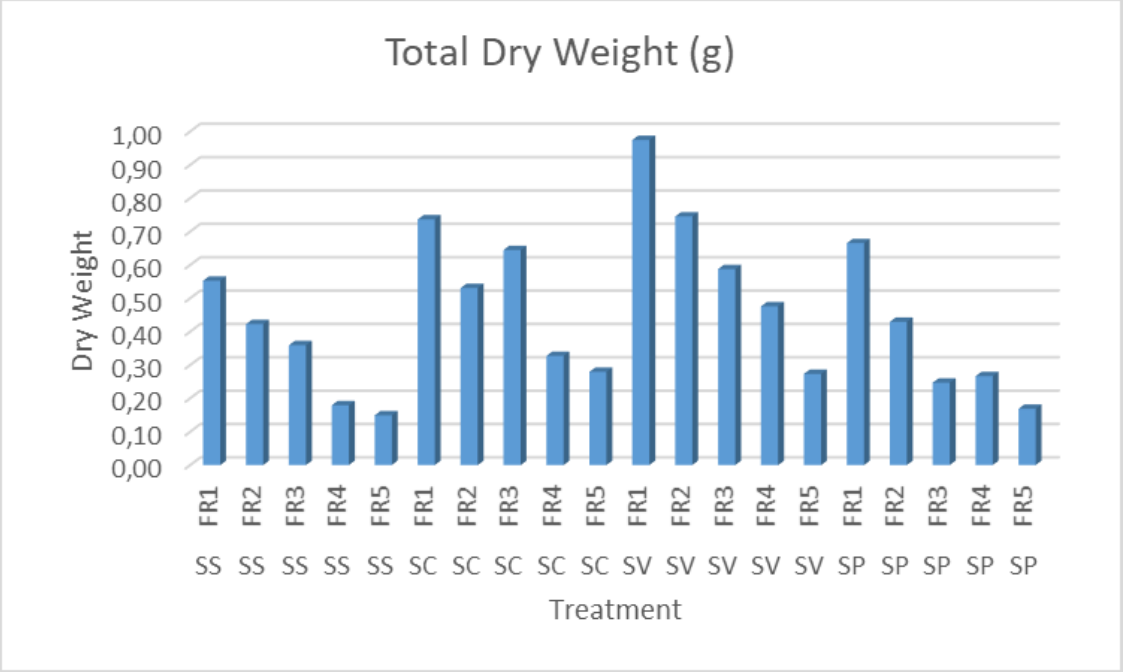

Figure 3.10

Dry weight of *S. tortuosum* in response to 4 different soilless media, and 5 different fertigation regimes in hydroponics. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4= fertigation regime 4; FR5= fertigation regime 5. Treatment SSFR3 was selected for the control.

3.5 Discussion & Conclusion

Treatments applied in this investigation had a significant effect on the vegetative root and shoot growth of *S. tortuosum*. Results obtained from this research suggest that well drained medium with high water holding capacity yielded better results in terms of vegetative growth (Benton Jones, 2014). Results obtained from this research in terms of fertigation regimes agrees with various previous studies which state that with a suitable quantity of water regarding to plant type improved overall plant growth (Begg & Turner, 1976; Wiedenfeld, 1995; Van Loon 1981; Mao *et al*., 2002; Yuan *et al*., 2003; Sezen *et al*., 2005; Kumar *et al*., 2007; Zeng *et al*., 2009). It was noticed plants responded better to mid-frequent fertigation intervals overall compared to low or high fertigation intervals.

To conclude, soilless media with higher water holding capacity (media containing coco-peat and vermiculite) had better results in terms of vegetative growth than media that does not (media containing pure silica sand, and perlite). Too frequent (every week) or infrequent (every fifth week) fertigation is not ideal for vegetative growth, instead plants reacted better to fertigation at 3 week intervals. One can

therefore suggest that well drained, yet high water holding capacity soilless media in conjunction with mid-frequent fertigation intervals would yield the best results in terms of vegetative growth. Further studies would be required on pH levels, EC levels, various different soilless media and combinations thereof in conjunction with various fertigation regimes and how *S. tortuosum* responds to these variables in order to further establish an optimal protocol for cultivation of the plant in hydroponics.

3.6 Acknowledgements

This study was funded by the Cape Peninsula University of Technology (CPUT), through the National Research Foundation and University Research Fund.

Thank you to Verve Dynamics (Pty) Ltd, Somerset West for supplying the initial mother plant for this research.

3.7 References

Begg, J.E. & Turner, N.C. 1976. Crop water deficits. *Advances in Agronomy*, 28: 161–217.

Benton Jones, J. 2014. *Growing Plants Hydroponically*. 1st ed. Boca Raton: CRC Press.

Brooking, I.R. 1976. Soilless potting media for controlled-environment facilities. New Zealand Journal of Experimental Agriculture, 4(2): 203–208.

Bunt, A.C. 1988. *Media Mixes for Container Grown Plants*. London, UK: Unwin Yyman.

Caron, J.C. & Nkongolo, V.K.N. 1999. Aeration in growing media: recent developments. *Acta Horticulturae*, 481: 545–551.

Fonteno, W.C. 1993. Problems & considerations in determining physical properties of horticultural substrates. *Acta Horticulturae*, (342): 197–204.

Gericke, N. & Viljoen, M. 2008. *Sceletium*–A review update. *Journal of Ethnopharmacology*, 119(3): 653–663.

Grafiadellis, I., Mattas, K., Maloupa, E., Tzouramani, I. & Galanopoulos, K. 2000. An economic analysis of soilless culture in *Gerbera* production. *HortScience*, 35(2): 300–303.

Greenwood, D.J. 1990. Production or productivity: the nitrate problem? *Annals of Applied Biology*, 117: 209–231.

Harvey, A.L., Young, L.C., Viljoen, A.M. & Gericke, N.P. 2011. Pharmacological actions of the South African medicinal and functional food plant *Sceletium tortuosum* and its principal alkaloids. *Journal of Ethnopharmacology*, 137(3): 1124–1129.

Jensen, M. 2017. The pros and cons of six soilless media options. *American Vegetable Grower*, 65(3): S2.

Kumar, S., Imtiyaz, M., Kumar, A., Singh, R. 2007. Response of onion (*Allium cepa* L.) to different levels of irrigation water. *Agricultural Water Management*, 89: 161–166.

Le Bot, J., Adamowicz, S. & Robin, P. 1998. Modelling plant nutrition of horticultural crops: A review. *Scientia Horticulturae*, 74(1–2): 47–82.

Mao, X., Liu, M., Wang, X., Liu, C., Hou, Z., Shi, J. 2002. Effects of deficit irrigation on yield and water use of greenhouse grown cucumber in the North China Plain. *Agricultural Water Management*, 61: 219–228.

Nejad, A.R. & Ismaili, A. 2014. Changes in growth, essential oil yield and composition of geranium (*Pelargonium graveolens* L.) as affected by growing media. *Journal of the Science of Food and Agriculture*, 94(5): 905–910.

Raviv, M., Wallach, R., Silber, A. & Bar-Tal, A. 2002. *Substrates and their analysis In: Hydroponic Production of Vegetables and Ornamentals*. Athens: Embryo Publications.

Reversat, G., Boyer, J., Sannier, C. & Pando-Bahuon, A. 1999. Use of a mixture of sand and water-absorbent synthetic polymer as substrate for the xenic culturing of plant-parasitic nematodes in the laboratory. *Nematology*, 1(2): 209–212.

Savvas, D., Gianquinto, G., Tuzel, Y., & Gruda, N., 2013. Soilless Culture. Good agricultural practices for greenhouse vegetable crops, Principles for Mediterranean climate areas. *FAO Plant Production and Protection Paper*, 217: 303–354.

Schultes, R.E., Hofmann, A. & Rätsch, C. 2001. *Plants of the Gods:Their Sacred , Healing, and Hallucinogenic Powers*. *2nd ed*. Lucerne: EMB-service for Publishers.

Sezen, S.M., Yazar, A., Canbolani, M., Eker, S. & Caikel, G. 2005. Effect of drip management on yield and quality of field grown green beans. *Agricultural Water Management,* 71 (3): 243–255.

Smith, M.T., Crouch, N.R., Gericke, N. & Hirst, M. 1996. Psychoactive constituents of the genus *Sceletium* N.E.Br. and other Mesembryanthemaceae: A review. *Journal of Ethnopharmacology*, 50(3): 119–130.

Steel, R.G.D. & Torrie, J.H. 1980. Principle and procedures of statistics: a biometrical approach. *2nd ed*. New York: McGraw-Hill.

Taiz, L & Zeiger, E. 2010. *Plant physiology*. *5th ed*. Sunderland: Sinauer Associates.

Van Loon, C.D. 1981. The effect of water stress on potato growth, development and yield. *American Potato Journal*, 58: 51–69.

Van Wyk, B. & Wink, M. 2012. *Medicinal Plants of the World*. 5th ed. Pretoria: Briza.

Van Wyk, B.E. & Wink, M. 2015. *Phytomedicines, Herbal Drugs and Plant Poisons*. *1st ed*. Pretoria: Briza Publications.

Wiedenfeld, R.P. 1995. Effects of irrigation and N fertilizer application on sugarcane yield and quality. *Field crops Research*, 43: 101–108.

Yuan, B.Z., Nishiyama, S., Kang, Y. 2003. Effects of different irrigation regimes on the growth and yield of drip – irrigated potato. *Agricultural Water Management,* 63 (3): 153–167.

Zeng, C.Z., Bie, Z.L., Yuan, B.Z. 2009. Determination of optimum irrigation water amount for drip irrigated muskmelon (*Cucumis melo L.)* in plastic greenhouse. *Agricultural Water Management*, 96: 597–601.

CHAPTER FOUR:

ALKALOID CONCENTRATION OF *S. TORTUOSUM* IN RESPONSE TO DIFFERENT SOILLESS GROWING MEDIA AND FERTIGATION REGIMES IN HYDROPONICS

ALKALOID CONCENTRATION OF *S. TORTUOSUM* IN RESPONSE TO DIFFERENT SOILLESS GROWING MEDIA AND FERTIGATION REGIMES IN HYDROPONICS

RJ Faber, CP Laubscher*, JC Coetzee, F Rautenbach

Department of Horticultural Sciences, Faculty of Applied Sciences, Cape Peninsula University of Technology, PO Box 1906, Bellville, 7535, South Africa.

*Email: laubscherc@cput.ac.za

4.1 Abstract

Sceletium tortuosum (L.) N.E. Br is a succulent medicinal crop plant in the family Mesembryanthemaceae, it was formerly known as *Mesembryanthemum tortuosum*. The plant is indigenous to South Africa where is it well known and utilized for its medicinal and psycho-active properties. Interest in *S. tortuosum* has been growing as mesembrine-type alkaloids found in the plant has tremendous potential as an alternative supplement for promoting health and treating a variety of psychological and psychiatric disorders. The purpose for this study was to investigate whether *S. tortuosum* was first of all suitable for hydroponic cultivation, as well as to test how the plant would respond to different soilless media and fertigation regimes in terms of alkaloid concentrations. Three hundred plants were cultivated from one initial mother plant obtained from Verve Dynamics (Pty) Ltd. Twenty treatments were evaluated with 15 sample replicates. Treatments were made up of 4 different soilless growing media, namely: pure silica sand, 50% silica sand with 50% coco-peat, 50% silica sand with 50% vermiculite, and 50% silica sand with 50% perlite. These growing media were tested in conjunction with 5 different fertigation regimes (FR), plants grown in FR1 received aqueous nutrient solution once every week, FR2 received aqueous nutrient solution once every second week, FR3 received aqueous nutrient solution once every third week, FR4 received aqueous nutrient solution once every fourth week and FR5 received aqueous nutrient solution once every fifth week respectively. Results from this experiment showed that different soilless growing media and fertigation regimes as well as soilless media in conjunction with fertigation regimes had varying effects on *S. tortuosum* and the plants alkaloid concentrations. It was observed that roots contained higher amounts of delta 7 mesembrenone and mesembrenone, while shoots contained higher amounts of the alkaloid mesembrine.

Keywords: alkaloids, delta 7 mesembrenone, fertigation regimes, hydroponics, mesembrenone, mesembrine, optimal, roots, shoots, soilless media

Abbreviations: fertigation regime (FR), silica sand (SS), silica sand and coco-peat (SC), silica sand and vermiculite (SV), silica sand and perlite (SP).

4.2 Introduction

4.2.1 Plant secondary metabolites

Primary metabolites serve a role in basic life functions, such as cell division, growth, respiration, storage, and reproduction as described in the two hundred years of modern chemistry and biology. The concept of secondary metabolites can be credited to Kossel (1891) who was the first to define these metabolites as opposed primary ones. Thirty years later Czapek (1921) dedicated his entire volume of “plant biochemistry”, which he named “endproduckt” making further progress as to explain the function of these products. As he suggests, these metabolites are derived from nitrogen metabolism, which he termed “secondary modifications”, deamination is a good example of such modifications. Secondary metabolites play a major role in the adaptation of plants to their environment. Apart from being described as possessing anti-bacterial, anti-fungal and anti-viral properties which can protect plants from pathogens they can also be anti-germinative, preventing other plants from growing in their area. Further they comprise UV absorbing properties, protecting plants from leaf damage caused by light intensity, they also have anti-feeding properties, protecting plants from insects and even larger animals. For example clover or alfalfa grass can express estrogenic effects, which interact with animal fertility (Li *et al*., 1993; Bourgaud *et al*., 2001)

Human infections, health disorders and illness has been treated using plants containing secondary metabolites since the beginning of mankind (Van Wyk & Wink, 2012). Plant structures have been in many cases the main lead to producing synthetic substances such as aspirin and salicylic acid, and have only been partly replaced by synthetic drugs over the last 100 years with advancements in modern medicine. Pharmaceutical, agrochemical, flavour and aroma are all industries that use secondary metabolites in plants. The present and future priority thus lies in finding new plant based chemicals, bearing sustainable conservation, rational utilization, and biodiversity in mind (Philipson, 1990).

Extracts and crude drugs are the main use of medicinal plants globally, with several active and potent substances being employed as isolated compounds. Examples of alkaloids in isolated form and their uses are, caffeine (stimulant), ephedrine (stimulant), scopolamine (travel sickness), capsaicin (rheumatic pains), morphine (pain killer), codeine (antitussive), papaverine (phosphodiesterase inhibitor), quinine (anti-malarial), colchicines (gout), yohimbine (aphrodisiac), pilocarpine (glaucoma), berberine (psoriasis), ajmaline (antiarrhythmic) and various cardiac glycosides (heart insufficiency) and the list goes on (Wink *et al*., 2005; Karuppusamy, 2009).

4.2.2 *Sceletium tortuosum* and its alkaloids

S. tortuosum is a succulent plant that belongs to the family Mesembryanthemaceae. It is indigenous to South Africa, where it is well known by the indigenous people, especially in Namaqualand where the plant is utilized regularly for its medicinal and psycho-active properties (Smith *et al*., 1996). According to Schultes *et al*. (2001) and Harvey *et al*. (2011) the main alkaloids responsible for these properties are mesembrine, mesembrenine (mesembrenone), and mesembrenol.

S. tortuosum is now considered a medicinal crop plant and is classified as mind-altering, sedative, euphoric, and not hallucinogenic (Van Wyk & Wink, 2012; Van Wyk & Wink, 2015). The alkaloids responsible for these psychoactive properties are mesembrine and mesembrenone. However, the concentration of alkaloids within individual plants may vary depending on their chemotype. Uses of the plant include the treatment of anxiety, stress, nervous tension, alcohol addiction, colic in infants and also for suppressing hunger and thirst. With clear ethno-pharmaceutical value it is also worthy to mention that the use of *S. tortuosum* develops no physical or psychological dependency (Van Wyk & Wink, 2015).

Sceletium spp. contain four classes of alkaloids, the 3a-aryl-*cis*-octahydroindole class, *C*-secomesembrine alkaloids, alkaloids containing a 2,3-disubstituted pyridine moiety and a ring *C*-seco *Sceletium* alkaloid A4 group (Gericke & Viljoen, 2008). *Sceletium* spp. contain high levels of the pharmacologically-active substance mesembrine, and is one of the only plant genera known to carry this alkaloid (Krstenansky, 2016). There are seven alkaloids attributed to *S. tortuosum*, namely mesembrine, mesembrenone, mesembranol, mesembrenol, alkaloid A4, chennanine and tortuosamine (Gericke & Viljoen, 2008).

Mesembrine serves a primary function as a monoamine releasing agent (MRA) and secondly as a selective serotonin reuptake inhibitor (SSRI), drug classes most commonly utilized in the treatment of anxiety and depression (Coetzee *et al*., 2015). Another alkaloid found in *S. tortuosum* is delta 7 mesembrenone, it is a potent antioxidant, and a powerful inhibitor of tyrosinase activity, kojic acid at high doses had an almost identical effect on this enzyme (Bennett *et al.*, 2018). Alkaloid composition in different *S. tortuosum* plant extracts vary, and higher concentrations of certain alkaloids can have different medical properties.

The aim of this study was to assist in determining an optimal protocol for cultivating *S. tortuosum* with an adequate concentration of total alkaloids, as well as the alkaloids delta 7 mesembrenone, mesembrenone, and mesembrine in order to produce consistent high quality medicinal plants.

