Cape Peninsula
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Effect of water stress and arbuscular mycorrhiza on the plant growth and antioxidant potential of *Pelargonium reniforme* Curtis and *Pelargonium sidoides* DC.

by

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DECLARATION

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

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ABSTRACT

Pelargoniums have been studied extensively for their medicinal properties. *P. reniforme* and *P. sidoides* in particular are proven to possess antimicrobial, antifungal and antibiotic abilities due to their high antioxidant potential from compounds isolated from their tuberous roots. These plants have now been added to the medicine trade market and this is now causing concern for conservationists and they are generally harvested from the wild populations. This study evaluated the effect of water stress alone and in conjunction with arbuscular mycorrhiza on two species of *Pelargoniums* grown in a soilless medium. The experiment consisted of five different watering regimes which were applied to one hundred plants of each species without inoculation with arbuscular mycorrhiza and to one hundred plants of each species in conjunction with inoculation with AM. All the plants in the experiment were fed with a half-strength, standard Hoagland nutrient solution at varying rates viz. once daily to pot capacity, every three days to pot capacity, every six days to pot capacity, every twelve days to pot capacity and every twenty-four days to pot capacity. The objectives of the study were to measure the nutrient uptake, SPAD-502 levels (chlorophyll production) and metabolite (phenolics) formation of both species, grown under various rates of irrigation and water stress, as well with or without the addition of arbuscular mycorrhiza at planting out. Each treatment consisted of 10 replicates. SPAD-502 levels were measured weekly using a hand held SPAD-502 meter. Determination of nutrient uptake of macronutrients N, K, P, Ca, Mg and Na and micronutrients Cu, Zn, Mn, Al and B were measured from dry plant material at the end of the experiment by Bemlab, 16 Van Der Berg Crescent, Gants Centre, Strand. Plant growth in terms of wet and dry shoot and root weight were measured after harvest. Determination of concentrations of secondary metabolites (phenolic compounds) were assayed and measured spectrophotometrically at the end of the experiment.

The highest significant reading of wet shoot weight for *P. reniforme* was taken in treatments 1 and 2 with and without mycorrhiza i.e. WF1, WF1M, WF2 and WF2M, with the highest mean found in WF1 with no mycorrhiza. This indicates that under high irrigation AM plays no part in plant growth, possibly due to leaching. More research is necessary in this regard. With regard to wet root weight, this was found to be not significant in any of the treatments, other than the longest roots being found in WF4. Measurements for dry root weight showed that WF1,2,3 and 5 were the most significant at $P \leq 0.001$ significance, with the highest weight found at treatment being WF3 and WF3M. The highest mean of shoot length of the plants was measured in treatment WF2 at moderate watering, but no statistical difference was found with water application and mycorrhiza addition. Nutrient uptake was increased in

P. sidoides in all the different watering levels in the experiment except in the uptake of Mg. AM inoculation showed an increase in the uptake of Ca, while absorption of N occurred at higher water availability. K uptake was enhanced by the addition of AM in high water availability and K utilisation decreased as water stress increased. Medium to low watering resulted in higher leaf content in *P. sidoides* while the interaction between water availability and AM inoculation increased chlorophyll production towards the end of the experiment. Antioxidant activity and accumulation of metabolites (courmarins and phenolics) were measured in the dry root material of the two species using assays for total polyphenols (Folin calcioteu), ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC). There was no presence of courmarins detected in the root material, possibly due to the immaturity of the plants. However, a high significance of polyphenols ($P \leq 0.05$) was found in *P. reniforme* compared to that of *P. sidoides* at $P \leq 0.001$. The addition of AM increased the FRAP values in the dry root material of *P. reniforme.* There was evidence in both species of increased polyphenol content and FRAP values in lower watering frequencies.

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CHAPTER ONE

PROBLEM STATEMENT, AIMS, HYPOTHESES AND OBJECTIVES

1.1 STATEMENT OF THE RESEARCH PROBLEM

A great number of plant species have been traditionally utilized for treatment against disease and illness for centuries. Much of the world's population uses traditional medicine to cure disease due to expense and lack of exposure to clinical medicine (Balunas & Kinghorn, 2005; Appidi et al., 2008). Indigenous plant species have been the traditional source of raw material for the manufacture of medicines and there is currently growing attention to natural plant-based medicines as a basis for commercial medication (Street & Prinsloo, 2012).

Two *Pelargonium* species, namely *P. reniforme* Curtis and *P.sidoides* DC have important sources of compounds which possess many pharmacological attributes (Saraswathi et al., 2011). Many South African medicinal plants, including these two *Pelargonium* species, are used in the production of internationally marketed natural remedies (Van Wyk & Gericke, 2000). The metabolites of *P. reniforme* and *P. sidoides*, which are the origin of the herbal medicine Umckaloabo®, possess a wide diversity and complexity in various phenolic compounds and other valuable metabolites (Kolodziej, 2007). *P. reniforme* has been found to possess significant antimicrobial and antioxidant properties which have led to its use in traditional medicine to treat liver disease (Adewusi & Afolayan, 2009).

The combination of traditional and commercial use of several plants has led to their overharvesting in the Eastern Cape, including *P. reniforme* and *P. sidoides* (Dold & Cocks, 2002). These authors also reported on informal trading in medicinal plants in the Eastern Cape province of South Africa and found that *P. reniforme* was heavily harvested and unsustainably harvested at the study sights. Trade in traditional medicines forms part of an informal economy in South Africa and is a direct result of increasing population numbers, urbanization, unemployment and the high cultural value of traditional medicines (Cunningham, 1997).

There has been very little commercial exploitation of *Pelargonium spp.* (Saraswathi *et al.*, 2011). This study aims at measuring the results of commercial cultivation of these two valuable Pelargonium species using different irrigation regimes and AM in order to optimise their yield and metabolites.

1.2 AIMS

The study aims to assess the effects of AM and various levels of water stress on the nutrient uptake, plant growth and antioxidant potential of *P. reniforme* CURTIS and *P. sidoides* DC.

1.3 HYPOTHESES

It is hypothesised that water stress will increase the antioxidant potential in *P. reniforme* and *P. sidoides* and have an unfavourable effect on nutrient uptake and plant growth. It is also hypothesised that nutrient uptake and plant growth will be positively influenced by the application of AM.