4.3 Materials and methods

4.3.1 Greenhouse Experiment

This investigation was conducted in the research greenhouse facility at the Cape Peninsula University of Technology, Bellville, Cape Town, South Africa; GPS co-ordinates - 33º 55’45.53S, 18º 38’ 31. 16E. The nature of the structure and the technology installed ensured control of the environment within the greenhouse.

4.3.2 Plant Preparation

One *Sceletium tortuosum* mother plant was obtained from Verve Dynamics, Somerset West. An additional ten mother plants were propagated and cultivated from this plant. Once these ten plants were large enough, ± 600 2.5-3 cm stem cuttings were propagated from the mother plants to ensure a supply of more than 300 rooted cuttings, which was the number of plant material required for the experiment.

Cuttings were treated with Dynaroot™ No 1 (Active ingredient: 0.1% I.B.A.) as rooting hormone and planted into 200 and 128 cell styrofoam trays containing a 50/50 mixture of silica sand, and coco-peat as rooting medium. The trays were placed in an environmentally controlled propagation greenhouse at the Cape Peninsula University of Technology, Bellville campus. The first sign of roots was observed after 2 weeks, and roots had fully developed after 2 months. After rooting, trays were moved to the

research greenhouse for acclimatization and watering was administered by hand. The best quality rooted cuttings in the batch were selected for the experiment.

4.3.3 Hydroponic experiment

Five identical hydroponic systems were constructed. Each system consisted of one 70 litre capacity low-density polyethylene (LDPE) reservoir; four polyvinyl chloride (PVC) square gutters (130 mm x 70 mm x 2 500 mm) secured onto wire mesh tables (900 mm x 1 250 m x 2 500 m); sixty 12,5 x 12,5 cm plastic pots, a 2 000 ℓ/h submersible water pump with a 2,5 m head capacity, ten-meter x 20 mm LDPE irrigation piping; four reducers (20 mm–15 mm); four 15 mm flow regulators; one air pump, one air stone (15cm) and eight 20 mm bulk head connectors. Reservoirs were kept filled to a level of approximately 50 ℓ with aqueous nutrient solution. Each gutter was sealed with PVC adhesive and fitted with an inlet guiding water towards the gutters and an outlet to return excess water to the reservoir. Each inlet consisted of LDPE irrigation piping converted from 20 mm to 15 mm diameter and each fitted with a 15 mm valve allowing maximum control of aqueous nutrient solution flow into gutters. All gutters were filled with silica sand to a depth of ± 1,5 cm. Fifteen pots (12,5 cm x 12,5 cm) were placed into each gutter on top of the silica sand. As water passes through the gutter the sand becomes fully saturated with aqueous nutrient solution which is then imbibed by soilless growing media in pots via capillary action. Every gutter for each system represented a different soilless substrate in 15 pots, as follows: 100% silica sand (SS); a 50/50 mixture of silica sand and coco-peat (SC); a 50/50 mixture of silica sand and vermiculite (SV) and a 50/50 mixture of silica sand and perlite (SP).

Each separate system was manually controlled to fertigate plants with the same amount of aqueous nutrient solution at different time intervals. The five systems were given aqueous nutrient solution amounts as follows: Fertigation regime 1 (FR1) was fertigated once a week at ± 350 ml per minute for 1 hour. Fertigation regime 2 (FR2) was fertigated every second week at ± 350 ml per minute for 1 hour. Fertigation regime 3 (FR3) was fertigated once every third week at ± 350 ml per minute for 1 hour. Fertigation regime 4 (FR4) was fertigated once every fourth week at ± 350 ml per minute for 1 hour. Fertigation regime 5 (FR5) was fertigated once every fifth week at ± 350 ml per minute for 1 hour. Fifteen plants, one per pot were placed into each soilless substrate of each system, which totals to 60 plants per hydroponic system and 300 plants for the entire experiment. Therefore 15 replicates in a randomized blocked design were tested for each media and fertigation regime combination.

All aqueous nutrient solutions used in the experiment were kept at a pH level of 6.0, and an electrical conductivity (EC) level of 0.5. EC levels of the aqueous nutrient solutions were monitored with a calibrated hand held digital EC meter (Hanna Instruments®™ HI 98312). Water reservoir pH levels were monitored with a calibrated handheld digital pH meter (Eurotech®™ pH 2 pen). For increasing pH potassium hydroxide was used, while phosphoric acid was used for decreasing pH of aqueous nutrient solutions. For decreasing the EC of aqueous nutrient solutions, reverse osmosis water was added into reservoirs, while adding Hoagland Solution to aqueous nutrient solutions in reservoirs increased the EC. Pots were individually numbered and arranged randomly.

All gutters were slightly tilted to allow aqueous nutrient solutions to flow from one end (inlet) to the other (outlet). Each outlet was guided to a separate smaller gutter which then returned the aqueous nutrient solution back to the reservoir, creating a circulating system.

4.3.4 Treatment preparation

Soilless medium treatments were prepared using four different soilless medium combinations, namely: 100% silica sand (SS); a 50/50 mixture of silica sand and coco-peat (SC); a 50/50 mixture of silica sand and vermiculite (SV) and a 50/50 mixture of silica sand and perlite (SP). For treatments SC, SV and SP equal parts silica sand and coco-peat/vermiculite/perlite was mixed together. All silica sand used was thoroughly rinsed with tap water until water poured through the sand ran clear.

Fertigation regimes were achieved by installing identical pumps to all hydroponic systems. The amount of aqueous nutrient solution delivered to each grow bed was controlled by adjusting all output valves until ±350 ml per minute was measured. Measurement was achieved by placing an empty bucket below each output for 1 minute, the liquid captured in the bucket was decanted and measured using a 500 ml Erlenmeyer flask until the amount of aqueous nutrient solution for each output was measured at ±350 ml for 1 minute.

Treatment SSFR3 was selected for the control, as it contained 100% silica sand, which was the base of all soilless mediums used in this experiment as well as a mid-frequent fertigation regime.

4.3.5 Treatment Application

Treatments applied consisted of 4 different soilless growth media and 5 different fertigation regimes in a circulating capillary action hydroponic system. The control consisted of treatment SSFR3 containing 100% silica sand growing media and a mid-frequent fertigation regime. Each treatment was numbered, as follows:

SSFR1 - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once a week.

SCFR1 – 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once a week.

SVFR1 – 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once a week.

SPFR1 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once a week.

SSFR2 - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every second week.

SCFR2 - 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every second week.

SVFR2 - 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every second week.

SPFR2 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every second week.

SSFR3 (control) - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every third week.

SCFR3 - 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every third week.

SVFR3 - 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every third week.

SPFR3 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every third week.

SSFR4 - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fourth week.

SCFR4 - 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fourth week.

SVFR4 - 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fourth week.

SPFR4 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fourth week.

SSFR5 - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fifth week.

SCFR5 - 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fifth week.

SVFR5 - 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fifth week.

SPFR5 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fifth week.

4.3.6 Data collection

4.3.6.1 Drying and milling of plant material

Plants were placed in brown paper bags post-harvest and dried at 30–31°C in a forced convection oven (Daihan Labtech LDO-150F) until the material was crisp dry. Plants were then weighed using an electronic balance (RADWAG® Model PS 750.R2) with 0.001 g readability and recorded. Dried plants of each treatment were combined together and milled to a powder using a 50 g capacity standard coffee grinder (Mellerware - Aromatic Coffee Mill & Grinder), and stored in transparent zip-lock plastic bags. The coffee grinder was cleaned thoroughly between the milling of each treatment.

4.3.6.2 Measuring total alkaloids

The total alkaloid content for roots and shoots were measured using the bromo cresol green method based on an atropine standard. For every treatment, 5 extractions were made. Extraction was done by placing 100 mg of plant material in 10 ml of methanol within a 15 ml lab grade plastic tube for 24 hours after which tubes were centrifuged at 4000 rpm for 2 minutes. One ml of methanol extract was transferred to a 50 ml lab grade plastic tube with 5 ml of buffering solution (pH 4.7), 12 ml of bromo cresol green and 12 ml of chloroform. The chloroform separates from the bromo cresol green and buffering solution and if a yellow colour is observed it means alkaloids are present in the extract. The yellow chloroform liquid was then placed in a 96 cell well plate using a pipette, 3 cells per extraction at 300 µl and placed in a multi scan spectrum reader. Extractions were observed at 417 nm, the wavelength for the colour yellow, and analysed with the software program SkanIt™ which measured the amount of yellow pigments in a sample, i.e. the total amount of alkaloids (Fadhil *et al*., 2007). This analysis did not specify which individual alkaloids are present in extract samples, but rather just the total amount of alkaloids present in a sample.

4.3.6.3 Measuring delta 7 mesembrenone, mesembrenone and mesembrine area %

The most commonly used technique for analysing natural products is high performance liquid chromatography (HPLC) with ultra violet light detection. Due to the commercial unavailability of *S. tortuosum* alkaloid standards the HPLC test for the

area % of delta 7 mesembrenone, mesembrenone and mesembrine was based on standard references for mesembrine-type alkaloids as seen by Patnala and Kanfer (2010). The liquid or mobile phase used for *S. tortuosum* is water plus acentronitile at a ratio of 72:28 plus 0.01% ammonia hydroxide. 20 µl of extractions were filtered into HPLC vials, the vials were then numerically placed in the sample collector according to treatment. From here the mobile phase and extract samples individually got injected into a column (C18 – kromasil 100-5-C18) at a flow rate of 1 µl/min, which separated the molecules and passed them through a UV detector and UV signatures at 280 nm were recorded and in turn determined the area % of the different alkaloids. Procedures were done at room temperature and repeated 5 times for each treatments extraction sample.

4.3.6.4 Measuring mesembrine

The pure mesembrine compound was injected into the HPLC system by itself, the peak observed as a result on the chromatograph represents mesembrine, which then draws a standard reference for other molecules to compare to, therefore if another molecule is injected and analysed in the same way and results are in the same peak on the chromatograph, they are the same molecule as the standard. The HPLC method with UV detection was again used for this analysis. 20 µl of extractions were filtered into HPLC vials, the vials were then numerically placed in the sample collector according to treatment. From here the mobile phase and extract samples individually got injected into a column (C18 – kromasil 100-5-C18) at a flow rate of 1 µl/min, which separated the molecules and passed them through a UV detector and UV signatures at 280 nm, molecules that were identical to the mesembrine standard were recorded. Procedures were done at room temperature and repeated 5 times for each treatments extraction sample (Patnala & Kanfer, 2010). The standard for mesembrine (Verve Dynamics (Pty) Ltd) was used as comparison (see Figure 4.1)

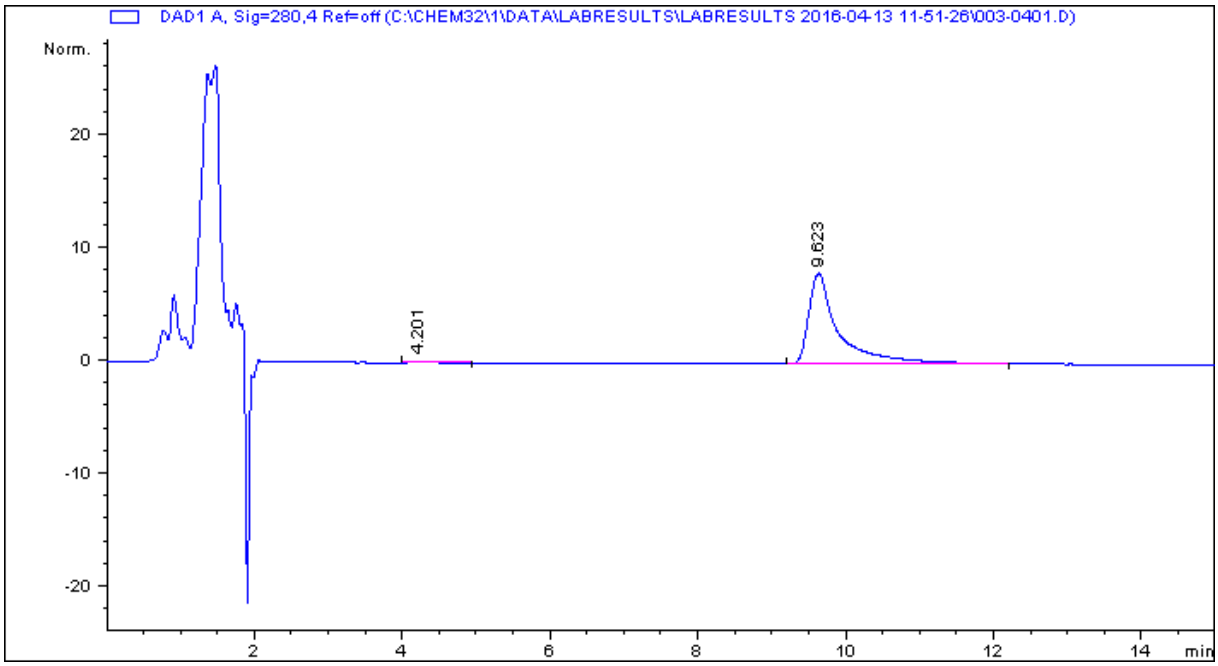

Figure 4.1

Chromatograph displaying Verve Dynamics (Pty) Ltd mesembrine standard.

4.3.7 Statistical analysis

All data collected was statistically analysed using two-way analysis of variance (ANOVA). Treatment means were compared using Fischer's Least Significant Difference (L.S.D) at values of $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$. The software program STATISTICA version 13 was used to perform all calculations on Windows 10 (Steel & Torrie, 1980).

4.4 Results

4.4.1 Total alkaloids

4.4.1.1 Roots

Soilless media

The effects of soilless media on root total alkaloid concentrations of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on root total alkaloid concentrations of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

Effects of soilless media in conjunction with fertigation regimes on root total alkaloid concentrations of *S. tortuosum* were found to be statistically significant at the value of $p \leq 0.001$. The highest individual mean value (1,68) of total alkaloids was observed in treatment SPFR4. Roots were found to have an overall lower concentration of total alkaloids than shoots (see Table 4.1 and Figure 4.2).

4.4.1.2 Shoots

Soilless media

The effects of soilless media on shoot total alkaloid concentrations of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on shoot total alkaloid concentrations of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

Effects of soilless media in conjunction with fertigation regimes on shoot total alkaloid concentrations of *S. tortuosum* were found to be statistically significant at the value of $p \leq 0.001$. Treatments SSFR3, SPFR2 and SPFR3 resulted in the highest mean values (2,30; 2,33; 2,37). Shoots were found to have an overall higher concentration of total alkaloids than the roots (see Table 4.1 and Figure 4.2).

Table 4.1

The total alkaloid concentration of *S. tortuosum* root and shoot extracts in response to four different soilless media and five fertigation regimes in hydroponics.

Treatment		Roots - Total Alkaloids		Shoots - Total Alkaloids	
Soilless Medium	Fertigation Regime	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group
SS	FR1	1,25	±0,06de	1,71	±0,07cd
SS	FR2	1,51	±0,03b	1,86	±0,04bcd
SS	FR3	1,51	±0,04b	2,30	±0,05a
SS	FR4	1,25	±0,04de	1,68	±0,11d
SS	FR5	1,34	±0,06cd	1,79	±0,08bcd
SC	FR1	1,25	±0,05de	1,78	±0,11bcd
SC	FR2	1,31	±0,03cde	1,77	±0,06bcd
SC	FR3	0,87	±0,06hi	1,89	±0,06bc
SC	FR4	0,97	±0,03gh	1,31	±0,09e
SC	FR5	1,45	±0,10bc	1,47	±0,03e
SV	FR1	1,04	±0,04fg	1,37	±0,05e
SV	FR2	0,94	±0,01gh	1,35	±0,07e
SV	FR3	0,71	±0,04j	1,48	±0,08e
SV	FR4	0,74	±0,02ij	1,07	±0,05f
SV	FR5	1,28	±0,08de	1,39	±0,04e
SP	FR1	1,17	±0,07ef	1,97	±0,07b
SP	FR2	1,18	±0,01ef	2,33	±0,11a
SP	FR3	1,30	±0,02de	2,37	±0,04a
SP	FR4	1,68	±0,08a	1,93	±0,09b
SP	FR5	1,34	±0,07cd	1,83	±0,02bcd

Two-way ANOVA F-Statistics

Soilless Medium		67,7***	101,2***
Fertigation Regime		13,3***	29,7***
Soilless Medium*Fertigation Regime		15,9***	3,4***

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤0.001 (***) as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4= fertigation regime 4; FR5= fertigation regime 5.

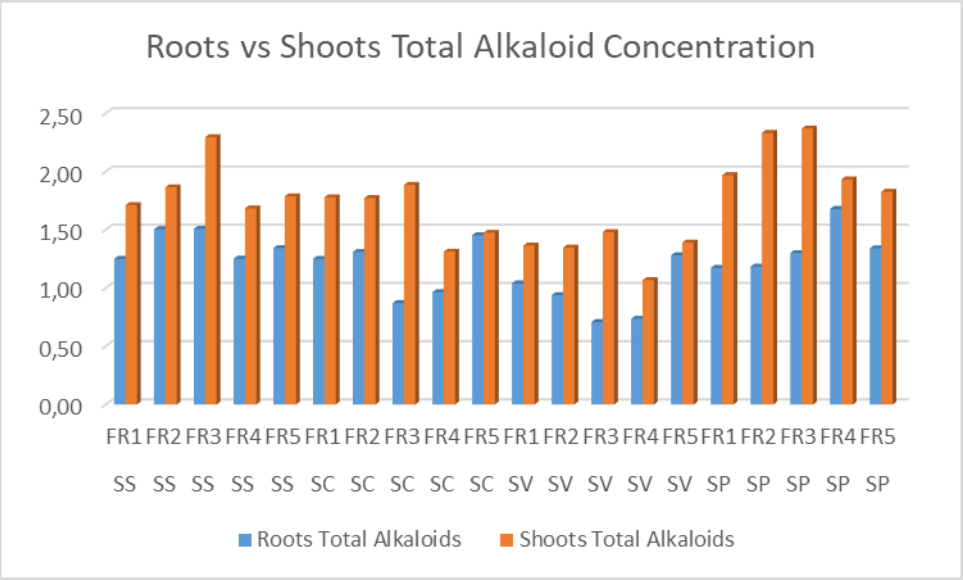

Figure 4.2

Comparing results of *S. tortuosum* root vs shoot total alkaloid concentration in response to 4 different soilless media and five fertigation regimes in hydroponics. Treatment SSFR3 was selected for the control.

4.4.2 Delta 7 mesembrenone area %

4.4.2.1 Roots

Soilless media

The response of *S. tortuosum* in terms of root delta 7 mesembrenone area % to soilless media was statistically significant at the value $p \leq 0.001$.

Fertigation regime

The response of *S. tortuosum* in terms of root delta 7 mesembrenone area % to fertigation regimes was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The response of *S. tortuosum* in terms of root delta 7 mesembrenone area % to soilless media in conjunction with fertigation regimes were found to be statistically significant at the value $p \leq 0.001$. The highest individual mean value (52,37) for root delta 7 mesembrenone area % was found in treatment SVFR4. All 20 treatments significantly differed from one another. Roots had an overall higher concentration of delta 7 mesembrenone in terms of area % than shoots (see Table 4.2 and Figure 4.3).

4.4.2.2 Shoots

Soilless media

The response of *S. tortuosum* in terms of shoot delta 7 mesembrenone area % to soilless media was statistically significant at the value $p \leq 0.001$.

Fertigation regime

The response of *S. tortuosum* in terms of shoot delta 7 mesembrenone area % to fertigation regimes was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The response of *S. tortuosum* in terms of shoot delta 7 mesembrenone area % to soilless media in conjunction with fertigation regimes were found to be statistically significant at the value $p \leq 0.001$. Treatment SVFR4 had the highest individual mean value (37,23) for shoots. All 20 treatments significantly differed from one another. Shoots had an overall lower concentration of delta 7 mesembrenone in terms of area % than roots (see Table 4.2 and Figure 4.3).

Table 4.2

The delta 7 mesembrenone area % of *S. tortuosum* root and shoot extracts in response to four different soilless media and five fertigation regimes in hydroponics.

Treatment		Roots delta 7 mesembrenone area %		Shoots delta 7 mesembrenone area %	
Soilless Medium	**Fertigation Regime**	**Mean**	**Std Err + Mean Group**	**Mean**	**Std Err + Mean Group**
SS	FR1	45,14	±0,17f	16,62	±0,08m
SS	FR2	46,44	±0,11e	23,29	±0,09f
SS	FR3	46,81	±0,08d	19,21	±0,07j
SS	FR4	37,88	±0,14k	20,47	±0,06h
SS	FR5	36,57	±0,14l	25,14	±0,06e
SC	FR1	30,69	±0,09o	15,49	±0,09n
SC	FR2	26,35	±0,07q	12,85	±0,06p
SC	FR3	28,04	±0,06p	11,47	±0,08q
SC	FR4	47,42	±0,11c	36,30	±0,03b
SC	FR5	39,42	±0,08j	30,78	±0,05c
SV	FR1	40,08	±0,11i	18,44	±0,09k
SV	FR2	46,95	±0,09d	25,89	±0,04d
SV	FR3	50,26	±0,13b	25,75	±0,07d
SV	FR4	52,37	±0,10a	37,23	±0,04a
SV	FR5	34,94	±0,07m	17,86	±0,06l
SP	FR1	30,78	±0,07o	14,50	±0,10o
SP	FR2	44,26	±0,09g	20,29	±0,06h
SP	FR3	30,62	±0,09o	10,85	±0,04r
SP	FR4	41,62	±0,12h	19,42	±0,06i
SP	FR5	31,79	±0,17n	22,39	±0,04g

Two-way ANOVA F-Statistics

Soilless Medium		10872***	10950***
Fertigation Regime		4440***	23755***
Soilless Medium*Fertigation Regime		4031***	9247***

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤0.001 (***) as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4= fertigation regime 4; FR5= fertigation regime 5.

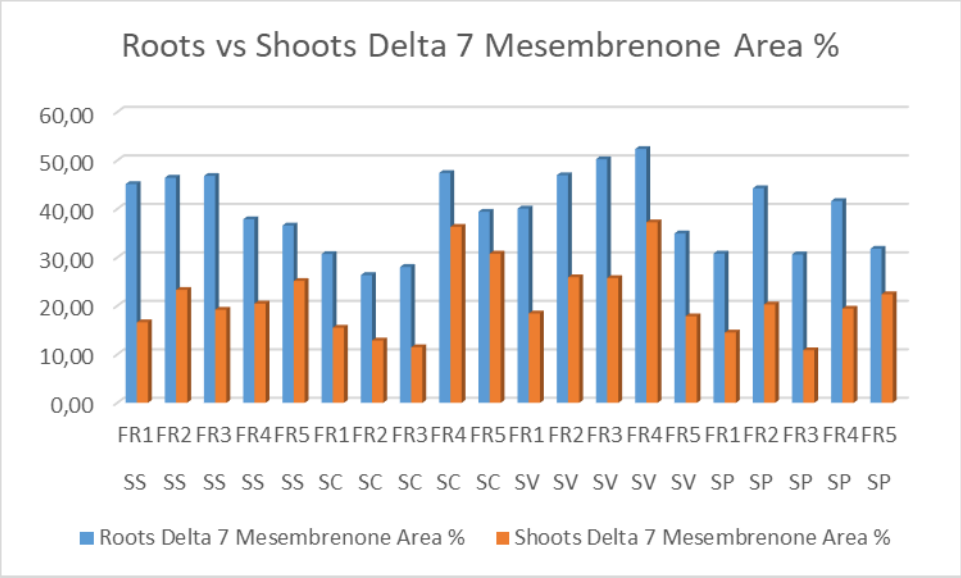

Figure 4.3

Comparing results of *S. tortuosum* root vs shoot delta 7 mesembrenone concentration in terms of area % in response to 4 different soilless media and five fertigation regimes in hydroponics. Treatment SSFR3 was selected for the control.

4.4.3 Mesembrenone area %

4.4.3.1 Roots

Soilless media

The response of *S. tortuosum* in terms of root mesembrenone area % to soilless media was statistically significant at the value $p \leq 0.001$.

Fertigation regime

The response of *S. tortuosum* in terms of root mesembrenone area % to fertigation regimes was statistically significant at the value $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The response of *S. tortuosum* in terms of root mesembrenone area % to soilless media in conjunction with fertigation regimes was statistically significant at the value $p \leq 0.001$. The highest mean values (38,16 and 38,32) for roots were found in treatments SCFR5 and SPFR2. Roots had an overall higher concentration of mesembrenone in terms of area % than shoots (see Table 4.3 and Figure 4.4).

4.4.3.2 Shoots

Soilless media

The response of *S. tortuosum* in terms of shoot mesembrenone area % to soilless media was statistically significant at the value $p \leq 0.001$ (Table 4.3).

Fertigation regimes

The response of *S. tortuosum* in terms of shoot mesembrenone area % to fertigation regimes was statistically significant at the value $p \leq 0.001$ (Table 4.3).

Interaction between soilless media and fertigation regimes

The response of *S. tortuosum* in terms of shoot mesembrenone area % to soilless media in conjunction with fertigation regimes were statistically significant at the value $p \leq 0.001$(Table 4.3). The highest individual mean value (12,35) for shoots were found in treatment SSFR1. Shoots had an overall lower concentration of mesembrenone in terms of area % than roots (Table 4.3 and Figure 4.4).

Table 4.3

The mesembrenone area % of *S. tortuosum* root and shoot extracts in response to four different soilless media and five fertigation regimes in hydroponics.

Treatment		Roots - Mesembrenone Area %		Shoots - Mesembrenone Area %	
Soilless Medium	**Fertigation Regime**	**Mean**	**Std Err + Mean Group**	**Mean**	**Std Err + Mean Group**
SS	FR1	33,70	±0,08d	12,35	±0,09a
SS	FR2	19,91	±0,15n	11,35	±0,09b
SS	FR3	29,91	±0,16h	6,10	±0,08f
SS	FR4	25,15	±0,10j	7,52	±0,08d
SS	FR5	21,15	±0,15m	6,59	±0,08e
SC	FR1	25,84	±0,11i	4,63	±0,07i
SC	FR2	22,55	±0,10l	3,18	±0,04l
SC	FR3	23,17	±0,09k	2,02	±0,05o
SC	FR4	33,40	±0,09d	5,08	±0,07h
SC	FR5	38,16	±0,18a	4,59	±0,05ij
SV	FR1	34,61	±0,06c	3,12	±0,05l
SV	FR2	35,38	±0,15b	5,57	±0,08g
SV	FR3	32,42	±0,12e	2,83	±0,05m
SV	FR4	31,18	±0,18f	6,11	±0,07f
SV	FR5	35,08	±0,17b	3,85	±0,05k
SP	FR1	22,96	±0,11k	6,57	±0,07e
SP	FR2	38,32	±0,16a	8,37	±0,10c
SP	FR3	17,56	±0,08o	2,49	±0,06n
SP	FR4	26,10	±0,09i	6,02	±0,06f
SP	FR5	30,48	±0,12g	4,41	±0,08j

Two-way ANOVA F-Statistics

Soilless Medium	3640***	5050,8***
Fertigation Regime	947***	1925,4***
Soilless Medium*Fertigation Regime	2691***	524,2***

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤0.001 (***) as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4= fertigation regime 4; FR5= fertigation regime 5.

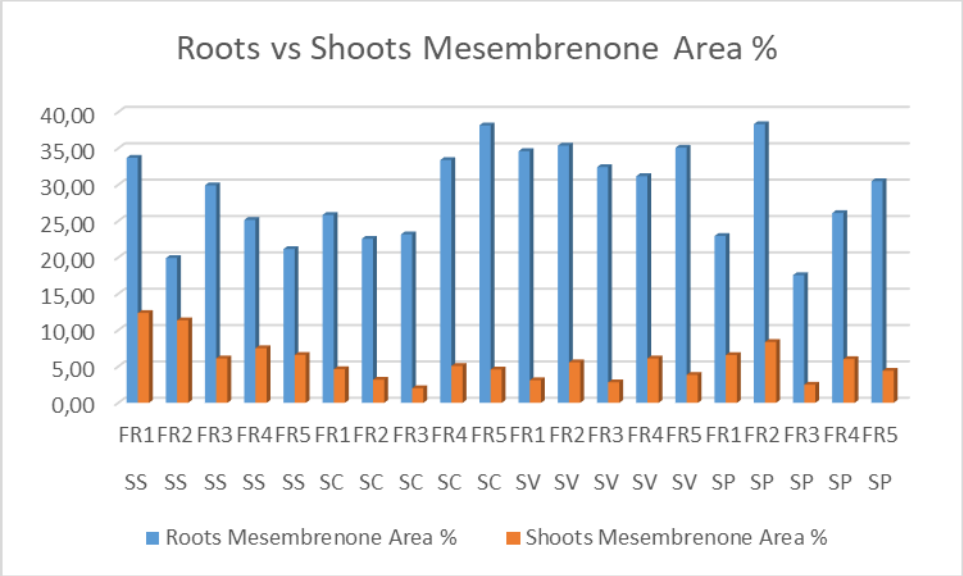

Figure 4.4

Comparing results of *S. tortuosum* root vs shoot mesembrenone concentration in terms of area % in response to 4 different soilless media and five fertigation regimes in hydroponics. Treatment SSFR3 was selected for the control.

4.4.4 Mesembrine area %

4.4.4.1 Roots

Soilless media

The response of *S. tortuosum* in terms of root mesembrine area % to soilless media was statistically significant at the value $p \leq 0.001$.

Fertigation regime

The response of *S. tortuosum* in terms of root mesembrine area % to fertigation regimes was statistically significant at the value $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The response of *S. tortuosum* in terms of root mesembrine area % to soilless media in conjunction with fertigation regimes were statistically significant at the value $p \leq 0.001$. The highest individual mean value (51,83) for roots were found in treatment SPFR3. Roots had an overall lower concentration of mesembrine in terms of area % than shoots (see Table 4.4 and Figure 4.5).

4.4.4.2 Shoots

Soilless media

The response of *S. tortuosum* in terms of shoot mesembrine area % to soilless media was statistically significant at the value $p \leq 0.001$.

Fertigation regime

The response of *S. tortuosum* in terms of shoot mesembrine area % to fertigation regimes was statistically significant at the value $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The response of *S. tortuosum* in terms of shoot mesembrine area % to soilless media in conjunction with fertigation regimes were statistically significant at the value $p \leq 0.001$. The highest mean values (86,52 and 86,65) for shoots were found in treatments SCFR3 and SPFR3. Shoots had an overall higher concentration of mesembrine in terms of area % than roots (see Table 4.4 and Figure 4.5).

Table 4.4

The mesembrine area % of *S. tortuosum* root and shoot extracts in response to four different soilless media and five fertigation regimes in hydroponics.

Treatment		Roots - Mesembrine Area %		Shoots - Mesembrine Area %	
Soilless Medium	**Fertigation Regime**	**Mean**	**Std Err + Mean Group**	**Mean**	**Std Err + Mean Group**
SS	FR1	21,16	±0,18o	71,03	±0,14j
SS	FR2	33,05	±0,49i	65,37	±0,10l
SS	FR3	23,27	±0,23m	74,68	±0,12f
SS	FR4	36,97	±0,23h	72,00	±0,04h
SS	FR5	42,28	±0,24f	68,28	±0,13k
SC	FR1	43,47	±0,12e	79,87	±0,13c
SC	FR2	51,09	±0,09b	83,96	±0,10b
SC	FR3	48,79	±0,11c	86,52	±0,08a
SC	FR4	19,18	±0,14p	58,62	±0,10n
SC	FR5	22,42	±0,17n	64,63	±0,07m
SV	FR1	25,31	±0,16l	78,44	±0,10e
SV	FR2	17,67	±0,08q	68,53	±0,05k
SV	FR3	17,32	±0,10q	71,43	±0,10i
SV	FR4	16,45	±0,13r	56,65	±0,09o
SV	FR5	29,98	±0,18k	78,29	±0,09e
SP	FR1	46,26	±0,15d	78,93	±0,14d
SP	FR2	17,42	±0,16q	71,33	±0,15i
SP	FR3	51,83	±0,12a	86,65	±0,09a
SP	FR4	32,28	±0,12j	74,56	±0,08f
SP	FR5	37,74	±0,24g	73,19	±0,11g

Two-way ANOVA F-Statistics

Soilless Medium		7410,1***	4792***
Fertigation Regime		1461,2***	11408***
Soilless Medium*Fertigation Regime		4216,1***	4890***

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤0.001 (***) as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4= fertigation regime 4; FR5= fertigation regime 5.

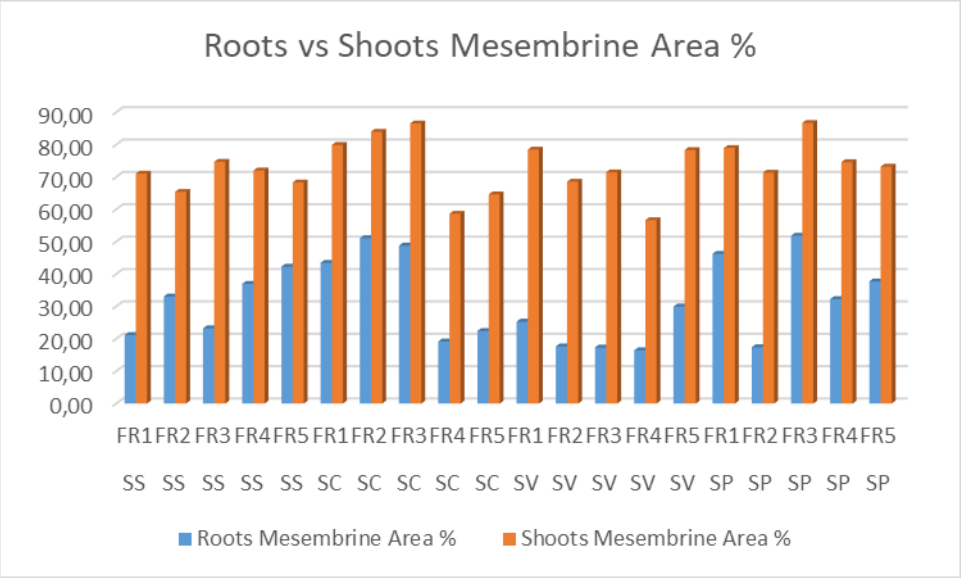

Figure 4.5

Comparing results of *S. tortuosum* root vs shoot mesembrenone concentration in terms of area % in response to 4 different soilless media and five fertigation regimes in hydroponics. Treatment SSFR3 was selected for the control.