1.4 OBJECTIVES

1.4.1 Main objective

To assess the effects of AM and various levels of water stress on the nutrient uptake, plant growth and accumulation of metabolites of *P. reniforme* and *P. sidoides*.

1.4.2 Specific objectives

1) To assess the effects of various levels of water stress on the nutrient uptake, plant growth and antioxidant potential of *P. reniforme* and *P. sidoides*.

2) To assess the effects of various levels of water stress without the application of AM on the nutrient uptake, plant growth and antioxidant potential of *P. reniforme* and *P. sidoides.*

3) To assess the effects of the application of AM with various levels of water stress on the nutrient uptake, plant growth and antioxidant potential of *P. reniforme* and *P. sidoides*.

4) To assess the effects of the application of AM and various levels of water stress on the wet root weight of *P. reniforme* and *P. sidoides.*

5) To assess the effects of the application of AM and various levels of water stress on the dry root weight of *P. reniforme* and *P. sidoides*.

6) To assess the effects of the application of AM and various levels of water stress on the wet shoot weight of *P. reniforme* and *P. sidoides.*

7) To assess the effects of AM and various levels of water stress on the dry shoot weight of *P. reniforme* and *P. sidoides*.

8) To investigate the effects of AM and various levels of water stress on the antioxidant potential in the roots of *P. reniforme* and *P.sidoides*.

9) To investigate the optimal watering regime for commercial production of *P. reniforme* and *P. sidoides* within an environment of decreasing annual rainfall.

10) To investigate the effectiveness of AM on the growth and antioxidant potential with respect to possible commercial cultivation and limiting of wild-harvesting of *P. reniforme* and *P. sidoides*.

CHAPTER TWO

THE COMMERCIAL POTENTIAL OF *PELARGONIUM RENIFORME* CURTIS AND *P. SIDOIDES* DC- A REVIEW.

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2.1 ABSTRACT

Of the great many southern African plants, the genus *Pelargonium* has been studied extensively for their medicinal and phytochemical properties. *Pelargonium reniforme* and *Pelargonium sidoides* in particular are proven to possess antimicrobial, antifungal and antibiotic abilities due to their high antioxidant potential and metabolite content, specifically found in the tuberous roots. Trade in traditional medicines in southern Africa represents a large part of a 'hidden economy' which is fuelled by unemployment, urbanization and the high cultural value of natural, traditional medicines. Demand for the raw material for these phytomedicines has extended across national boundaries, which has resulted in the trade in medicinal plant species expanding over the last decade. *P. reniforme* and *P. sidoides* now added to the medicinal trade market has presenting a potential concern for conservationists and action for sustainable management of habitats. Seventy to eighty per cent of African people consult traditional healers and most plants used in this treatment are wild harvested. This increasing demand for wild harvested tubers for medicinal use in South Africa and internationally results in a need for commercially viable cultivation methods by small scale farmers. There also exists the necessity to investigate drought-tolerant crops in a country facing on-going drought.

Keywords: antiviral activity, antifungal activity, phytomedicine, traditional medicine, commercial cultivation, antioxidant, antimicrobial, phenolic compounds

2.2 Introduction

The genus Pelargonium (Pelargos in Greek) belongs to the family Geraniaceae along with four other species, *Geranium*, *Erodium*, *Monsonia* and *Sarcocaulon* (Lawrence, 2001). Approximately 80% of the 280 *Pelargonium* species are indigenous to South Africa, with the largest concentration of species is found in the south-western Cape (Lalli *et al.*, 2008). *Pelargoniums* have a long history as ornamental and medicinal plants however, only a few studies have revealed some phenolic acids, proanthocyanidins, flavonoids, tannins and some coumarin potential (Makunga, 2015). Pelargonium species are commonly used for a wide range of ailments as infusions and concoctions of the tuberous roots by traditional healers in certain parts of South Africa (Latté & Kolodziej, 2004).

Pelargonium reniforme is a shrublet or sub-shrub with pink to purple flowers, small, cordate, velvety leaves and tuberous roots (Jones & Adams, 2011). It is found in the grasslands and dry flats between Knysna and Umtata in the Eastern Cape Province of South Africa (Van Der Walt, 1997). *P. sidoides* is an aromatic sub-shrub with evergreen foliage when cultivated dying back to varying levels when grown in the wild. The plant forms a rosette-like arrangement shape and is distinguishable by its small almost black flowers, long-stalked crowded, cordate, slightly aromatic leaves and thick, dark brown underground roots (Lawrence, 2001: Lewu *et al.*, 2007). The species has a wide distribution throughout South Africa, and occurs from the Eastern Cape to Mpumalanga and Lesotho with a distribution range of 600,000 km² (Newton *et al.*, 2011). *P. sidoides* is considered extremely valuable to traditional healers and commercial producers of natural medicine alike (Maree & Viljoen, 2012). *P. reniforme* and *P. sidoides* are very similar in growth apart from flower differences; however *P. sidoides* appears to have a greater value for medicinal properties (Solomon, 2015).

Traditional medicine is important culturally to a large South African population as it is utilised for physical and mental health purposes (Cunningham, 1998). The past twenty years have seen a dramatic change in the international trade in natural products for both medicinal and other uses, with the commercialisation of products by richer western countries; however, this has not necessarily benefited the people of the country of origin (Van Niekerk & Wynberg, 2012). Due to the efficiency of these two species there is a continued increase in use of these medicinal plants and as a result the trade in medicinal plants threatens these species with extinction in the wild (Locher *et al.*, 1995; Cunningham, 1998; Lange, 1997). As the demand for natural medicine increases by the west, and the indiscriminate harvesting of

plant material from the wild continues, a real possibility exists for these *Pelargoniums* to be lost to future generations (Schippmann *et al.*, 2002). As long as wild-harvesting of valuable medicinal plants continues, the feasibility of commercial production has to be addressed in order to satisfy the growing demand for natural medicine from the developed of the world (Moyo *et al.*, 2013).