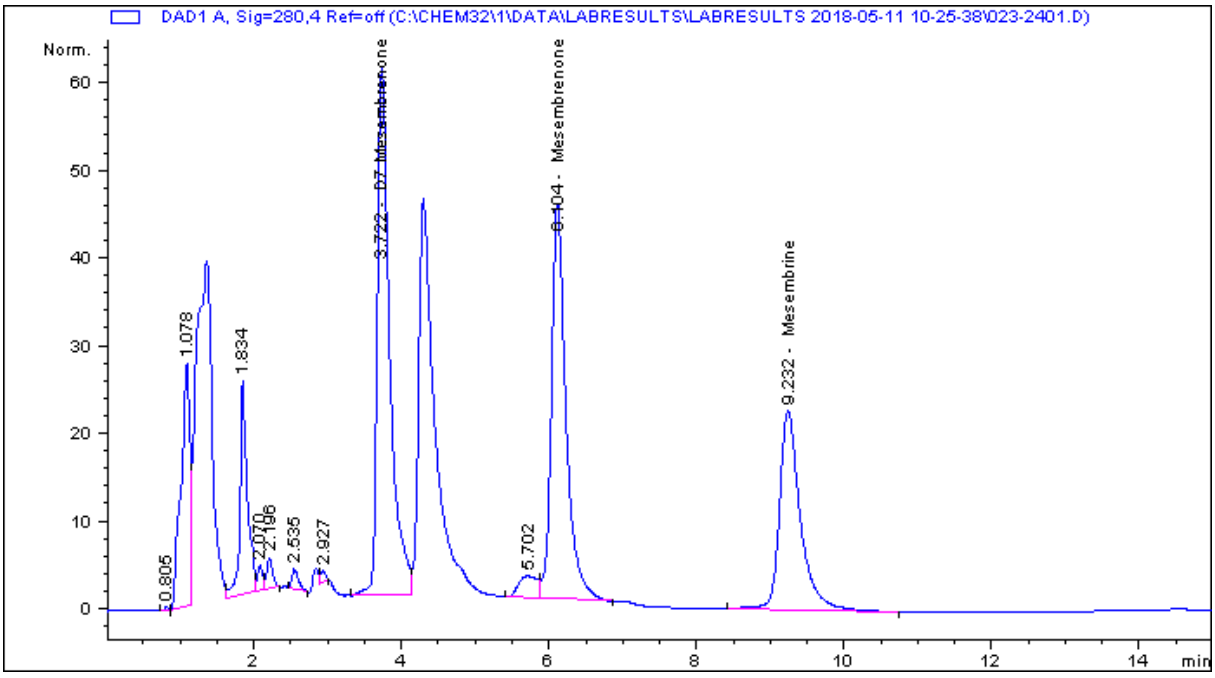

Figure 4.6

HPLC chromatograph results for treatment SSFR3 root extract displaying delta 7 mesembrenone, mesembrenone and mesembrine area %.

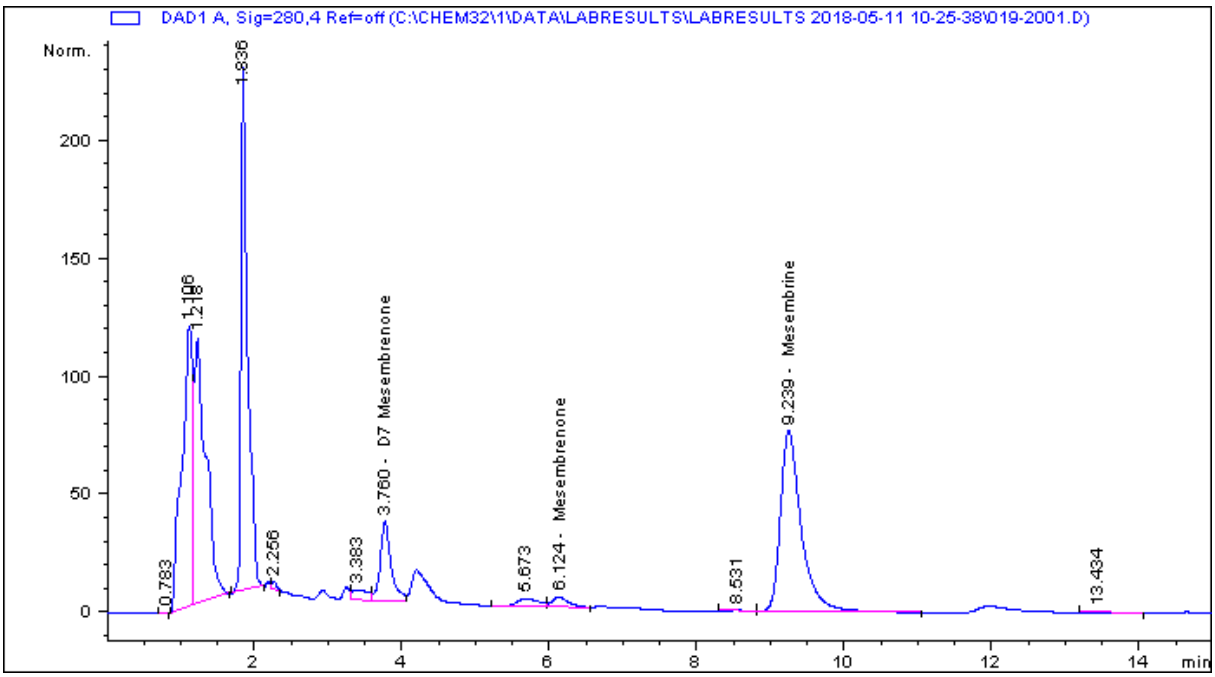

Figure 4.7

HPLC chromatograph results for treatment SPFR4 shoot extract displaying delta 7 mesembrenone, mesembrenone and mesembrine area %.

4.4.5 Mesembrine standard

4.4.5.1 Roots

Soilless media

The response of *S. tortuosum* in terms of root mesembrine concentrations to soilless media was statistically significant at the value of $p \leq 0.001$.

Fertigation regime

The response of *S. tortuosum* in terms of root mesembrine concentrations to fertigation regimes was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The response of *S. tortuosum* in terms of root mesembrine concentrations to the effects of soilless media in conjunction with fertigation regimes were statistically significant at the value of $p \leq 0.001$. For roots the highest individual mean value (1,14) was observed in treatment SCFR2. Roots overall had lower mesembrine concentrations than roots. All 20 treatments significantly differed from one another (see Table 4.5 and Figure 4.8).

4.4.5.2 Shoots

Soilless media

The response of *S. tortuosum* in terms of shoot mesembrine concentrations to soilless media was statistically significant at the value of $p \leq 0.001$.

Fertigation regime

The response of *S. tortuosum* in terms of shoot mesembrine concentrations to fertigation regimes was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The response of *S. tortuosum* in terms of shoot mesembrine concentrations to the effects of soilless media in conjunction with fertigation regimes were statistically significant at the value of $p \leq 0.001$. The highest individual mean value (4,19) was observed in treatment SCFR3. Shoots had an overall higher mesembrine concentration than roots. All 20 treatments significantly differed from one another (see Table 4.5 and Figure 4.8).

Table 4.5

The mesembrine concentration of *S. tortuosum* root and shoot extracts in response to four different soilless media and five fertigation regimes in hydroponics.

Treatment		Roots Mesembrine %		Shoots Mesembrine %	
Soilless Medium	Fertigation Regime	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group
SS	FR1	0,39	±0,002i	1,60	±0,007k
SS	FR2	0,36	±0,004j	1,67	±0,005j
SS	FR3	0,46	±0,008g	2,55	±0,004e
SS	FR4	0,54	±0,008f	1,70	±0,014j
SS	FR5	0,47	±0,007g	1,05	±0,010n
SC	FR1	1,08	±0,016b	2,27	±0,011g
SC	FR2	1,14	±0,006a	2,61	±0,014d
SC	FR3	0,82	±0,009e	4,19	±0,016a
SC	FR4	0,17	±0,002n	0,78	±0,013p
SC	FR5	0,29	±0,007l	0,95	±0,007o
SV	FR1	0,42	±0,009h	1,97	±0,005i
SV	FR2	0,24	±0,006m	1,27	±0,013m
SV	FR3	0,14	±0,004o	1,47	±0,006l
SV	FR4	0,10	±0,002p	0,59	±0,009q
SV	FR5	0,29	±0,005l	1,50	±0,012l
SP	FR1	1,02	±0,005c	2,85	±0,010c
SP	FR2	0,28	±0,002l	2,31	±0,013f
SP	FR3	0,88	±0,006d	3,72	±0,016b
SP	FR4	0,32	±0,009k	2,13	±0,013h
SP	FR5	0,38	±0,006i	1,62	±0,009k

Two-way ANOVA F-Statistics

Soilless Medium		4028.4***	10758,3***
Fertigation Regime		2557,9***	16377,2***
Soilless Medium*Fertigation Regime		1500,8***	3063,6***

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤0.001 (***) as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4= fertigation regime 4; FR5= fertigation regime 5.

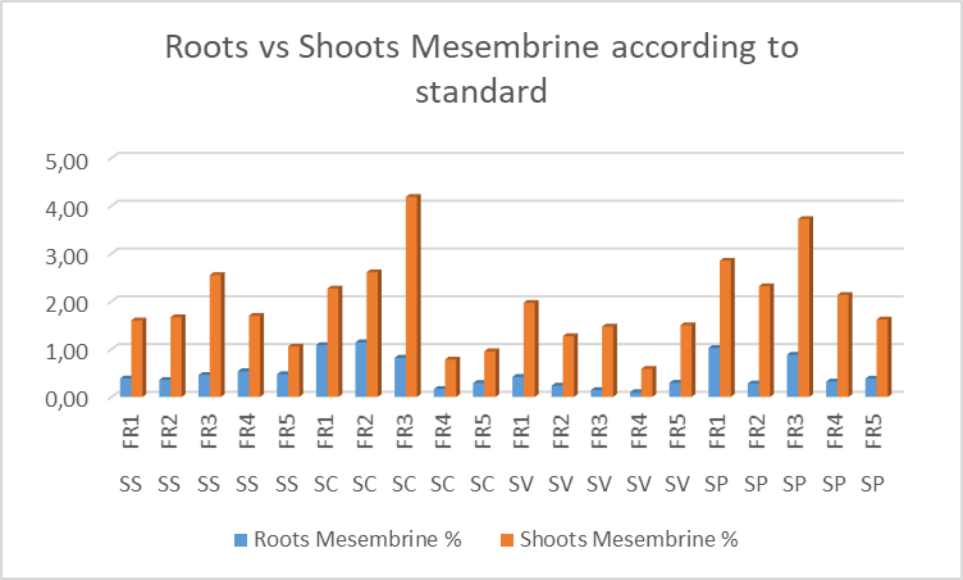

Figure 4.8

Comparing results of *S. tortuosum* root vs shoot mesembrenone concentration in terms of area % in response to 4 different soilless media and five fertigation regimes in hydroponics. Treatment SSFR3 was selected for the control.

4.5 Discussion & conclusion

Treatments applied in this investigation had a significant effect on the alkaloid concentrations *S. tortuosum.* The results obtained in this research agrees with Bourgaud *et al*. (2001) which states that plants such as *S. tortuosum* respond to biotic and abiotic stresses by controlling their influx of secondary metabolite concentrations. It was further noticed that root extracts of plants samples had more delta 7 mesembrenone and mesembrenone concentrations than shoots, while shoots had more mesembrine. Shoots had a higher amount of total alkaloids than the roots.

S. tortuosum is a one of a kind medicinal crop plant, containing various alkaloids for application in the pharmacological industry (Van Wyk & Wink, 2012; Van Wyk & Wink, 2015; Gericke & Viljoen, 2008). This study has shown there is a clear effect on alkaloid concentration in *S. tortuosum* root and shoot extracts in response to different soilless media, fertigation regimes as well as soilless media in conjunction with fertigation regimes (Choudhury & Gupta, 2002; Shukla *et al.,* 2013*)*.Shoot extracts contained a higher concentration of total alkaloids than root extracts, however root extracts had an overall higher amount of delta 7 mesembrenone, and mesembrenone in terms of area %, while shoots had higher amounts of mesembrine.

Further looking at the mesembrine standard shoots clearly had an overall higher concentration of mesembrine than the roots. These results can suggest that roots of

S. tortuosum should be harvested for the purpose of extracting delta 7 mesembrenone and mesembrenone molecules, while the shoots should be harvested for extracting mesembrine. These findings agree with Patnala & Kanfer (2013), which state that mesembrine is the major alkaloid in *S. tortuosum* along with minor alkaloids delta 7 mesembrenone and mesembrenone. Further studies would be needed to test how other methods of fertigation, various organic/soilless media and a combination of both would affect the concentration yields of mesembrine-type alkaloids when cultivating *S. tortuosum* in soil or hydroponics. More research is also required on how fermentation of *S. tortuosum* plant material affects alkaloid concentrations.

4.6 Acknowledgements

This study was funded by Cape Peninsula University of Technology (CPUT), through the National Research Foundation and University Research Fund.

Richard Davies, Managing Director of Verve Dynamics (Pty) Ltd who supplied the initial *Sceletium tortuosum* mother plant for this study and gave special permission for the use of their mesembrine standard.

4.7 References

Bennett, A.C., Van Camo, A., Lopez, L. & Smith, C. 2018. *Sceletium tortuosum* may delay chronic disease progression via alkaloid-dependent antioxidant or anti-inflammatory action. *Journal of Physiology and Biochemistry*, 74(4): 1–9.

Bourgaud, F., Gravot, A., Milesi, S. & Gontier, E. 2001. Production of plant secondary metabolites: A historical perspective. *Plant Science*, 161(5): 839–851.

Coetzee, D.D., Lopez, V. & Smith, C. 2015. High-Mesembrine *Sceletium* extract (TrimesimineTM) is a monoamine releasing agent, rather than only a selective serotonin reuptake inhibitor. *Journal of Ethnopharmacology*, 177: 111–116.

Choudhury, S., Gupta, K. 2002. Influence of seasons on biomass and alkaloid productivity in *Catharanthus roseus. Journal of Medicinal and Aromatic Plant Sciences*, 24: 664-668.

Czapek, F. 1921. Spezielle Biochemie. *Biochemie der Pflanzen*, 3: 369.

Fadhil, S., Reza, M.H., Rouhollah, G. & Reza, V.R.M. 2007. Spectrophotometric Determination of Total Alkaloids in Peganum harmala L. Using Bromocresol Green. *Research Journal of Phytochemistry*, 1(2): 79–82.

Gericke, N. & Viljoen, a. M. 2008. *Sceletium*-A review update. *Journal of Ethnopharmacology*, 119(3): 653–663.

Harvey, A.L., Young, L.C., Viljoen, A.M. & Gericke, N.P. 2011. Pharmacological actions of the South African medicinal and functional food plant *Sceletium tortuosum* and its principal alkaloids. *Journal of Ethnopharmacology*, 137(3): 1124–1129.

Karuppusamy, S. 2009. A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures. *Journal of Medicinal Plants Research*, 3(13): 1222–1239.

Krstenansky, J. 2016. Mesembrine alkaloids: Review of their occurrence, chemistry and pharmacology. *Journal of Ethnopharmacology*, 195: 10–19.

Li, J., Ou-Lee, T.M., Raba, R., Amundson, R.G. & Last, R.L. 1993. Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. *Plant Cell*, 5(2): 171–179.

Patnala, S. & Kanfer, I. 2010. HPLC analysis of mesembrine-type alkaloids in *Sceletium* plant material used as an african traditional medicine. *Journal of Pharmacy and Pharmaceutical Sciences*, 13(4): 558–570.

Patnala, S. & Kanfer, I. 2013. Chemotaxonomic studies of mesembrine-type alkaloids in Sceletium plant species. *South African Journal of Science*, 109(3–4): 5–9.

Philipson, J.D. 1990. *Plants as source of valuable products. In: Secondary products from plant tissue culture.* B. V. Chalwood & M. J. Rhodes, eds. Oxford: Clarendon Press.

Schultes, R.E., Hofmann, A., Rätsch, C. 2001. *Plants of the Gods: Their Sacred, Healing, and Hallucinogenic Powers*. 2nd ed. Lucerne: EMB-service for Publishers.

Shukla, A.K., Mall, M., Rai, S.K., Singh, S., Nair, P., Parashar, G., Shasany, A.K., Singh, S.C., Joshi, V.K. & Khanuja, S.P.S. 2013. A transcriptomic approach for exploring the molecular basis for dosha-balancing property-based classification of plants in Ayurveda. *Molecular Biology Reports*, 40: 3255-3262.

Smith, M.T., Crouch, N.R., Gericke, N. & Hirst, M. 1996. Psychoactive constituents of the genus *Sceletium* N.E.Br. and other Mesembryanthemaceae: A review. *Journal of Ethnopharmacology*, 50(3): 119–130.

Steel, R.G.D. & Torrie, J.H. 1980. *Principle and procedures of statistics: a biometrical approach*. *2nd ed*. New York: McGraw-Hill.

Van Wyk, B. & Wink, M. 2012. *Medicinal Plants of the World*. 5th ed. Pretoria: Briza.

Van Wyk, B.-E. & Wink, M. 2015. *Phytomedicines, Herbal Drugs, and Plant Poisons*. 1st ed. Pretoria: Briza Publications.

Wink, M., Alfermann, A.W., Franke, R., B, W., Distl, M., Windhovel, J., Krohn, O., Fuss, E., Garden, H Mohagheghzaden, A., Wildi, E. & Ripplinger, P. 2005. Sustainable bioproduction of phytochemicals by plant in vitro cultures: anticancer agents. *Plant Genetic Resource*, 3: 90–100.

CHAPTER FIVE:

THE INTERACTION BETWEEN VEGETATIVE GROWTH AND ALKALOID CONCENTRATION OF *S. TORTUOSUM* IN RESPONSE TO DIFFERENT SOILLESS GROWING MEDIA AND FERTIGATION REGIMES IN HYDROPONICS

THE INTERACTION BETWEEN VEGETATIVE GROWTH AND ALKALOID CONCENTRATION OF *S. TORTUOSUM* IN RESPONSE TO DIFFERENT SOILLESS GROWING MEDIA AND FERTIGATION REGIMES IN HYDROPONICS

RJ Faber, CP Laubscher*, JC Coetzee, F Rautenbach

Department of Horticultural Sciences, Faculty of Applied Sciences, Cape Peninsula University of Technology, PO Box 1906, Bellville, 7535, South Africa.

*Email: laubscherc@cput.ac.za

5.1 Abstract

Sceletium tortuosum (L.) N.E. Br is a succulent medicinal crop plant in the family Mesembryanthemaceae, it was formerly known as *Mesembryanthemum tortuosum*. The plant is indigenous to South Africa where is it well known and utilized for its medicinal and psycho-active properties. Interest in *S. tortuosum* has been growing as mesembrine-type alkaloids found in the plant has tremendous potential as an alternative supplement for promoting health and treating a variety of psychological and psychiatric disorders. The purpose for this study was to investigate whether *S. tortuosum* was first of all suitable for hydroponic cultivation, as well as to test how the plant would respond to different soilless media and fertigation regimes in terms of alkaloid concentrations.

Three hundred plants were cultivated from one initial mother plant obtained from Verve Dynamics (Pty) Ltd. Twenty treatments were evaluated with 15 sample replicates. Treatments were made up of 4 different soilless growing media, namely: pure silica sand, 50% silica sand with 50% coco-peat, 50% silica sand with 50% vermiculite, and 50% silica sand with 50% perlite. These growing media were tested in conjunction with 5 different fertigation regimes (FR), plants grown in FR1 received aqueous nutrient solution once every week, FR2 received aqueous nutrient solution once every second week, FR3 received aqueous nutrient solution once every third week, FR4 received aqueous nutrient solution once every fourth week and FR5 received aqueous nutrient solution once every fifth week respectively.

Results from this experiment showed that different soilless growing media and fertigation regimes as well as soilless media in conjunction with fertigation regimes had varying effects on *S. tortuosum* and the plants vegetative growth and alkaloid concentrations. It was observed that more optimal vegetative growth was not necessarily desirable in terms of alkaloid concentration, which suggests that reasonable amounts of stress could increase alkaloid concentrations in the plant.

Keywords: alkaloids, delta 7 mesembrenone, fertigation regimes, hydroponics, mesembrenone, mesembrine, soilless media, vegetative growth

Abbreviations: fertigation regime (FR), silica sand (SS), silica sand and coco-peat (SC), silica sand and vermiculite (SV), silica sand and perlite (SP)

5.2 Introduction

Relevant natural compounds, mainly secondary metabolite concentration and composition, determines the quality of medicinal plants. Water availability, light intensity, and temperature are examples of various environmental conditions which affect the quality and quantity of secondary metabolites (Kleinwächter *et al*., 2014).

Any method of growing plants without the use of soil is known as soilless plant cultivation (Savvas *et al*., 2013). Soilless plant cultivation can produce higher yields and allow for more frequent harvests, making it more cost-effective in comparison to soil-based cultivation (Grafiadellis *et al*., 2000; Nejad & Ismaili, 2014).

Depending on the plant and the growers desired outcome with regards to quality, quantity, and rate of growth, the amount of water applied should be carefully considered as there is no prevalent recommendation that can be made for all plants to increase plant quality, quantity, and rate of growth by deliberately applying drought-stress without first thoroughly investigating how different plants react to different amounts of water and means of application (Kleinwächter & Selmar, 2014).

The key driver for the development of soilless growing media is the inability of soil to provide such a balance in minimal volumes. Creation and alteration of soilless media has allowed for the guarded control of water, air and nutrients to root systems while also excluding soil borne pathogens (Raviv *et al*., 2002).

The challenge lies therefore in finding the right medium to nutrient combination for different plant species in order to achieve optimal vegetative growth (Brooking, 1976; Jensen, 2017).

Investigating the effect different soilless growth media and fertigation regimes have on the vegetative growth, alkaloid production, and nutrient uptake of *S. tortuosum* will contribute to developing optimal growing protocols for cultivating high quality medicinal plants of *S. tortuosum* in hydroponics for the ethno-pharmaceutical industry.

The aim of this study was to assist in determining an optimal protocol for the vegetative growth and alkaloid concentrations of *S. tortuosum* in order to produce consistent high yields as well as good quality medicinal plants in hydroponics.

5.3 Materials and methods

5.3.1 Greenhouse Experiment

This investigation was conducted in the research greenhouse facility at the Cape Peninsula University of Technology, Bellville, Cape Town, South Africa; GPS co-ordinates - 33º 55'45.53S, 18º 38' 31. 16E. The nature of the structure and the technology installed ensured control of the environment within the greenhouse.

5.3.2 Plant Preparation

One *Sceletium tortuosum* mother plant was obtained from Verve Dynamics, Somerset West. An additional ten mother plants were propagated and cultivated from this plant. Once these ten plants were large enough, ± 600 2.5-3 cm stem cuttings were propagated from the mother plants to ensure a supply of more than 300 rooted cuttings, which was the number of plant material required for the experiment.

Cuttings were treated with Dynaroot™ No 1 (Active ingredient: 0.1% I.B.A.) as rooting hormone and planted into 200 and 128 cell styrofoam trays containing a 50/50 mixture of silica sand, and coco-peat as rooting medium. The trays were placed in an environmentally controlled propagation greenhouse at the Cape Peninsula University of Technology, Bellville Campus. The first sign of roots was observed after 2 weeks, and roots had fully developed after 2 months. After rooting, trays were moved to the research greenhouse for acclimatization and watering was administered by hand. The best quality rooted cuttings in the batch were selected for the experiment.

5.3.3 Hydroponic experiment

Five identical hydroponic systems were constructed. Each system consisted of one 70 litre capacity low-density polyethylene (LDPE) reservoir; four polyvinyl chloride (PVC) square gutters (130 mm x 70 mm x 2 500 mm) secured onto wire mesh tables (900 mm x 1 250 m x 2 500 m); sixty 12,5 x 12,5 cm plastic pots, a 2 000 ℓ/h submersible water pump with a 2,5 m head capacity, ten-meter x 20 mm LDPE irrigation piping; four reducers (20 mm–15 mm); four 15 mm flow regulators; one air

pump, one air stone (15 cm) and eight 20 mm bulk head connectors. Reservoirs were kept filled to a level of approximately 50 ℓ with aqueous nutrient solution. Each gutter was sealed with PVC adhesive and fitted with an inlet guiding water towards the gutters and an outlet to return excess water to the reservoir. Each inlet consisted of LDPE irrigation piping converted from 20 mm to 15 mm diameter and each fitted with a 15mm valve allowing maximum control of aqueous nutrient solution flow into gutters. All gutters were filled with silica sand to a depth of ± 1,5 cm. Fifteen pots (12,5 cm x 12,5 cm) were placed into each gutter on top of the silica sand. As water passes through the gutter the sand becomes fully saturated with aqueous nutrient solution which is then imbibed by soilless growing media in pots via capillary action. Every gutter for each system represented a different soilless substrate in 15 pots, as follows: 100% silica sand (SS); a 50/50 mixture of silica sand and coco-peat (SC); a 50/50 mixture of silica sand and vermiculite (SV) and a 50/50 mixture of silica sand and perlite (SP).

Each separate system was manually controlled to fertigate plants with the same amount of aqueous nutrient solution at different time intervals.

The five systems were given aqueous nutrient solution amounts as follows: Fertigation regime 1 (FR1) was fertigated once a week at ± 350 ml per minute for 1 hour. Fertigation regime 2 (FR2) was fertigated every second week at ± 350 ml per minute for 1 hour. Fertigation regime 3 (FR3) was fertigated once every third week at ± 350 ml per minute for 1 hour. Fertigation regime 4 (FR4) was fertigated once every fourth week at ± 350 ml per minute for 1 hour. Fertigation regime 5 (FR5) was fertigated once every fifth week at ± 350 ml per minute for 1 hour. Fifteen plants, one per pot were placed into each soilless substrate of each system, which totals to 60 plants per hydroponic system and 300 plants for the entire experiment. Therefore 15 replicates in a randomized blocked design were tested for each media and fertigation regime combination.

All aqueous nutrient solutions used in the experiment were kept at a pH level of 6.0, and an electrical conductivity (EC) level of 0.5. EC levels of the aqueous nutrient solutions were monitored with a calibrated hand held digital EC meter (Hanna Instruments®™ HI 98312). Water reservoir pH levels were monitored with a calibrated handheld digital pH meter (Eurotech®™ pH 2 pen). For increasing pH potassium hydroxide was used, while phosphoric acid was used for decreasing pH of aqueous nutrient solutions. For decreasing the EC of aqueous nutrient solutions, reverse osmosis water was added into reservoirs, while adding Hoagland Solution to

aqueous nutrient solutions in reservoirs increased the EC. Pots were individually numbered and arranged randomly.

All gutters were slightly tilted to allow aqueous nutrient solutions to flow from one end (inlet) to the other (outlet). Each outlet was guided to a separate smaller gutter which then returned the aqueous nutrient solution back to the reservoir, creating a circulating system.

5.3.4 Treatment preparation

Soilless medium treatments were prepared using four different soilless medium combinations, namely: 100% silica sand (SS); a 50/50 mixture of silica sand and coco-peat (SC); a 50/50 mixture of silica sand and vermiculite (SV) and a 50/50 mixture of silica sand and perlite (SP). For treatments SC, SV and SP equal parts silica sand and coco-peat/vermiculite/perlite was mixed together. All silica sand used was thoroughly rinsed with tap water until water poured through the sand ran clear.

Fertigation regimes were achieved by installing identical pumps to all hydroponic systems. The amount of aqueous nutrient solution delivered to each grow bed was controlled by adjusting all output valves until ±350 ml per minute was measured. Measurement was achieved by placing an empty bucket below each output for 1 minute, the liquid captured in the bucket was decanted and measured using a 500 ml Erlenmeyer flask until the amount of aqueous nutrient solution for each output was measured at ±350 ml for 1 minute.

Treatment SSFR3 was selected for the control, as it contained 100% silica sand, which was the base of all soilless mediums used in this experiment as well as a mid-frequent fertigation regime.

5.3.5 Treatment Application

Treatments applied consisted of 4 different soilless growth media and 5 different fertigation regimes in a circulating capillary action hydroponic system. The control consisted of treatment SSFR3 containing 100% silica sand growing media and a mid-frequent fertigation regime. Each treatment was numbered, as follows:

SSFR1 - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once a week.

SCFR1 – 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once a week.

SVFR1 – 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once a week.

SPFR1 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once a week.

SSFR2 - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every second week.

SCFR2 - 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every second week.

SVFR2 - 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every second week.

SPFR2 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every second week.

SSFR3 (control) - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every third week.

SCFR3 - 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every third week.

SVFR3 - 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every third week.

SPFR3 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every third week.

SSFR4 - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fourth week.

SCFR4 - 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fourth week.

SVFR4 - 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fourth week.

SPFR4 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fourth week.

SSFR5 - 100% Silica sand growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fifth week.

SCFR5 - 50% Silica sand + 50% coco-peat growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fifth week.

SVFR5 - 50% Silica sand + 50% vermiculite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fifth week.

SPFR5 - 50% Silica sand + 50% perlite growth media combined with a fertigation regime of ±350 ml aqueous nutrient solution per minute for 1 hour once every fifth week.

5.3.6 Data collection

5.3.6.1 Root & shoot growth

Three hundred *S. tortuosum* specimens were grown hydroponically in an environmentally controlled green house for 7 weeks. The treatments were made up of 4 soilless mediums and 5 different fertigation regime combinations, with 15 sample replicates for all 20 treatments. Root and shoot lengths were measured prior to transplanting into the hydroponic systems and again at the end of the experiment. Measurements were done in millimetres using a standard ruler and recorded.

5.3.6.2 Dry weight

Plants were placed in brown paper bags and dried at 30-31 °C inside a forced convection oven (Daihan Labtech LDO-150F) until the material was crisp dry. Plants were then individually weighed in grams using an electronic scale (RADWAG® Model PS 750.R2) and recorded.

5.3.6.3 Drying and milling of plant material

Plants were placed in brown paper bags and dried at 30-31°C inside a forced convection oven (Daihan Labtech LDO-150F) until the material was crisp dry. Plants were then individually weighed in grams using an electronic scale (RADWAG® Model PS 750.R2) and recorded. Dried plants for each treatment were placed together and milled to powder using a standard coffee grinder (Mellerware - Aromatic Coffee Mill & Grinder, 50g capacity, wattage: 120W, Voltage: 220 -230V), and placed in a transparent, zip-lock plastic bag. The coffee grinder was cleaned thoroughly between the milling of each treatments' plant material.

5.3.6.4 Measuring total alkaloids.

Total alkaloid content for roots and shoots were measured using the bromo cresol green method based on the atropine standard (Fadhil *et al*., 2007). For every treatment, 5 extractions were made. Each extraction was done by placing 100 mg of plant material in 10 ml of methanol within a 15 ml tube for 24 hours. Tubes were placed in a centrifuge at 4000 rpm for 2 minutes (Eppendorf Centrifuge 5810 R). 1 ml of methanol extract was transferred to a 50 ml tube. 5 ml of buffering solution (pH 4.7), 12 ml of bromo cresol green and 12 ml of chloroform was added to the methanol extract. The chloroform separates from the bromo cresol green and buffer, if a yellow colour is observed it means alkaloids are present in the extract. The yellow chloroform liquid was then placed in a 96 cell well plate, 3 cells per extraction at 300 µl and placed in a multi scan spectrum reader. Extractions were observed at 417 nm, the wavelength for the colour yellow, and analysed with the software program SkanIt™ which captures the amount of yellow pigments in a sample, hence the amount of alkaloids (Fadhil *et al*., 2007). This analysis does not specify which alkaloids are present, but rather just the total amount of alkaloids in a sample.

5.3.6.5 Measuring Delta 7 mesembrenone, mesembrenone and mesembrine area %

The most commonly used technique for analysing natural products is high performance liquid chromatography (HPLC) with ultra violet light detection. Due to the

commercial unavailability of *S. tortuosum* alkaloid standards the HPLC test for the area % of delta 7 mesembrenone, mesembrenone and mesembrine was based on standard references for mesembrine-type alkaloids as seen by Patnala and Kanfer (2010). The liquid or mobile phase used for *S. tortuosum* is water plus acentronitile at a ratio of 72:28 plus 0.01% ammonia hydroxide. 20 µl of extractions were filtered into HPLC vials, the vials were then numerically placed in the sample collector according to treatment. From here the mobile phase and extract samples individually got injected into a column (C18 – kromasil 100-5-C18) at a flow rate of 1 µl/min, which separated the molecules and passed them through a UV detector and UV signatures at 280nm were recorded and in turn determined the area % of the different alkaloids. Procedures were done at room temperature and repeated 5 times for each treatments extraction sample.

5.3.6.6 Measuring mesembrine

The pure mesembrine compound was injected into the HPLC system by itself, the peak observed as a result on the chromatograph represents mesembrine, which then draws a standard reference for other molecules to compare to, therefore if another molecule is injected and analysed in the same way and results are in the same peak on the chromatograph, they are the same molecule as the standard. The HPLC method with UV detection was again used for this analysis. 20 µl of extractions were filtered into HPLC vials, the vials were then numerically placed in the sample collector according to treatment. From here the mobile phase and extract samples individually got injected into a column (C18 – kromasil 100-5-C18) at a flow rate of 1 µl/min, which separated the molecules and passed them through a UV detector and UV signatures at 280 nm, molecules that were identical to the mesembrine standard were recorded. Procedures were done at room temperature and repeated 5 times for each treatments extraction sample (Patnala & Kanfer, 2010).

5.3.7 Statistical analysis

All data collected was statistically analysed using two-way analysis of variance (ANOVA). Treatment means were compared using Fischer's Least Significant Difference (L.S.D) at values of $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$, with ns being not significant. The software program STATISTICA version 13 was used to perform all calculations on Windows 10 (Steel & Torrie, 1980).

5.4 RESULTS

5.4.1 Vegetative growth and total alkaloid concentration of *S. tortuosum* in response to four different soilless media and five different fertigation regimes in hydroponics

5.4.1.1 Roots

Soilless media

The effects of soilless media on root growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The effects of soilless media on root total alkaloid concentrations of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on root growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The effects of fertigation regimes on root total alkaloid concentrations of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on root growth of *S. tortuosum* were found to be statistically significant at the value of $p \leq 0.01$. The highest individual mean value (197,93 mm) for root growth was observed in treatment SCFR3. Effects of soilless media in conjunction with fertigation regimes on shoot total alkaloid concentrations of *S. tortuosum* were found to be statistically significant at the value of $p \leq 0.001$. Treatments SSFR3, SPFR2 and SPFR3 resulted in the highest mean values (2,30; 2,33; 2,37). Shoots were found to have an overall higher concentration of total alkaloids than the roots (see Table 5.1).