Figure 2.1: *Pelargonium reniforme* flower
(Picture: http;\\ pza.sanbi.org\ perlargonium- reniforme)

Figure 2.2: *Pelargonium sidoides* flower
(Picture: https:\\www.crocus.co.uk\plants_\
perlargonium-sidoides\classid.2000010252\)

This review aims to investigate the extent of the threats posed to *P. reniforme* Curtis and *P. sidoides* DC and their commercial potential for both national and international markets in order to preserve them for future generations.

2.3 Medicinal importance of *Pelargonium reniforme*

Organisms such as fungi and bacteria have long been responsible for many of the diseases which plague humankind (Rittman, 2012). They have also been known to escalate the progression of other diseases such as liver disease related to alcoholism (Adewusi & Afolayan, 2009). In spite of the common use of antibiotics, bacterial infections are behind almost 25% of deaths in patients with liver disease (Wyke, 1987). Adewusi & Afolayan (2009) discovered that three different extracts (acetone, methanol and water) of the roots and leaves of *P. reniforme* showed inhibitory activity against a wide range of commonly found bacteria, particularly against the gram-positive organisms. The same extracts showed significant activity against most of the fungi species tested, especially those extracts from the

leaves. The roots of *P. reniforme* are widely used to treat diarrhoea, chest infections and liver disease (Mattys et al., 2003: Bladt & Wagner, 2007). The therapeutic benefits of these two *Pelargoniums* have been extensively documented and studies on their efficacy against infections, in particular upper respiratory tract infections and acute bronchitis (Kolodzeij & Kayser, 1998; Kolodzeij, 2002). In an earlier study, Adewusi & Afolayan (2009) demonstrated that the root extract of *P. reniforme*, when administered to rats, resulted in an increase in total white blood cell count, which would enhance the function of phagocytes in the blood. These findings could prove a basis for the significant antifungal, antitubercular and antibacterial abilities of *P. reniforme* (Mativandlela *et al.*, 2006).

Alcohol abuse is an on-going problem in parts of the Eastern Cape Province of South Africa and with this abuse, is associated liver disease (Adewusi, 2009). Alcohol dependence and toxicity has become a major health issue in the world today (Singha *et al.*, 2007), and there is now mounting evidence that oxidative stress has an important role in the progress of alcohol induced liver disease (Zima *et al.*, 2001). Heavy and prolonged alcohol consumption is directly linked to alcohol-related liver damage as 80% of ingested alcohol is metabolized by the liver into cytotoxic acetaldehyde resulting in the formation of reactive oxygen species (ROS) which are chemically reactive molecules containing oxygen (Tuma & Cassey, 2003). A survey conducted in the Nkonkobe district in the Eastern Cape showed that *P. reniforme* is used frequently by the local Xhosa people for the treatment of alcohol-induced liver damage (Adewusi, 2009). Studies conducted on male Wistar rats using the aqueous root extract of *P. reniforme* showed significant antioxidant activity on the liver tissue which displayed alcohol-induced hepatotoxicity (Adewusi & Afolayan, 2009). Phenolic compounds (tannins and flavonoids) isolated from *P. reniforme* in a study (2004) conducted by Latte & Kolodzeij were proven to possess marked antioxidant effects which showed encouraging possibilities for the treatment of liver disorders. Several studies have been conducted on medicinally valuable plants which have known antioxidant capacities and all have proven hepatoprotective qualities (Latte & Kolodziej, 2004). Verbal information from traditional healers from the Eastern Cape province of South Africa confirms that the fleshy roots of *P. reniforme* have been used for centuries for the treatment of liver disease as a result of alcohol abuse (Adewusi, 2009). A study by Latté & Kolodziej, (2004), *P. reniforme* demonstrated considerable antioxidant activity with hepatoprotective potential. There is therefore a strong basis for further studies into specifically *P.reniforme* with the emphasis being on the identification of the antioxidant capacity it possesses and therefore the potential use as phytomedicinal agents.

2.4 Medicinal importance of *Pelargonium sidoides*

Plants have been recognised as having medical significance since ancient times and have enormous potential for traditional medicine and for modern pharmaceutical drugs (Saraswathi et al., 2011). Much of the world population relies on traditional plant-based medicines for their primary medical care due to poverty and distance from clinical medicine (Balunas & Kinghom, 2005). The importance of *Pelargonium* species, in particular *P. sidoides* has been studied and documented extensively over the past three decades (Kolodziej & Kiderlen, 2007). *Pelargonium* species are used on a large scale in South Africa by traditional healers for a variety of ailments such as respiratory tract infections, diarrhoea and liver disease. *Pelargonium sidoides* is highly valued in South Africa as well as internationally as it has made a large and important contribution to the medical care of a large number of the indigenous population of South Africa,

The roots of *P. sidoides* DC are widely used to treat dysentery, diarrhoea, chest infections and liver disease (Watt & Breyer-Brandwijk, 1962; Mattys *et al*., 2003; Bladt & Wagner, 2007; Kolodziej, 2011). The therapeutic benefits of this species have been extensively documented and there have been many studies on their efficacy against infections, in particular upper respiratory tract infections and acute bronchitis (Kolodzeij & Kayser, 1998; Kolodzeij, 2002. The tuberous roots of this species are sold to a German phytopharmaceutical company for the preparation of an ethanolic extract known and patented as Umckaloabo® (Maree & Viljoen, 2012). This extract is widely used throughout the first world for the treatment of upper respiratory tract infections (URTI) and bronchitis (Kolodziej & Kiderlen, 2007). There is now an aqueous-ethanolic preparation of the tuberous roots of *P. sidoides* which has been patented as EPs® 7630, and EPs® 7630 is made up mainly of polyphenols and anthocyanidins. Both these compounds have demonstrated antibacterial activity (Schötz and Nöldner, 2007). Clinical investigations have demonstrated the efficacy of this extract in the treatment of URTIs. EPs® 7630 was found to develop anti-infective properties against group A-streptococci and host epithelia which provides a strong rationale for its use in the treatment of diseases such as bronchitis and URTIs (Conrad *et al*., 2007). In a study by Kolodziej & Kinderlen in 2007, phenolic and methanolic root extracts of *P. sidoides* were found to show inhibitory action (96%) against *Mycobacterium tuberculosis.* This study also demonstrated that, as respiratory tract infections are often associated with viruses, and the control thereof is initiated by interferons (IFNs) produced by the host cells, the direct antibacterial activity suggests that the action of the hosts immune system could be one of the contributing factors to the efficacy of EPs® 7630. This ethanolic root extract has

been authorised to be marketed by the German regulatory agency for patented drugs (Conrad *et al*., 2007).