5.4.1.2 Shoots

Soilless media

The effects of soilless media on shoot growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The effects of soilless media on shoot total alkaloid concentrations of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on shoot growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The effects of fertigation regimes on shoot total alkaloid concentrations of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on shoot growth of *S. tortuosum* were found to be not significant. The highest individual mean value (27,07 mm) for shoot growth was observed in treatment SVFR1. Effects of soilless media in conjunction with fertigation regimes on shoot total alkaloid concentrations of *S. tortuosum* were found to be statistically significant at the value of $p \leq 0.001$. Treatments SSFR3, SPFR2 and SPFR3 resulted in the highest mean values (2,30; 2,33; 2,37). Shoots were found to have an overall higher concentration of total alkaloids than the roots (see Table 5.1).

Table 5.1

The results of root and shoot vegetative growth and totoal alkaloids of *S. tortuosum*.

Treatment		Root Growth (mm)		Shoot Growth (mm)		Roots - Total Alkaloids		Shoots - Total Alkaloids	
Soilless Medium	Fertigation Regime	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group
SS	FR1	41,07	±6,82i	15,33	±1,95cde	1,25	±0,06de	1,71	±0,07cd
SS	FR2	62,27	±13,17ghi	10,20	±1,79efg	1,51	±0,03b	1,86	±0,04bcd
SS	FR3	77,20	±13,64fgh	8,27	±1,20ghi	1,51	±0,04b	2,30	±0,05a
SS	FR4	53,80	±13,36hi	9,40	±6,65fgh	1,25	±0,04de	1,68	±0,11d
SS	FR5	58,13	±13,15hi	3,47	±0,83i	1,34	±0,06cd	1,79	±0,08bcd
SC	FR1	114,20	±14,96def	21,13	±2,19abc	1,25	±0,05de	1,78	±0,11bcd
SC	FR2	109,40	±16,26def	19,67	±3,02bc	1,31	±0,03cde	1,77	±0,06bcd
SC	FR3	197,93	±25,26a	22,87	±2,04ab	0,87	±0,06hi	1,89	±0,06bc
SC	FR4	150,67	±22,54bcd	12,80	±0,96def	0,97	±0,03gh	1,31	±0,09e
SC	FR5	67,47	±10,80fgh	10,00	±1,44efg	1,45	±0,10bc	1,47	±0,03e
SV	FR1	91,87	±14,16efg	27,07	±2,31a	1,04	±0,04fg	1,37	±0,05e
SV	FR2	135,47	±19,18cde	16,47	±1,60bcde	0,94	±0,01gh	1,35	±0,07e
SV	FR3	186,93	±18,55ab	16,27	±2,63cde	0,71	±0,04j	1,48	±0,08e
SV	FR4	171,40	±27,09abc	12,40	±1,33efg	0,74	±0,02ij	1,07	±0,05f
SV	FR5	54,53	±20,36hi	9,40	±1,99fgh	1,28	±0,08de	1,39	±0,04e
SP	FR1	89,33	±13,12efg	19,27	±2,10bcd	1,17	±0,07ef	1,97	±0,07b
SP	FR2	111,73	±14,58def	12,40	±2,09efg	1,18	±0,01ef	2,33	±0,11a
SP	FR3	95,20	±13,45efg	10,60	±1,49efg	1,30	±0,02de	2,37	±0,04a
SP	FR4	109,67	±22,40def	6,47	±0,97ghi	1,68	±0,08a	1,93	±0,09b
SP	FR5	33,00	±10,22i	4,47	±1,36hi	1,34	±0,07cd	1,83	±0,02bcd

Two-way ANOVA F-Statistics

Soilless Medium	20,0***	14,7***	67,7***	101,2***
Fertigation Regime	15,5***	20,1***	13,3***	29,7***
Soilless Medium*Fertigation Regime	2,5**	1,1ns	15,9***	3,4***

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤ 0.01(**), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4 = fertigation regime 4; FR5 = fertigation regime 5.

5.4.2 Vegetative growth and delta 7 mesembrenone area % of *S. tortuosum* in response to four different soilless media and five different fertigation regimes in hydroponics.

5.4.2.1 Roots

Soilless media

The effects of soilless media on root growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of root delta 7 mesembrenone area % to soilless media was statistically significant at the value $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on root growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of root delta 7 mesembrenone area % to fertigation regimes was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on root growth of *S. tortuosum* were found to be statistically significant at the value of $p \leq 0.01$. The response of *S. tortuosum* in terms of root delta 7 mesembrenone area % to soilless media in conjunction with fertigation regimes were found to be statistically significant at the value $p \leq 0.001$. The highest individual mean value (52,37) for root delta 7 mesembrenone area % was found in treatment SVFR4. All 20 treatments significantly differed from one another. Roots had an overall higher concentration of delta 7 mesembrenone in terms of area % than shoots (see Table 5.2).

5.4.2.2 Shoots

Soilless media

The effects of soilless media on shoot growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of shoot delta 7 mesembrenone area % to soilless media was statistically significant at the value $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on shoot growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of shoot delta 7 mesembrenone area % to fertigation regimes was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on shoot growth of *S. tortuosum* were found to be not significant. The highest individual mean value (27,07 mm) for shoot growth was observed in treatment SVFR1. The response of *S. tortuosum* in terms of shoot delta 7 mesembrenone area % to soilless media in conjunction with fertigation regimes were found to be statistically significant at the value $p \leq 0.001$. Treatment SVFR4 had the highest individual mean value (37,23) for shoots. All 20 treatments significantly differed from one another. Shoots had an overall lower concentration of delta 7 mesembrenone in terms of area % than roots (see Table 5.2).

Table 5.2
The results of root and shoot vegetative growth and delta 7 mesembrenone area % of *S. tortuosum*.

Treatment		Root Growth (mm)		Shoot Growth (mm)		Roots delta 7 mesembrenone area %		Shoots delta 7 mesembrenone area %	
Soilless Medium	Fertigation Regime	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group
SS	FR1	41,07	±6,82i	15,33	±1,95cde	45,14	±0,17f	16,62	±0,08m
SS	FR2	62,27	±13,17ghi	10,20	±1,79efg	46,44	±0,11e	23,29	±0,09f
SS	FR3	77,20	±13,64fgh	8,27	±1,20ghi	46,81	±0,08d	19,21	±0,07j
SS	FR4	53,80	±13,36hi	9,40	±6,65fgh	37,88	±0,14k	20,47	±0,06h
SS	FR5	58,13	±13,15hi	3,47	±0,83i	36,57	±0,14l	25,14	±0,06e
SC	FR1	114,20	±14,96def	21,13	±2,19abc	30,69	±0,09o	15,49	±0,09n
SC	FR2	109,40	±16,26def	19,67	±3,02bc	26,35	±0,07q	12,85	±0,06p
SC	FR3	197,93	±25,26a	22,87	±2,04ab	28,04	±0,06p	11,47	±0,08q
SC	FR4	150,67	±22,54bcd	12,80	±0,96def	47,42	±0,11c	36,30	±0,03b
SC	FR5	67,47	±10,80fgh	10,00	±1,44efg	39,42	±0,08j	30,78	±0,05c
SV	FR1	91,87	±14,16efg	27,07	±2,31a	40,08	±0,11i	18,44	±0,09k
SV	FR2	135,47	±19,18cde	16,47	±1,60bcd	46,95	±0,09d	25,89	±0,04d
SV	FR3	186,93	±18,55ab	16,27	±2,63cde	50,26	±0,13b	25,75	±0,07d
SV	FR4	171,40	±27,09abc	12,40	±1,33efg	52,37	±0,10a	37,23	±0,04a
SV	FR5	54,53	±20,36hi	9,40	±1,99fgh	34,94	±0,07m	17,86	±0,06l
SP	FR1	89,33	±13,12efg	19,27	±2,10bcd	30,78	±0,07o	14,50	±0,10o
SP	FR2	111,73	±14,58def	12,40	±2,09efg	44,26	±0,09g	20,29	±0,06h
SP	FR3	95,20	±13,45efg	10,60	±1,49efg	30,62	±0,09o	10,85	±0,04r
SP	FR4	109,67	±22,40def	6,47	±0,97ghi	41,62	±0,12h	19,42	±0,06i
SP	FR5	33,00	±10,22i	4,47	±1,36hi	31,79	±0,17n	22,39	±0,04g

Two-way ANOVA F-Statistics

Soilless Medium	20,0***	14,7***	10872***	10950***
Fertigation Regime	15,5***	20,1***	4440***	23755***
Soilless Medium*Fertigation Regime	2,5**	1,1ns	4031***	9247***

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤ 0.01(**), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4 = fertigation regime 4; FR5 = fertigation regime 5.

5.4.3 Vegetative growth and mesembrenone area % of *S. tortuosum* in response to four different soilless media and five different fertigation regimes in hydroponics.

5.4.3.1 Roots

Soilless media

The effects of soilless media on root growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of root mesembrenone area % to soilless media was statistically significant at the value $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on root growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of root mesembrenone area % to fertigation regimes was statistically significant at the value $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on root growth of *S. tortuosum* were found to be statistically significant at the value of $p \leq 0.01$. The response of *S. tortuosum* in terms of root mesembrenone area % to soilless media in conjunction with fertigation regimes was statistically significant at the value $p \leq 0.001$. The highest mean values (38,16 and 38,32) for roots were found in treatments SCFR5 and SPFR2. Roots had an overall higher concentration of mesembrenone in terms of area % than shoots (see Table 5.3).

5.4.3.2 Shoots

Soilless media

The effects of soilless media on shoot growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of shoot mesembrenone area % to soilless media was statistically significant at the value $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on shoot growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of shoot mesembrenone area % to fertigation regimes was statistically significant at the value $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on shoot growth of *S. tortuosum* were found to be not significant. The highest individual mean value (27,07 mm) for shoot growth was observed in treatment SVFR1. The response of *S. tortuosum* in terms of shoot mesembrenone area % to soilless media in conjunction with fertigation regimes were statistically significant at the value $p \leq 0.001$(Table 4.3). The highest individual mean value (12,35) for shoots were found in treatment SSFR1. Shoots had an overall lower concentration of mesembrenone in terms of area % than roots (see Table 5.3).

Table 5.3

The results of root and shoot vegetative growth and mesembrenone area % of *S. tortuosum*.

Treatment		Root Growth (mm)		Shoot Growth (mm)		Roots - Mesembrenone Area %		Shoots - Mesembrenone Area %	
Soilless Medium	Fertigation Regime	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group
SS	FR1	41,07	±6,82i	15,33	±1,95cde	33,70	±0,08d	12,35	±0,09a
SS	FR2	62,27	±13,17ghi	10,20	±1,79efg	19,91	±0,15n	11,35	±0,09b
SS	FR3	77,20	±13,64fgh	8,27	±1,20ghi	29,91	±0,16h	6,10	±0,08f
SS	FR4	53,80	±13,36hi	9,40	±6,65fgh	25,15	±0,10j	7,52	±0,08d
SS	FR5	58,13	±13,15hi	3,47	±0,83i	21,15	±0,15m	6,59	±0,08e
SC	FR1	114,20	±14,96def	21,13	±2,19abc	25,84	±0,11i	4,63	±0,07i
SC	FR2	109,40	±16,26def	19,67	±3,02bc	22,55	±0,10l	3,18	±0,04l
SC	FR3	197,93	±25,26a	22,87	±2,04ab	23,17	±0,09k	2,02	±0,05o
SC	FR4	150,67	±22,54bcd	12,80	±0,96def	33,40	±0,09d	5,08	±0,07h
SC	FR5	67,47	±10,80fgh	10,00	±1,44efg	38,16	±0,18a	4,59	±0,05ij
SV	FR1	91,87	±14,16efg	27,07	±2,31a	34,61	±0,06c	3,12	±0,05l
SV	FR2	135,47	±19,18cde	16,47	±1,60bcd	35,38	±0,15b	5,57	±0,08g
SV	FR3	186,93	±18,55ab	16,27	±2,63cde	32,42	±0,12e	2,83	±0,05m
SV	FR4	171,40	±27,09abc	12,40	±1,33efg	31,18	±0,18f	6,11	±0,07f
SV	FR5	54,53	±20,36hi	9,40	±1,99fgh	35,08	±0,17b	3,85	±0,05k
SP	FR1	89,33	±13,12efg	19,27	±2,10bcd	22,96	±0,11k	6,57	±0,07e
SP	FR2	111,73	±14,58def	12,40	±2,09efg	38,32	±0,16a	8,37	±0,10c
SP	FR3	95,20	±13,45efg	10,60	±1,49efg	17,56	±0,08o	2,49	±0,06n
SP	FR4	109,67	±22,40def	6,47	±0,97ghi	26,10	±0,09i	6,02	±0,06f
SP	FR5	33,00	±10,22i	4,47	±1,36hi	30,48	±0,12g	4,41	±0,08j

Two-way ANOVA F-Statistics

Soilless Medium	20,0***	14,7***	3640***	5050,8***
Fertigation Regime	15,5***	20,1***	947***	1925,4***
Soilless Medium*Fertigation Regime	2,5**	1,1ns	2691***	524,2***

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤ 0.01(**), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4 = fertigation regime 4; FR5 = fertigation regime 5.

5.3.4 Vegetative growth and mesembrine area % of *S. tortuosum* in response to four different soilless media and five different fertigation regimes in hydroponics.

5.3.4.1 Roots

Soilless media

The effects of soilless media on root growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of root mesembrine area % to soilless media was statistically significant at the value $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on root growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of root mesembrine area % to fertigation regimes was statistically significant at the value $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on root growth of *S. tortuosum* were found to be statistically significant at the value of $p \leq 0.01$. The response of *S. tortuosum* in terms of root mesembrine area % to soilless media in conjunction with fertigation regimes were statistically significant at the value $p \leq 0.001$. The highest individual mean value (51,83) for roots were found in treatment SPFR3. Roots had an overall lower concentration of mesembrine in terms of area % than shoots (see Table 5.4).

5.3.4.2 Shoots

Soilless media

The effects of soilless media on shoot growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of shoot mesembrine area % to soilless media was statistically significant at the value $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on shoot growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of shoot mesembrine area % to fertigation regimes was statistically significant at the value $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on shoot growth of *S. tortuosum* were found to be not significant. The highest individual mean value (27,07 mm) for shoot growth was observed in treatment SVFR1. The response of *S. tortuosum* in terms of shoot mesembrine area % to soilless media in conjunction with fertigation regimes were statistically significant at the value $p \leq 0.001$. The highest mean values (86,52 and 86,65) for shoots were found in treatments SCFR3 and SPFR3. Shoots had an overall higher concentration of mesembrine in terms of area % than roots (see Table 5.4).

Table 5.4

The results of root and shoot vegetative growth and mesembrine area % of *S. tortuosum*.

Treatment		Root Growth (mm)		Shoot Growth (mm)		Roots - Mesembrine Area %		Shoots - Mesembrine Area %	
Soilless Medium	Fertigation Regime	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group
SS	FR1	41,07	±6,82i	15,33	±1,95cde	21,16	±0,18o	71,03	±0,14j
SS	FR2	62,27	±13,17ghi	10,20	±1,79efg	33,05	±0,49i	65,37	±0,10l
SS	FR3	77,20	±13,64fgh	8,27	±1,20ghi	23,27	±0,23m	74,68	±0,12f
SS	FR4	53,80	±13,36hi	9,40	±6,65fgh	36,97	±0,23h	72,00	±0,04h
SS	FR5	58,13	±13,15hi	3,47	±0,83i	42,28	±0,24f	68,28	±0,13k
SC	FR1	114,20	±14,96def	21,13	±2,19abc	43,47	±0,12e	79,87	±0,13c
SC	FR2	109,40	±16,26def	19,67	±3,02bc	51,09	±0,09b	83,96	±0,10b
SC	FR3	197,93	±25,26a	22,87	±2,04ab	48,79	±0,11c	86,52	±0,08a
SC	FR4	150,67	±22,54bcd	12,80	±0,96def	19,18	±0,14p	58,62	±0,10n
SC	FR5	67,47	±10,80fgh	10,00	±1,44efg	22,42	±0,17n	64,63	±0,07m
SV	FR1	91,87	±14,16efg	27,07	±2,31a	25,31	±0,16l	78,44	±0,10e
SV	FR2	135,47	±19,18cde	16,47	±1,60bcd	17,67	±0,08q	68,53	±0,05k
SV	FR3	186,93	±18,55ab	16,27	±2,63cde	17,32	±0,10q	71,43	±0,10i
SV	FR4	171,40	±27,09abc	12,40	±1,33efg	16,45	±0,13r	56,65	±0,09o
SV	FR5	54,53	±20,36hi	9,40	±1,99fgh	29,98	±0,18k	78,29	±0,09e
SP	FR1	89,33	±13,12efg	19,27	±2,10bcd	46,26	±0,15d	78,93	±0,14d
SP	FR2	111,73	±14,58def	12,40	±2,09efg	17,42	±0,16q	71,33	±0,15i
SP	FR3	95,20	±13,45efg	10,60	±1,49efg	51,83	±0,12a	86,65	±0,09a
SP	FR4	109,67	±22,40def	6,47	±0,97ghi	32,28	±0,12j	74,56	±0,08f
SP	FR5	33,00	±10,22i	4,47	±1,36hi	37,74	±0,24g	73,19	±0,11g

Two-way ANOVA F-Statistics

Soilless Medium	20,0***	14,7***	7410,1***	4792***
Fertigation Regime	15,5***	20,1***	1461,2***	11408***
Soilless Medium*Fertigation Regime	2,5**	1,1ns	4216,1***	4890***

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤ 0.01(**), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4 = fertigation regime 4; FR5 = fertigation regime 5.

5.3.5 Vegetative growth and mesembrine % of *S. tortuosum* in response to four different soilless media and five different fertigation regimes in hydroponics.

5.3.5.1 Roots

Soilless media

The effects of soilless media on root growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of root mesembrine concentrations to soilless media was statistically significant at the value of $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on root growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of root mesembrine concentrations to fertigation regimes was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on root growth of *S. tortuosum* were found to be statistically significant at the value of $p \leq 0.01$. The response of *S. tortuosum* in terms of root mesembrine concentrations to the effects of soilless media in conjunction with fertigation regimes were statistically significant at the value of $p \leq 0.001$. For roots the highest individual mean value (1,14) was observed in treatment SCFR2. Roots overall had lower mesembrine concentrations than roots. All 20 treatments significantly differed from one another (see Table 5.5).

5.3.5.2 Shoots

Soilless media

The effects of soilless media on shoot growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of shoot mesembrine concentrations to soilless media was statistically significant at the value of $p \leq 0.001$.

Fertigation regime

The effects of fertigation regimes on shoot growth of *S. tortuosum* was statistically significant at the value of $p \leq 0.001$. The response of *S. tortuosum* in terms of shoot mesembrine concentrations to fertigation regimes was statistically significant at the value of $p \leq 0.001$.

Interaction between soilless media and fertigation regimes

The effects of soilless media in conjunction with fertigation regimes on shoot growth of *S. tortuosum* were found to be not significant. The highest individual mean value (27,07 mm) for shoot growth was observed in treatment SVFR1. The response of *S. tortuosum* in terms of shoot mesembrine concentrations to the effects of soilless media in conjunction with fertigation regimes were statistically significant at the value of $p \leq 0.001$. The highest individual mean value (4,19) was observed in treatment SCFR3. Shoots had an overall higher mesembrine concentration than roots. All 20 treatments significantly differed from one another (see Table 5.5).

Table 5.5

The results of root and shoot vegetative growth and mesembrine %.

Treatment		Root Growth (mm)		Shoot Growth (mm)		Roots Mesembrine %		Shoots Mesembrine %	
Soilless Medium	Fertigation Regime	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group	Mean	Std Err + Mean Group
SS	FR1	41,07	±6,82i	15,33	±1,95cdef	0,39	±0,002i	1,60	±0,007k
SS	FR2	62,27	±13,17ghi	10,20	±1,79efg	0,36	±0,004j	1,67	±0,005j
SS	FR3	77,20	±13,64fgh	8,27	±1,20ghi	0,46	±0,008g	2,55	±0,004e
SS	FR4	53,80	±13,36hi	9,40	±6,65fgh	0,54	±0,008f	1,70	±0,014j
SS	FR5	58,13	±13,15hi	3,47	±0,83i	0,47	±0,007g	1,05	±0,010n
SC	FR1	114,20	±14,96def	21,13	±2,19abc	1,08	±0,016b	2,27	±0,011g
SC	FR2	109,40	±16,26def	19,67	±3,02bc	1,14	±0,006a	2,61	±0,014d
SC	FR3	197,93	±25,26a	22,87	±2,04ab	0,82	±0,009e	4,19	±0,016a
SC	FR4	150,67	±22,54bcd	12,80	±0,96def	0,17	±0,002n	0,78	±0,013p
SC	FR5	67,47	±10,80fgh	10,00	±1,44efg	0,29	±0,007l	0,95	±0,007o
SV	FR1	91,87	±14,16efg	27,07	±2,31a	0,42	±0,009h	1,97	±0,005i
SV	FR2	135,47	±19,18cde	16,47	±1,60bcd	0,24	±0,006m	1,27	±0,013m
SV	FR3	186,93	±18,55ab	16,27	±2,63cde	0,14	±0,004o	1,47	±0,006l
SV	FR4	171,40	±27,09abc	12,40	±1,33efg	0,10	±0,002p	0,59	±0,009q
SV	FR5	54,53	±20,36hi	9,40	±1,99fgh	0,29	±0,005l	1,50	±0,012l
SP	FR1	89,33	±13,12efg	19,27	±2,10bcd	1,02	±0,005c	2,85	±0,010c
SP	FR2	111,73	±14,58def	12,40	±2,09efg	0,28	±0,002l	2,31	±0,013f
SP	FR3	95,20	±13,45efg	10,60	±1,49efg	0,88	±0,006d	3,72	±0,016b
SP	FR4	109,67	±22,40def	6,47	±0,97ghi	0,32	±0,009k	2,13	±0,013h
SP	FR5	33,00	±10,22i	4,47	±1,36hi	0,38	±0,006i	1,62	±0,009k

Two-way ANOVA F-Statistics

Soilless Medium		20,0***	14,7***	4028.4***	10758,3***
Fertigation Regime		15,5***	20,1***	2557,9***	16377,2***
Soilless Medium*Fertigation Regime		2,5**	1,1ns	1500,8***	3063,6***

Mean values ±SE are shown in columns. The mean values followed by different letters are significantly different at P ≤ 0.01(**), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference. SS = silica sand; SC = silica sand + coco-peat; SV = silica sand + vermiculite; SP = silica sand + perlite. FR1 = fertigation regime 1; FR2 = fertigation regime 2; FR3 = fertigation regime 3; FR4 = fertigation regime 4; FR5 = fertigation regime 5.

5.5. Discussion & conclusion

Treatments applied in this investigation had a significant effect on the vegetative root and shoot growth of *S. tortuosum.* Results obtained from this research agrees with various previous studies which state that with a suitable quantity of water regarding to plant type improved overall plant growth (Begg & Turner, 1976; Wiedenfeld, 1995; Van Loon 1981; Mao *et al*., 2002; Yuan *et al*., 2003; Sezen *et al*., 2005; Kumar *et al*., 2007; Zeng *et al*., 2009). It was noticed plants responded better to mid-frequent fertigation intervals compared to low or high fertigation intervals.

Treatments applied in this investigation also had a significant effect on the alkaloid concentrations of *S. tortuosum.* The results obtained in this research agrees with Bourgaud *et al*. (2001) which states that plants such as *S. tortuosum* respond to biotic and abiotic stresses by controlling their influx of secondary metabolite concentrations. It was further noticed that root extracts of plants samples had more delta 7 mesembrenone and mesembrenone concentrations than shoots, while shoots had more mesembrine. Shoots also had a higher amount of total alkaloids than roots.

The results of this investigation also agrees with other research papers which follows the common belief that environmental conditions or seasons have a great impact on the biosynthesis of secondary metabolites in plants (Choudhury & Gupta, 2002; Shukla *et al.,* 2013).

S. tortuosum responded in various ways to different soilless media and fertigation regimes as well as soilless treatments in conjunction with fertigation regimes. There appears to be a clear trend that displays higher concentrations of mesembrine where shoot growth was more optimal, however more optimal growth did not display a higher concentration of total alkaloids, which suggests that a reasonable amount of stress increases total alkaloid concentrations (Selmar & Kleinwächter, 2013). Shoots contained more mesembrine overall than roots, suggesting shoots should be harvested for the extraction of mesembrine molecules rather than roots. In terms of root growth and total alkaloid concentration, it does not appear more optimal growth induces higher concentrations of total root alkaloids, meaning reasonable stress on plant root growth could promote higher concentrations of total alkaloids (Ashraf et al., 2018). It is also clear that overall roots contain more delta 7 mesembrenone and mesembrenone than shoots, suggesting roots should be harvest for extracting these molecules specifically. These findings can be related to Birecka *et al.* (1988) who also found roots and shoots had different alkaloid concentrations in the plants *Senecio riddellii* and *Crotolaria retusa.* In most cases high concentrations of delta 7

mesembrenone in roots also had similar amounts of mesembrenone, however in other instances certain treatments resulted in higher concentrations of either one of the compounds, therefore their similar molecular structure does not always permit similar manifestation in the plant material.

Further studies would be required to determine optimal growing protocols for producing these two individual molecules. Mesembrine concentrations are consistently higher in plants with better shoot growth, however it is not clear when these concentrations could become diluted in terms of over fertigation. More studies also need to be done on the interaction between certain nutrients and alkaloid concentrations in *S. tortuosum,* as alkaloids are nitrogen based, more or less nitrogen could certainly affect concentrations of alkaloids in the plant (Patnala & Kanfer, 2013).

For vegetative growth more research would be required on various soilless media and combinations thereof, as well as how pH, EC levels and methods of fertigation would affect the root and shoot growth of *S. tortuosum.*

5.6 Acknowledgements

This study was funded by Cape Peninsula University of Technology (CPUT), through the National Research Foundation and University Research Fund.

Richard Davies, Managing Director of Verve Dynamics (Pty) Ltd who supplied the initial *Sceletium tortuosum* mother plant for this study and gave special permission for the use of their mesembrine standard.

5.7 References

Ashraf, M.A., Iqbal, M., Rasheed, R., Hussain, I., Riaz, M. & Arif, M.S. 2018. *Environmental Stress and Secondary Metabolites in Plants: An Overview*. London: Academic Press.

Begg, J.E., Turner, N.C. 1976. Crop water deficits. *Advances in Agronomy*, 28: 161–217.

Birecka, H., Birecki, M., Cohen, E.J., Bitonti, A.J. & Mccann, P.P. 1988. Alkaloids in *Senecio* and *Crotalaria; Plant Physiology 86*: 224–230.

Brooking, I.R. 1976. Soilless potting media for controlled-environment facilities. *New Zealand Journal of Experimental Agriculture*, 4(2): 203–208.

Bourgaud, F., Gravot, A., Milesi, S. & Gontier, E. 2001. Production of plant secondary metabolites: A historical perspective. *Plant Science*, 161(5): 839–851.

Choudhury, S., Gupta, K. 2002. Influence of seasons on biomass and alkaloid productivity in *Catharanthus roseus. Journal of Medicinal and Aromatic Plant Sciences*, 24: 664-668.

Fadhil, S., Reza, M.H., Rouhollah, G. & Reza, V.R.M. 2007. Spectrophotometric Determination of Total Alkaloids in *Peganum harmala* L. Using Bromocresol Green. *Research Journal of Phytochemistry*, 1(2): 79–82.

Grafiadellis, I., Mattas, K., Maloupa, E., Tzouramani, I. & Galanopoulos, K. 2000. An economic analysis of soilless culture in gerbera production. *HortScience*, 35(2): 300–303.

Jensen, M. 2017. The Pros and Cons of Six Soilless Media Options. *American Vegetable Grower*, 65(3): S2.

Kleinwächter, M., Paulsen, J., Bloem, E., Schnug, E. & Selmar, D. 2014. Moderate drought and signal transducer induced biosynthesis of relevant secondary metabolites in thyme (*Thymus vulgaris*), greater celandine (*Chelidonium majus*) and parsley (*Petroselinum crispum*). *Industrial Crops & Products*, 64: 158–166.

Kleinwächter, M. & Selmar, D. 2014. Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. *Industrial Crops & Products*, 42: 558–566.

Kumar, S., Imtiyaz, M., Kumar, A., Singh, R. 2007. Response of onion (*Allium cepa* L.) to different levels of irrigation water. *Agricultural Water Management*, 89: 161–166.

Mao, X., Liu, M., Wang, X., Liu, C., Hou, Z. & Shi, J. 2002. Effects of deficit irrigation on yield and water use of greenhouse grown cucumber in the North China Plain. *Agricultural Water Management*, 61: 219–228.

Nejad, A.R. & Ismaili, A. 2014. Changes in growth, essential oil yield and composition of geranium (*Pelargonium graveolens* L.) as affected by growing media. *Journal of the Science of Food and Agriculture*, 94(5): 905–910.

Patnala, S. & Kanfer, I. 2009. Investigations of the phytochemical content of *Sceletium tortuosum* following the preparation of 'Kougoed' by fermentation of plant material. *Journal of Ethnopharmacology*, 121(1): 86–91.

Patnala, S. & Kanfer, I. 2013. Chemotaxonomic studies of mesembrine-type alkaloids in *Sceletium* plant species. *South African Journal of Science*, 109(3–4): 5–9.

Raviv, M., Wallach, R., Silber, A. & Bar-Tal, A. 2002. *Substrates and their analysis In: Hydroponic Production of Vegetables and Ornamentals.* D. and H. P. Savvas, ed. Athens: Embryo Publications.

Savvas, D., Gianquinto, G., Tuzel, Y. & Gruda, N., 2013. Soilless Culture. Good Agricultural Practices for Greenhouse Vegetable Crops, Principles for Mediterranean Climate Areas. *FAO Plant Production and Protection Paper, 217*: 303–354.

Selmar, D. & Kleinwächter, M. 2013. Stress enhances the synthesis of secondary plant products: The impact of stress-related over-reduction on the accumulation of natural products. *Plant and Cell Physiology*, 54(6): 817–826.

Sezen, S.M., Yazar, A., Canbolani, M., Eker, S. & Caikel, G. 2005. Effect of drip management on yield and quality of field grown green beans. *Agricultural Water Management,* 71 (3): 243–255.

Shukla, A.K., Mall, M., Rai, S.K., Singh, S., Nair, P., Parashar, G., Shasany, A.K., Singh, S.C., Joshi, V.K. & Khanuja, S.P.S. 2013. A transcriptomic approach for exploring the molecular basis for dosha-balancing property-based classification of plants in Ayurveda. *Molecular Biology Reports*, 40: 3255-3262.

Steel, R.G.D. & Torrie, J.H. 1980. *Principle and procedures of statistics: a biometrical approach*. *2nd ed*. New York: McGraw-Hill.

Van Loon, C.D. 1981. The effect of water stress on potato growth, development and yield. *American Potato Journal*, 58: 51–69.

Wiedenfeld, R.P. 1995. Effects of irrigation and N fertilizer application on sugarcane yield and quality. *Field crops Research*, 43: 101–108.

Yuan, B.Z., Nishiyama, S. & Kang, Y. 2003. Effects of different irrigation regimes on the growth and yield of drip – irrigated potato. *Agricultural Water Management.* 63 (3): 153–167

Zeng, C.Z., Bie, Z.L., Yuan, B.Z. 2009. Determination of optimum irrigation water amount for drip irrigated muskmelon (*Cucumis melo L.)* in plastic greenhouse. *Agricultural Water Management*, 96: 597–601.

CHAPTER SIX:

GENERAL DISCUSSION AND CONCLUSION AND RECOMMENDATIONS

6.1 General discussion, conclusion and recommendations

Chapter 2 found that *Sceletium tortuosum* is a relevant Traditional African Medicinal Plant with pharmaceutical and economic viability and that the vegetative growth and alkaloid concentrations could be affected by applying various treatments when cultivating the plant.

Chapter 3 found soilless media with higher water holding capacity (media containing coco-peat and vermiculite) yielded more desirable results in terms of vegetative growth than media that did not (media containing pure silica sand and/or perlite). Too frequent (every week) or infrequent (every fifth week) fertigation is not ideal for vegetative growth, instead plants reacted significantly to fertigation at 3 week intervals. One can therefore suggest that well drained, yet high water holding capacity soilless media in conjunction with mid-frequent fertigation intervals would yield the best results in terms of vegetative growth. Further studies would be required on pH levels, EC levels, various different soilless media and combinations thereof in conjunction with various fertigation regimes and how *S. tortuosum* responds to these variables in order to further establish an optimal protocol for cultivation of the plant in hydroponics.

Chapter 4 found there is a clear effect on alkaloid concentration in *S. tortuosum* root and shoot extracts in response to different soilless media, fertigation regimes as well as soilless media in conjunction with fertigation regimes. Results from this study has shown that shoot extracts contain a higher concentration of total alkaloids than root extracts, however root extracts had an overall higher amount of delta 7 mesembrenone, and mesembrenone in terms of area %, while shoots had higher amounts of mesembrine. Further looking at the mesembrine standard from Verve Dynamics (Pty) Ltd shoots clearly have an overall higher concentration of mesembrine than roots. These results suggest that roots of *S. tortuosum* should be harvested for the purpose of extracting delta 7 mesembrenone and mesembrenone molecules, while the shoots should be harvested for extracting mesembrine. Further studies would be needed to test how other methods of fertigation, various organic/soilless media and a combination of both would affect the concentration of mesembrine-type alkaloids when cultivating *S. tortuosum* in soil or hydroponics. More research is also required on how fermentation of *S. tortuosum* plant material affects alkaloid concentrations.

Chapter 5 found *S. tortuosum* responded in various ways to different soilless media and fertigation regimes as well as soilless treatments in conjunction with fertigation

regimes. There is a clear trend that displays higher concentrations of mesembrine where shoot growth was more optimal, however more optimal growth did not display a higher concentration of total alkaloids, which suggests that a reasonable amount of stress increases total alkaloid concentrations. Shoots contained more mesembrine overall than roots, suggesting shoots should be harvested for the extraction of mesembrine molecules rather than roots. In terms of root growth and total alkaloid concentrations, it does not appear more optimal growth induces higher concentrations of total root alkaloids, meaning reasonable stress on plant root growth could promote higher concentrations of total alkaloids. It is also clear that overall roots contain more delta 7 mesembrenone and mesembrenone than shoots, suggesting roots should be harvested for extracting these molecules specifically. In most cases high results of delta 7 mesembrenone in roots also had similar amounts of mesembrenone, however in some results certain treatments resulted in higher concentrations of either one of the compounds, therefore their similar molecular structure does not always permit similar manifestation in the plant material. Further studies would be required to determine optimal growing protocols for producing these two individual molecules. Mesembrine concentrations are consistently higher in plants with better shoot growth, however it is not clear when these concentrations could become diluted in terms of over fertigation. More studies also need to be done on the interaction between certain nutrients and alkaloid concentrations in *S. tortuosum,* as alkaloids are nitrogen based, more or less nitrogen availability could certainly affect concentrations of alkaloids in the plant.