The root extract from *P. sidoides* has also been found to significantly limit the severity of URTIs and also reduce the frequency of asthma attacks in a study by Tahan and Yaman (2013) involving children aged 1 to 14 years old. There exists a need for new anti-influenza drugs as matrix protein inhibitors are not advisable for use against epidemic and seasonal influenza as a result of resistance (Fiore *et al*., 2011). Much phytochemical work exists on the constituents of *P. sidoides* and its relevance in the treatment of respiratory tract infections and significant antibacterial activity was proven at minimum inhibitory concentrations of 220-2000 µg/ml (Kolodziej *et al*., 2003). Current and historical research and studies have shown pharmacological activity such as moderate broad-spectrum antibacterial potential and marked immune modulatory potential which provides strong evidence for the medicinal use of EPs 7630® in the treatment of respiratory tract infections and lung infections (Kolodziej *et al.*, 2003). With the current swing to more naturally based medication in many parts of the world it is important to understand the medical potential of these *Pelargoniums.*

2.5 Threatened wild status of P*. reniforme* and P*. sidoides*

South Africa's first official list of endangered or threatened plant species was completed in 2009, with over 300 plant species listed as "in danger". Many of these species were found to exist in areas which were well known to botanists, such as the Western Cape. Many of them also were found to occur in specialized micro-habitats ranging as far as the Overberg (Williams *et al.*, 2013). Over 40% of these species are now in danger of extinction, with five of them considered already extinct. This emphasises the importance of on-going research and botanical updates, particularly of the national plant Red Data List (Williams *et al.*, 2013). *P. reniforme and P. sidoides* are currently listed as LC (Least Concern). However, a study conducted by Dold & Cocks in 2002 concluded that *P. reniforme* was, at that stage, heavily traded and unsustainably harvested in the Eastern Cape. *P. sidoides* is listed as "declining" on the Red Data List of South African plant species and is therefore in danger of becoming extinct as a result of overharvesting from wild populations (Moyo *et al*., 2013). In a study conducted in the Eastern Cape Province of South Africa in 2007 by Lewu et al., all respondents to the survey on wild harvesting of *P. reniforme* and *P. sidoides*, agreed that these species have become scarce in the wild and more difficult to find. Population decline of *P. sidoides* is taking place as a result of habitat destruction for agriculture and overgrazing

(Brassine, 2011). These threats are more serious than the problem of overharvesting (De Castro *et al.*, 2012). Added to this, wild plants are being increasing threatened by logging, agriculture and human settlement (Cunningham, 1993). Consequently, there remains a strong recommendation that threatened plant species be commercially cultivated (Lambert *et al.*, 1997). Africa, in particular southern Africa, possesses a wide diversity of plants, many of whom are medicinally valuable. Until the present, only a handful of these species have been commercially cultivated (Van Wyk, 2008). This has resulted in a discussion as to the practicality of large scale cultivation of medicinal plants to supply the growing South African and international market for plant-based products (Moyo *et al.*, 2013).

German based pharmaceutical companies produce an ethanolic extract from the roots of *P. reniforme* and *P. sidoides* which is sold in many countries around the world under the brand name Umckaloabo®. This product affords huge profit for this company, most of which does not find its way back to the local communities who they use to harvest from the wild (Jordan, 2007). A significant method of protecting over exploited plant species is to emphasise cultivation and propagation, especially in rural communities. This would in turn help to lessen poverty and its ultimate pressure on natural plant populations (Lewu *et al.*, 2006). Low viability of seed and consequent germination rates are a problem for the regeneration of wild populations (Lewu *et al*., 2006). Furthermore, the survival in the wild of these two species of medicinally valuable Pelargoniums would be guaranteed for the benefit of future biodiversity as it is important that the wild populations be carefully monitored and that steps are taken to ensure their survival to continue supply a growing market for *P. reniforme* and *P. sidoides.*

2.6 A growing market for *P. reniforme* and *P. sidoides*

Today, millions of people rely heavily upon gathered plant and animal products (Schippmann, 2002) and the use of herbal medicines is increasing significantly (Canter, 2005). The resultant demand for a wide variety of medicinal plant species is growing, along with the increase in human need and trade in these plants. This has been accompanied by an increase in research into new medicinal plant-based products as is attested to by the increase in scientific publications on the subject (Van Wyk, 2008). In order to satisfy local and international markets, the source material for the expanding markets is harvested more and more from wild populations (Lange, 1997). Growing populations of human beings and the uncontrolled collection of medicinal plant material from the wild has resulted in certain plants becoming threatened. The protection of some medicinal South African plants has become urgent (Zschocke *et al.*, 2000). Sales of commercially produced herbal medicine

and remedies has increased dramatically over the last decade while the preference for over-the-counter preparations has increased in popularity with 12% of the world's population using herbal medicine (Ndhlala *et al.*, 2011). The World Health Organisation (WHO) has developed guidelines to encourage scientific methods of evaluating herbal medicine through various declarations such as the Alma-Ata declaration (Navarro, 1984). Trends and fashions in anything change and evolve over time and it has been predicted that the major areas of growth in the herbal medicine market will be in specific areas such as fitness, weight loss and mental health products (Makunga *et al.*, 2008).

Today, South Africa is facing an upsurge in "lifestyle diseases" which are a result of an increase in non-traditional habits. Natural preparations used for the treatment of heart disease and others such as diabetes are becoming increasingly valuable (Brendler & van Wyk, 2008). In addition to these diseases, sub-Saharan Africa has been ravaged by the effects of the HIV/AIDS epidemic and the exploration of natural plant-based pharmaceutical solutions is now considered vital for developing countries with a superior technology base (Ma *et al*., 2005). The agricultural market in South Africa has always been concerned about usage of traditional crops for agricultural use (Reinten & Coetzee, 2002). New and viable crops now have to be found in order for young rural farmers to compete on a commercial level with historically traditional crops. According to Simbi and Aliber (2000), job opportunities in the agricultural sector are declining rapidly, with 140,000 permanent jobs being shed between 1988 and 1998. The growth requirements of some medicinal plants are very specific and they often demonstrate low germination rates due to fungal infections (Vines, 2004).

There are various international agreements which influence the possible commercial cultivation of indigenous plants, however, there is very little regulation with respect to Indigenous Knowledge Systems which makes working on indigenous plants difficult (Reinten & Coetzee, 2002). Apart from these issues, research and development of the process is expensive and drawn out (Wessels *et al.*, 1998). There is also a general lack of knowledge regarding the importance of cultivating medicinal indigenous plants in both the public and private sectors (Mander *et al.*, 1998). There is certainly a market in South Africa for smaller scale growers who cultivate non-traditional crops with higher potential value than the usual agricultural crops. These alternative crops are usually grown for medicinal, cosmetic or industrial purposes and generally receive a better price in the market place especially if deemed to be fair trade or organic (Makanga *et al.*, 2008). Field cultivation of medicinal *Pelargoniums* in South Africa is limited, a situation which has resulted in an increasing

demand on wild populations. While phyto-pharmaceutical companies are dependent on agricultural field production of the plant material, the serious depletion of the species in the wild continue to be a threat (Makunga, 2015). A large number of the gatherers of these species in the Eastern Cape come out of poor rural communities and have no defined income and limited access to any employment. They therefore rely on wild resources such as plant collecting for survival, either via personal use or sale (Lewu *et al.*, 2007). The market for *Pelargoniums* has grown to over 4 million tubers exported annually to United Kingdom, United States, Europe and Australia (Makunga, 2015). It is time that these species, commercial cultivation be expanded to supply the growing demand to benefit all.

2.7 Commercial cultivation of *P. reniforme* and *P. sidoides*

Commercial cultivation of any crop ensures control with respect to manipulation of phenotypes and the concentration of medicinal material, as well as maintaining a consistent product (Canter *et al.*, 2005). The desirable compounds in the plants are generally secondary metabolites which are affected in the wild by any number of environmental situations, for example water availability, temperature and soil pH (Bjorkman, 2011). This can lead to variations in quality and medicinal efficacy from wild-harvested plants (Canter *et al.*, 2005). However, cultivation of medicinal plants appears to the correct strategy for meeting the cultural perspectives in some countries which may prove to be problematic (Schippmann *et al.*, 2002). For example, in China wild ginseng roots are considered to be ten times more valuable than artificially propagated roots and in Botswana traditional healers feel that cultivated plant material is unacceptable as it does not have the effectiveness of wild-harvested plant material (Cunningham, 1998).

Pelargoniums are very robust plants and can withstand poor soil and drought conditions as they are grow naturally in arid to dry climates where they go dormant or will withstand frosts (Solomon, 2015). Some Pelargoniums have thickened, underground branch-like tubers which can withstand fires in their natural habitat (Solomon, 2015). About 80% of the South African *Pelargonium* species are confined to the winter rainfall south-western Cape (Lawrence, 2001). *P. sidoides* occurs naturally in areas with moderate summer temperatures and winter frost to possible snow. The species requires less water in winter, while watering is more necessary during the summer months. Plants respond well to slow release fertilizers and mulching to keep the roots cool (Lawrence, 2001). As for most Pelargoniums*, P. reniforme* is propagated from cuttings throughout the year (Jones & Adams, 2011). Lawrence (2001) indicated that autumn is more preferable to propagate *P. sidoides.*

Pelargonium roots easily stem from cuttings using a rooting hormone and planted in river sand in a shaded cold frame area for a period of 4 weeks (Mithila *et al.*, 2001; Jones & Adams, 2011). However, this process involves the maintenance of a large number of healthy stock plants and could lead to the spread of fungal and bacterial disease (Mastalerz, 1971). Rooted cuttings prefer a sunning position once planted out and respond positively to liquid organic fertilizers at monthly intervals (Jones & Adams, 2011).

The benefits of commercial production of medicinal plants are well documented. Techniques for *in vitro* propagation of valuable plant species for commercial production have improved significantly over the last two decades to a point where this method can be used successfully in commercial cultivation where more conventional techniques have been less successful (Lakshmana Rao, 1994). Makunga (2015) reported that tissue culture plantlets of medicinal *Pelargonium* would establish tubers within 6 months and could offer a continuous year-round production in a shorter possible time with mass production in automated systems. Furthermore, plants can be maintained to produce top quality phyto-pharmaceutical material to access key compounds for commercial product formulation and thus reduce the threat on wild harvesting (Makunga, 2015). Pelargoniums can also be propagated from seed sown in late summer to autumn and will germinate in 4 weeks (Jones & Adams, 2011) although plants propagated vegetatively from selected stock is more likely to produce similar coumarin profiles for phyto-pharmaceutical products and also disease- free plants (Jones & Adams, 2011; Makunga, 2015). Cultivation and harvesting of medicinal *Pelargonium* species would help to support local livelihoods and ensure meaningful participation by those with the traditional knowledge. This would ensure that the production of Umckaloabo® be limited to South Africa and Lesotho who would make it available to the international market to the benefit of the South African economy (Jordan, 2007).

Hydroculture cultivation could have several benefits to produce medicinal plants. Even though no record could be found on successful cultivation of Pelargoniums in hydroponics, several other studies reported positive results for medicinal species. Jaziri *et al.*, (1993) highlighted the success of obtaining the highest artemisinin in hydroponic cultivated *Artemisia annua*. *Ocimum basilicum* L. responded well in hydroponic cultivation with an adequate content of rosmarinic acid. While it is also beneficial to harvest the roots which contained higher levels of rosmarinic acid compared to the leaves (Maggini *et al.*, 2011). Dorais *et al.*, (2001) reported in a study of 6 medicinal species (*Achillea millefolium*, *Artemisia vulgaris*, *Inula helenium*, *Stellaria media*, *Taraxacum officinalis*, and *Valeriana officinalis*) grown hydroponically, using a floating raft system, that all species showed higher

dry root and shoot weight compared to field grown plants. Hydroponics is recommended to be widely used in future medicinal plant studies (Zeng *et al.*, 2007). The possibilities of increasing commercial cultivation of *Pelargonium* species such as *P. reniforme* and *P. sidoides* have great potential to benefit both humans and plant species.