Overall this study has found that *S. tortuosum* is suitable for cultivation in hydroponics, and that soilless media, fertigation regimes as well as soilless media in conjunction with fertigation regimes affected the vegetative growth and alkaloid concentrations of *S. tortuosum.* The research has shown that some soilless media and fertigation regime treatments had more desirable results in terms of vegetative growth and/or alkaloid concentration of the plant.

CHAPTER SEVEN:

REFERENCES

REFERENCES:

Agung Putra, P. & Yuliando, H. 2015. Soilless Culture System to Support Water Use Efficiency and Product Quality: a Review. *Agriculture and Agricultural Science Procedia*, 3: 283–288.

Ashraf, M.A., Iqbal, M., Rasheed, R., Hussain, I., Riaz, M. & Arif, M.S. 2018. *Environmental Stress and Secondary Metabolites in Plants: An Overview*. London: Academic Press.

Begg, J.E., Turner, N.C. 1976. Crop water deficits. *Advances in Agronomy*, 28: 161–217.

Bennett, A.C., Van Camo, A., Lopez, L. & Smith, C. 2018. *Sceletium tortuosum* may delay chronic disease progression via alkaloid-dependent antioxidant or anti-inflammatory action. *Journal of Physiology and Biochemistry*, 74(4): 1–9.

Benton Jones, J. 2014. *Growing Plants Hydroponically*. 1st ed. Boca Raton: CRC Press.

Birecka, H., Birecki, M., Cohen, E.J., Bitonti, A.J. & Mccann, P.P. 1988. Alkaloids in Seneclo and Crotalaria. : 224–230.

Bourgaud, F., Gravot, A., Milesi, S. & Gontier, E. 2001. Production of plant secondary metabolites: A historical perspective. *Plant Science*, 161(5): 839–851.

Brooking, I.R. 1976. Soilless potting media for controlled-environment facilities. *New Zealand Journal of Experimental Agriculture*, 4(2): 203–208.

Bunt, A.C. 1988. *Media Mixes for Container Grown Plants*. London, UK: Unwin Yyman.

Caron, J.C. & Nkongolo, V.K.N. 1999. Aeration in growing media: recent developments. *Acta Horticulturae*, 481: 545–551.

Clements, E.J. 2000. Silica Sand. *Mining Engineering*, 52(6): 60–61.

Coetzee, D.D., Lopez, V. & Smith, C. 2015. High-Mesembrine *Sceletium* extract (TrimesimineTM) is a monoamine releasing agent, rather than only a selective serotonin reuptake inhibitor. *Journal of Ethnopharmacology*, 177: 111–116.

Czapek, F. 1921. Spezielle Biochemie. *Biochemie der Pflanzen*, 3: 369.

Fadhil, S., Reza, M.H., Rouhollah, G. & Reza, V.R.M. 2007. Spectrophotometric Determination of Total Alkaloids in Peganum harmala L. Using Bromocresol Green. *Research Journal of Phytochemistry*, 1(2): 79–82.

Fageria, N.K. 2009. *The use of nutrients in crop plants*. 1st ed. Boca Raton: CRC Press.

Fennell, C.W., Light, M.E., Sparg, S.G., Stafford, G.I. & Van Staden, J. 2004. Assessing African medicinal plants for efficacy and safety: Agricultural and storage practices. *Journal of Ethnopharmacology*, 95(2–3): 113–121.

Fonteno, W.C. 1993. Problems & Considerations in Determining Physical Properties of Horticultural Substrates. *Acta Horticulturae*, (342): 197–204.

Frink, C.R., P.E. Waggoner and J.H. Ausubel, 1999. Nitrogen fertilizer: retrospect and prospect. *Proceedings of the National Academy of Sciences*, USA. 96: 1175-1180.

Gericke, N. & Viljoen, a. M. 2008. *Sceletium*-A review update. *Journal of Ethnopharmacology*, 119(3): 653–663.

Grafiadellis, I., Mattas, K., Maloupa, E., Tzouramani, I., Galanopoulos, K. 2000. An economic analysis of soilless culture in Gerbera production. *HortScience*, 35(2): 300–303.

Greenwood, D.J. 1990. Production or productivity: the nitrate problem? *Ann. Appl. Biol.*, 117: 209–231.

Hartmann, H.E.K. 2001. *Illustrated Handbook of Succulent Plants: Aizoaceae A-E; Aizoaceae F-Z.* Berlin: Springer.

Harvey, A.L., Young, L.C., Viljoen, A.M. & Gericke, N.P. 2011. Pharmacological actions of the South African medicinal and functional food plant *Sceletium tortuosum* and its principal alkaloids. *Journal of Ethnopharmacology*, 137(3): 1124–1129.

Hoagland, D.R. & Arnon, D.I. 1950. The water-culture method for growing plants without soil. *California Agricultural Experiment Station Circular*, 347: 1–32.

Hutchings, A., Scott, A.H., Lewis, G., Cunningham, A. 1996. *Zulu Medicinal Plants*. 1st ed. Pietermaritzburg: Natal University Press.

Jensen, M. 2017. The Pros and Cons of Six Soilless Media Options. *American Vegetable Grower*, 65(3): S2.

Karuppusamy, S. 2009. A review on trends in production of secondary metabolites from higher plants by in vitro tissue, organ and cell cultures. *Journal of Medicinal Plants Research*, 3(13): 1222–1239.

Keirungi, J. & Fabricius, C. 2005. Selecting medicinal plants for cultivation at Nqabara on the Eastern Cape Wild Coast, South Africa. *South African Journal of Science*, 101(11–12): 497–501.

Kirakosyan, A., Kaufman, P., Warber, S., Zick, S., Aaronson, K., Bolling, S. & Chang, S.C. 2004. Applied environmental stresses to enhance the levels of polyphenolics in leaves of hawthorn plants. *Physiologia Plantarum*, 121(2): 182–186.

Klak, C., Bruyns, P. V. & Hedderson, T. J. 2007. A phylogeny and new classification for Mesembryanthemoideae (Aizoaceae). *Taxon*, 56: 737–756.

Kleinwächter, M., Paulsen, J., Bloem, E., Schnug, E. & Selmar, D. 2014. Moderate drought and signal transducer induced biosynthesis of relevant secondary metabolites in thyme (*Thymus vulgaris*), greater celandine (Chelidonium majus) and parsley (*Petroselinum crispum*). *Industrial Crops & Products*, 64: 158–166.

Kleinwächter, M. & Selmar, D. 2014. Influencing the product quality by deliberately applying drought stress during the cultivation of medicinal plants. *Industrial Crops & Products*, 42: 558–566.

Krstenansky, J. 2016. Mesembrine alkaloids: Review of their occurrence, chemistry and pharmacology. *Journal of Ethnopharmacology*, 195: 10–19.

Kumar, S., Imtiyaz, M., Kumar, A., Singh, R. 2007. Response of onion (*Allium cepa* L.) to different levels of irrigation water. *Agricultural Water Management*, 89: 161–166.

Le Bot, J., Adamowicz, S. & Robin, P. 1998. Modelling plant nutrition of horticultural crops: A review. *Scientia Horticulturae*, 74(1–2): 47–82.

Li, J., Ou-Lee, T.M., Raba, R., Amundson, R.G. & Last, R.L. 1993. Arabidopsis flavonoid mutants are hypersensitive to UV-B irradiation. *Plant Cell*, 5(2): 171–179.

Liu, J. & Zhu, J.K. 1997. An Arabidopsis mutant that requires increased calcium for potassium nutrition and salt tolerance. *Proceedings of the National Academy of Sciences*. USA, 94 (26), 14960-14964.

Liopa-Tsakalidi, a., Barouchas, P. & Salahas, G. 2015. Response of Zucchini to the Electrical Conductivity of the Nutrient Solution in Hydroponic Cultivation. *Agriculture and Agricultural Science Procedia*, 4: 459–462.

Makunga, N.P., Philander, L.E. & Smith, M. 2008. Current perspectives on an emerging formal natural products sector in South Africa. *Journal of Ethnopharmacology*, 119(3): 365–375.

Mander, M. 1998. Marketing of indigenous medicinal plants in South Africa - a case study in Kwazulu-Natal. *FAO - Food and Agriculture Organization of the United Nations*: 1–158.

Manukyan, A. 2011. Effect of growing factors on productivity and quality of lemon catmint, lemon balm and sage under soilless greenhouse production: I. drought stress. *Med Aromat Plant Sci Biotechnol*, 5(2): 119–125.

Mao, X., Liu, M., Wang, X., Liu, C., Hou, Z., Shi, J. 2002. Effects of deficit irrigation on yield and water use of greenhouse grown cucumber in the North China Plain. *Agricultural Water Management*, 61: 219–228.

Marschner, P. & Rengel, Z., 2007. *Nutrient Cycling in Terrestrial Ecosystems.* Berlin: Springer.

Nejad, A.R., Ismaili, A. 2014. Changes in growth, essential oil yield and composition of geranium (*Pelargonium graveolens* L.) as affected by growing media. *Journal of the Science of Food and Agriculture*, 94(5): 905–910.

Olivier, F.C. & Singels, A. 2015. Increasing water use efficiency of irrigated sugarcane production in South Africa through better agronomic practices. *Field Crops Research*, 176: 87–98.

Patnala, S. & Kanfer, I. 2009. Investigations of the phytochemical content of *Sceletium tortuosum* following the preparation of 'Kougoed' by fermentation of plant material. *Journal of Ethnopharmacology*, 121(1): 86–91.

Patnala, S. & Kanfer, I. 2010. HPLC analysis of mesembrine-type alkaloids in *Sceletium* plant material used as an african traditional medicine. *Journal of Pharmacy and Pharmaceutical Sciences*, 13(4): 558–570.

Patnala, S. & Kanfer, I. 2013. Chemotaxonomic studies of mesembrine-type alkaloids in Sceletium plant species. *South African Journal of Science*, 109(3–4): 5–9.

Philipson, J.D. 1990. *Plants as source of valuable products. In: Secondary products from plant tissue culture.* B. V. Chalwood & M. J. Rhodes, eds. Oxford: Clarendon Press.

Pichersky, E. & Gang, D.R. 2000. Genetics and biochemistry of secondary metabolites in plants: An evolutionary perspective. *Trends in Plant Science*, 5(10): 439–445.

Raviv, M., Wallach, R., Silber, A. & Bar-Tal, A. 2002. *Substrates and their analysis In: Hydroponic Production of Vegetables and Ornamentals.* D. and H. P. Savvas, ed. Athens: Embryo Publications.

Reversat, G., Boyer, J., Sannier, C. & Pando-Bahuon, A. 1999. Use of a mixture of sand and water-absorbent synthetic polymer as substrate for the xenic culturing of plant-parasitic nematodes in the laboratory. *Nematology*, 1(2): 209–212.

Santamaria, P., Campanile, G., Parente, A. & Elia, A. 2003. Sub irrigation vs drip-irrigation: Effects on yield and quality of soilless grown cherry tomato. *Journal of Horticultural Science and Biotechnology*, 78(3): 290–296.

Savvas, D., Gianquinto, G., Tuzel, Y., Gruda, N., 2013. 2013. Soilless Culture. Good Agricultural Practices for Greenhouse Vegetable Crops, Principles for Mediterranean Climate Areas. *FAO Plant Production and Protection Paper, 217*: 303–354.

Scherer, H.W. 2001. Sulphur in crop production - Invited paper. *European Journal of Agronomy*, 14(2): 81–111.

Schnitzler, W.H., Trüggelmann, L., Toth, A., Woitke, M., Tüzel, Y., Tüzel, H., Hanafi, A., Jaquet, F., Le Grusse, P., Junge, H., Giuffrida, F., Leonardi, C., Wadid Awad,

M.M., El-Behairy, Fort, F., Codron, J.M. & Qaryouti, M.M. 2003. Efficient Water Use for High Quality Vegetables through the Environmentally Sound Hydroponic Production 'Ecoponics'. *Acta Horticulturae*, (609): 493–495.

Schultes, R.E., Hofmann, A., Rätsch, C. 2001. *Plants of the Gods: Their Sacred, Healing, and Hallucinogenic Powers*. 2nd ed. Lucerne: EMB-service for Publishers.

Schultes, R.E. 1976. *Hallucinogenic Plants*. New York: Golden Press.

Selmar, D. & Kleinwächter, M. 2013. Stress enhances the synthesis of secondary plant products: The impact of stress-related over-reduction on the accumulation of natural products. *Plant and Cell Physiology*, 54(6): 817–826.

Sezen, S.M., Yazar, A., Canbolani, M., Eker, S. & Caikel, G. 2005. Effect of drip management on yield and quality of field grown green beans. *Agricultural Water Management,* 71 (3): 243–255.

Shukla, A.K., Mall, M., Rai, S.K., Singh, S., Nair, P., Parashar, G., Shasany, A.K., Singh, S.C., Joshi, V.K. & Khanuja, S.P.S. 2013. A transcriptomic approach for exploring the molecular basis for dosha-balancing property-based classification of plants in Ayurveda. *Molecular Biology Reports*, 40, 3255-3262.

Smith, G.F., Chesselet, P., van Jaarsveld, E.J., Hartmann, H., Hammer, S., van Wyk, B.-E., Burgoyne, P., Klak, C. & Kurzweil, H. 1998. *Mesembs of the World*. 1st ed. Pretoria, South Africa: Briza Publications.

Smith, M.T., Crouch, N.R., Gericke, N. & Hirst, M. 1996. Psychoactive constituents of the genus *Sceletium* N.E.Br. and other Mesembryanthemaceae: A review. *Journal of Ethnopharmacology*, 50(3): 119–130.

Song, M.C., Kim, E.J., Kim, E., Rathwell, K., Nam, S. & Yoon, Y.J. 2014. Microbial biosynthesis of medicinally important plant secondary metabolites. *Natural Product Reports*, 00: 1–13.

Steel, R.G.D. & Torrie, J.H. 1980. *Principle and procedures of statistics: a biometrical approach*. *2nd ed*. New York: McGraw-Hill.

Suzuki, M., Umeda, H., Matsuo, S., Kawasaki, Y., Ahn, D., Hamamoto, H. & Iwasaki, Y. 2015. Effects of relative humidity and nutrient supply on growth and nutrient uptake in greenhouse tomato production. *Scientia Horticulturae*, 187: 44–49.

Taiz, L. & Zeiger, E. 2010. *Plant Physiology*. 5th ed. Sunderland, MA USA: Sinauer Associates.

Trejo-téllez, L.I. & Gómez-merino, F.C. 2012. *Nutrient Solutions for Hydroponic Systems - A Standard Methodology for Plant Biological Researches*.

Van Andel, T. & Havinga, R. 2008. Sustainability aspects of commercial medicinal plant harvesting in Suriname. *Forest Ecology and Management*, 256(8): 1540–1545.

Van Loon, C.D. 1981. The effect of water stress on potato growth, development and yield. *American Potato Journal*, 58: 51–69.

Van Wyk, B.E. 2008. A broad review of commercially important southern African medicinal plants. *Journal of Ethnopharmacology*, 119(3): 342–355.

Van Wyk, B. & Wink, M. 2012. *Medicinal Plants of the World*. 5th ed. Pretoria: Briza.

Van Wyk, B.E, Van Oudtshoorn, B. & Gericke, N. 1997. *Medicinal Plants of South Africa*. Pretoria: Briza Publications.

Van Wyk, B.-E. & Wink, M. 2015. *Phytomedicines, Herbal Drugs, and Plant Poisons*. 1st ed. Pretoria: Briza Publications.

Venter, G. 2010. *Successful hydroponics, a comprehensive practical guide to scientifically based hydroponic crop production*. USA: Xlibris Corporation.

Wiedenfeld, R.P. 1995. Effects of irrigation and N fertilizer application on sugarcane yield and quality. *Field crops Research*, 43: 101–108.

Wink, M., Alfermann, A.W., Franke, R., B, W., Distl, M., Windhovel, J., Krohn, O., Fuss, E., Garden, H Mohagheghzaden, A., Wildi, E. & Ripplinger, P. 2005. Sustainable bioproduction of phytochemicals by plant in vitro cultures: anticancer agents. *Plant Genetic Resource*, 3: 90–100.

Wink, M. & van Wyk, B.-E. 2008. *Mind-altering & Poisonous Plants of the World*. 1st ed. Pretoria, South Africa: Briza Publications.

White, P.J. & Broadley, M.R. 2003. Calcium in plants. *Annals of Botany*, 92, 487-511.

Yuan, B.Z., Nishiyama, S. & Kang, Y. 2003. Effects of different irrigation regimes on the growth and yield of drip – irrigated potato. *Agricultural Water Management.* 63 (3): 153–167

Zeng, C.Z., Bie, Z.L. & Yuan, B.Z. 2009. Determination of optimum irrigation water amount for drip irrigated muskmelon (*Cucumis melo L.)* in plastic greenhouse. *Agricultural Water Management*, 96: 597–601.