2.8 Conclusion

A great amount of cultural significance is attached to medicinal plants worldwide, especially in developing countries. Today the trend is moving away from clinical western medicine and towards more natural and organic methods of treatment. Recently, there has been an increase in research and development into medicinal plants which has resulted in various new products and plant discoveries. Approximately 10.8% of the indigenous African flora is currently used as traditional medicine (Neuwinger, 2000). Ethanolic extracts of *P. reniforme* and *P. sidoides* have demonstrated significant medicinal and antioxidant potential in numerous studies (Street & Prinsloo, 2013). In a country such as South Africa where a large part of the rural population exist below the poverty line there is a great need to create new opportunities for the disadvantaged small scale farmer (Van Wyk, 2011). Given the fact that these plants are facing possible extinction due to habitat destruction and overharvesting, there exists an urgent need in South Africa for more research into commercial cultivation of these valuable species, and investigation into optimal propagation and growth requirements.

2.9 Acknowledgements

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CHAPTER THREE

EFFECT OF DIFFERENT WATERING FREQUENCIES AND ARBUSCULAR MYCORRHIZA ON THE GROWTH, LEAF CHLOROPHYLL AND NUTRIENT UPTAKE OF *PELARGONIUM RENIFORME* CURTIS

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3.1 ABSTRACT

Pelargonium reniforme Curtis is an herbaceous groundcover indigenous to the Western Cape of South Africa with economic importance as a traditional medicinal species. This study was carried out to measure the effects of different applications of irrigation frequencies on plant growth of *Pelargonium reniforme* Curtis as well as the effects on plant growth with and without the addition of arbuscular mycorrhiza (AM) to the growing medium. The experiment was conducted over a period of 15 weeks when a total of ten treatments were applied to ten replicates. Irrigation frequencies comprised of 320 mls of water from once a day to every twenty- four days. Half of the treatments were inoculated with 30 g of AM and the other half were not. Results of the of the water frequency test showed statistical significant variance at P≤ 0.001 in all variables but the wet root weight. Significant difference was observed in the mycorrhiza inoculation in only the total wet weight measure (P≤ 0.001). A more significant (P≤ 0.05) observation was seen in the results with water frequency and mycorrhiza treatment in the wet root weight. Results on measuring C, K, Mg, N and P were all significant (P ≤0.001) in the watering frequency treatments without mycorrhiza but only significant with mycorrhiza (P ≤0.05) in Mg availability. Similarly, there was also a higher significant interaction (P ≤0.05) between the watering frequency without and with mycorrhiza in the availability of Mg. Total dry weight was the highest in the highest watering frequency for *P. reniforme*. Increased chlorophyll production was seen at the higher watering frequencies. The addition of arbuscular mycorrhiza generally increased growth parameters, photosynthesis and water assimilation.

Key words: irrigation, plant growth, traditional medicine, water deficit, water stress, arbuscular mycorrhiza, AM

Clarification of terms: The terms 'fresh' and 'wet' are used interchangeably when referring to plant weight.

3.2 INTRODUCTION

Traditional healing medicines are used by a great proportion of South Africa's indigenous people to address their health requirements. The indigenous knowledge and medicinally valuable plants have therefore become important considerations for future scientific developments (Rabe & van Staden, 1997; Cunningham, 1988). While herbal preparations form a vital part of the daily lives of many South African urban and rural populations (Fennell et al., 2004), medicinal plant research and related discovery of new compounds continue to provide challenges for possible treatments against cancers, HIV aids, arthritis, liver disease and many other conditions (Balunas & Kinghorn, 2005). Recordings of a global move towards the use of more natural medicine in the West have shown 62% of Americans using complimentary medicines by 2002 (Makunga *et al*., 2008). It was further estimated by the World Health Organisation that as many as 70-80% of developing countries use traditional medicines for health conditions (Olsen & Helles, 1997). This applies equally to South Africa, where traditional treatments are considered by many indigenous people as complimentary to or even superior to western medicine (Cunningham, 1988).

The focus on indigenous plants as a re-emerging source of primary health care has been largely attributed to the rising cost of clinical medication and health care (Hoareau & Da Silva, 1999). Furthermore, traditional medicine encompasses many different types of health practices and various cultural beliefs and inherited knowledge (Aziz & Wright, 2005). It approaches health problems in a holistic manner, contrary to Western clinical medicine (Anyinam, 1987: Hedberg & Hedberg, 1982). A global resurgence of the use of more natural medicines has recently emerged and alternative medicines are fast gaining popularity in Western countries (Makunga *et al*. 2008). Approximately 80% of the South African population make use of natural traditional preparations as their primary health care (Street & Prinsloo, 2012). In context, Africa possesses an extremely high number of indigenous plant species, with about 25% of higher plants of the world being native to sub-Saharan Africa (Klopper *et al*., 2006). This diversity is a valuable resource for continued scientific studies high reaching commercial potential (Van Wyk, 2008).

Various medicinal species in South Africa have shown promising scientific potential. The genus *Pelargonium* (Geraniaceae) is one such important genera which has been documented to possess many medicinal properties (Watt & Breyer-Brandwijk, 1962). Data based studies describe the genus *Pelargonium* to consist of over 250 species of shrubs and perennial sub-shrubs with specific geographical distribution, with 80% of species indigenous to the southern parts of Africa (Van der Walt & Vorster, 1985). Two *Pelargonium* species, namely *P. reniforme* Curtis and *P. sidoides* DC, are important sources of a wide diversity and complexity of phenolic compounds and other valuable metabolites (Kolodziej, 2007). Both species contain many pharmacological attributes which are used to supply international markets. Metabolites of *P. reniforme* and *P. sidoides* are used for the manufacturing of a herbal medicine Umckaloabo® as one such example (Van Wyk & Gericke, 2000; Saraswathi *et al*., 2011).

3.2.1 Medicinal value of P. reniforme

P. reniforme has been found to possess significant antimicrobial and antioxidant properties which have led to its use in traditional medicine to treat liver disease (Adewusi & Afolayan, 2009). Root extracts of *P. reniforme* have been used traditionally for many years by South African indigenous people to treatment tuberculosis and there is sufficient evidence of the effectiveness of the treatments (Seidel & Taylor, 2003). Tuberculosis is recognised to be one of the major causes of human death, with a resurgence of the disease during the past two decades. There seems to be a growing number, approximately eight million diagnosed cases and 200,000 reported deaths annually worldwide possibly due to the emergence of drug resistant strains of tuberculosis which has impacted negatively on its treatment and control (WHO, 2001).

A study by Adewusi & Afolayan in 2009 demonstrated that the root extract of *P. reniforme,* when administered to rats, resulted in an increase in total white blood cell count, which would enhance the function of phagocytes in the blood i.e. that of ingesting and destroying microorganisms. These findings could prove a basis for the significant antifungal, antitubercular and antibacterial abilities of *P. reniforme* (Mativandlela *et al*., 2006). Considering the medicinal importance of this species, further cultivation studies are warranted to improve plant quality to support future commercial market demands. Little is known about the relationship of medicinal species and mycorrhiza benefits to enhance these species. Arbuscular Mycorrhiza (AM) is a widely spread symbiotic relationship between host plants and fungi belonging the phylum Glomeromycota. It enables the host plant to take up

water and nutrients such as nitrogen and phosphorus more efficiently. In this relationship, 20% of plant-stored carbon is utilised by the fungus (Parniske, 2008). A mycorrhizal association is a symbiotic interaction between the roots of plants and certain fungi in the soil which plays a pivotal part in cycling of nutrients in soil ecosystems. The majority of the plant families are capable of forming mycorrhizal relationships with the most common type being the AM type (Azcon-Aguilar & Barea, 1996). The root zones of almost all plants live symbiotically with soil fungi, some of which are termed mycorrhiza. These fungi colonise the cortex of the roots developing a mycelium which enables the plant to take up extra mineral nutrients (Jeffries & Barea, 1994). The mycorrhizal mycelium and various other soil flora form water-stable aggregates which improve the soil quality (Bethlenfalvay & Schüepp, 1994). Many valuable horticultural crops and ornamental plants form mycorrhizal associations with these plant species often displaying mycotrophy. As a result, their optimal growth and development becomes dependant on early AM establishment (Gianinazzzi, 1996). AM can also increase the amount of soil that is available to plant root zones and as a result, colonisation of roots with AM frequently results in increased uptake of immobile nutrients. AM aid with the uptake of macro and micro nutrients which are important for vital metabolic processes and growth (Feldmann *et al*., 1989).

The use of natural resources, or biological control, in agriculture today is a key practice and certain organisms which exist naturally in the root zone of plants have been reported to possess antagonistic activity against harmful organisms such as nematodes, bacterial and fungal spores. AM has been found to significantly limit the damage caused by these types of soil-borne pathogens, some species being more effective than others (Azcon-Aguilar & Barea, 1996). Simultaneously large quantities of nutrients are lost in soils due to leaching and as gases. These losses can negatively impact on production (Cavagnaro *et al*., 2015). AM have been shown to improve nutrient acquisition and reduce nutrient loss by expanding the availability zone of nutrients to the root zone and helping to lessen nutrient loss after leaching following rain or over-irrigation (Cavagnaro *et al*., 2015). It has also been documented that most vegetable plants are able to host AM fungi, which has been shown to improve the supplies of water and nutrients, improve tolerance of various environmental stress factors and aid in resistance of nematode damage and root diseases (Baum *et al*., 2015).

This study therefore aimed to investigate the effect of water stress in cultivation and AM on the growth parameters of medicinal species *P. reniforme* Curtis.

3.3 MATERIALS AND METHODS

3.3.1 Greenhouse experiment

The experiment was conducted from June to September 2014. It was located in the research greenhouse at the Cape Peninsula University of Technology, Cape Town, South Africa with the GPS co-ordinates - 33° 55ˈ 58.27S, 18° 25ˈ 57.04E. This facility ensured that the environment in which the experiment was conducted was controlled. Minimum and maximum temperatures and relative humidity were recorded 3 times daily. Temperatures within the greenhouse ranged between 14 - 34°C during daytime and 10 - 19°C at night. Relative humidity of the facility averaged 37%. The water used in the experiment was municipal water filtered through a Hager IP 65 Water Filtration Plant de-ioniser.

3.3.2 Plant preparation

The plant sample consisted of *P. reniforme* stock plants obtained from Trevor Adams, horticulturist at Kirstenbosch Botanical Gardens, Cape Town, South Africa. One hundred and fifty uniform cuttings were made using the stock material and placed in cutting trays containing washed and sterilized coarse river sand (Lawrence, 2001). The cutting trays were then placed in the main greenhouse on the Cape Town campus of the Cape Peninsula University of Technology on heated propagation beds. Once rooted, one hundred *P. reniforme* plants of uniform size were then planted into individual 12.5cm pots containing an inert, sterilized medium (2:1:1:1) consisting of two parts coco peat, one part foamalite, one part Consol® silica sand grade 6/17 and one part perlite. The plants were then placed under 40% shade cloth for one week to harden off before being planted out into the experimental site. The plants were placed onto galvanized steel tables covered in black plastic sheeting with ten replicates of each treatment.

3.3.3 Experimental treatments

The experiment consisted of five watering regimes which were applied to fifty plants of the species *P. reniforme* without inoculation with arbuscular mycorrhiza and to fifty plants of *P. reniforme* in conjunction with inoculation with arbuscular mycorrhiza. There were therefore a total of one hundred plants of species *P. reniforme* with fifty receiving mycorrhiza and fifty not receiving mycorrhiza upon planting out into the experiment. A randomized block design, made up of 10 replicates each with 10 individual plants was used to study the effects of

various irrigation frequencies with and without mycorrhiza inoculation on *P. reniforme.* Drip irrigation was used with the following irrigation frequencies based on experiments conducted by Wang *et al*., (2003) on the effect of different drip irrigation frequencies on potato growth and yield:

1) WF1 (320 mls once every day to PC). Control treatment (Kirnak *et al*., 2001).
2) WF2 (320 mls once every three days to PC
3) WF3 (320 mls once every six days to PC)
4) WF4 (320 mls once every twelve days to PC)
5) WF5 (320 mls once every twenty-four days to PC)

The experiment consisted of the following treatments:

Group A: *P. reniforme* without mycorrhiza receiving WF1, WF2, WF3, WF4 and WF5.

Group B: *P. reniforme* with mycorrhiza receiving WF1M, WF2M, WF3M, WF4M and WF5M.

Plants which received mycorrhiza were inoculated with 30 grams of AM (commercially available product Mycoroot™ obtained from the Horti Shop, 125 Belvedere Road, Claremont, Cape Town) on planting. Each individual pot was irrigated with half-strength Hoaglands solution (Recipe for Hoaglands Solution : nitrogen 210 ppm, potassium 235 ppm, calcium 200 ppm, phosphorus 31 ppm, sodium 64 ppm, magnesium 48 ppm, boron 0.5 ppm, iron 1-5 ppm, manganese 0.5 ppm, zinc 0.05 ppm, copper 0.02 ppm and molybdenum 0.01 ppm) (Anonymous, 2017). A half-strength nutrient solution was used due to the reduced nutrient requirements of South African fynbos species in the wild (Midgley *et al*., 1995). Pot capacity was determined at 320 millilitres.

3.3.4 Acclimatization of experimental plants

Rooted cuttings from *P. reniforme* gradually acclimatized in the sterilized growing medium (2:1:1:1) of two parts coco peat, one part foamalite, one part Consol® silica sand, grade 6/17 sand and one part perlite under 40% shade cloth for one week to harden off before being moved to the experimental site.

3.3.5 Hardening-off and growing period

The plants were kept for one week adapting to the greenhouse situation after hardening off. The experiment was conducted from the second of June 2014 until the eighteenth of September 2014. After this period all plants were harvested and various postharvest measurements were measured. Parameters measured included shoot length, root and shoot wet weights and root and shoot dry weights.

3.3.6 Determination of plant growth

3.3.6.1 Plant weight

The weight of plants was measured using a standard laboratory scale before planting out on 2 June 2014 to ensure homogeneity within the sample. Post-harvest, shoot and root systems were separated and individual samples' fresh/ wet weights were recorded. The plant material was then oven dried at 55 ^{o}C in a LABTECH™ model LDO 150F (Daihan Labtech India. Pty. Ltd. 3269 Ranjit Nagar, New Dehli, 110008) oven until all water was removed from the material and a constant weight was reached; the dry weights were measured and recorded. The difference between the wet and dry weights correlates with the amount of water held within the plants' tissues.

3.3.6.2 Shoot length

The shoot length was used as a variable to determine height and internode growth. Shoot length was measured (mm) weekly with a standard ruler and recorded on a data sheet.

3.3.6.3 Chlorophyll content of leaves

The chlorophyll content was measured weekly using a SPAD-502 meter supplied by Konica-Minolta. This device measures transmission of red light at 650 nm, (the frequency at which chlorophyll absorbs light) and transmission of infrared light at 940 nm (at which no absorption occurs). Using these two transmission values the instrument calculates a SPAD (Soil Plant Analysis Development) level which is indicative of chlorophyll content. The readings of two fully formed leaves were taken from each plant and the figures were averaged out by the SPAD-502 meter to produce a final number. The readings were taken between 11 am and midday from weeks 11 to 15 of the experiment with average daylight levels of 10 kLux (light intensity).

3.3.7 Determination of nutrient uptake from dry plant material

In order to determine the nutrient uptake of each set of replicates in the experiment, three plants were randomly selected from each set at the end of week 16 the vegetative material

was removed and labelled and sent to Bemlab Laboratory, 16 van der Berg Crescent, Gant's Centre, Strand, Cape Town. The methodology to determine macronutrients (N, K, P, Ca, Mg and Na) and micronutrients (Cu, Zn, Mn, Fe, Al and B) was conducted by ashing 1g ground sample of plant material in a porcelain crucible at 500°C overnight. This was followed by dissolving the ash in 5mL of HCl and placing it in an oven at 50°C for 30 min. Thirty-five millilitres of deionised water was then added and the extract filtered through Whatman no, 1 filter paper. Nutrient concentrations in plant extracts were determined using an inductively-coupled plasma (ICP) emission spectrophotometer (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusettes, USA), (Giron, 1973).

3.3.8 Statistical analysis

All data was analysed using two-way analysis of variance (ANOVA), using the computing software program STATISTICA 13.2. Occurrence of statistical difference was determined by using the Fisher Least Significance Difference (L.S.D.) at values of P ≤0.05; P ≤0.01 and P ≤0.001 levels of significance (Steel & Torrie, 1980).

Figure 3.1 Experimental layout showing replicates.

Figure 3.2 Plant response to WF2 at week 5 of the experiment.

3.4 RESULTS AND DISCUSSION

The experiment was conducted to determine the relationship between different water frequencies with mycorrhiza (50 plants) and without mycorrhiza inoculation (50 plants) and revealed varied significant results. Within the drip irrigation the five watering regimes varied between daily applications (control), every 3 days, every 6 days, every 12 days and every 24 days (at 320mls per treatment) which were conducted on the species *P. reniforme.* Results of the water frequency test showed statistical significant variance at P≤ 0.001 in all variables with the exception of the wet root weight (see Table 3.1).

3.4.1 Wet shoot weight

The wet shoot weight showed significance at P≤ 0.001 in the water frequency treatments 1 and 2 with and without mycorrhiza (WF1, WF1M, WF2, WF2M) additions. The means in the treatment WF1M indicated the highest measurement of 13.77 however the highest wet shoot measurement was obtained at 34.40 g in the WF1 treatment with no mycorrhiza. A study in 2008 by Eman *et al.* revealed that *Thymus vulgaris* plants produced the highest wet weights of shoot material under a frequent irrigation regime in a field experiment. Both the mycorrhiza and water frequency vs mycorrhiza showed no significance (Table 3.1).

3.4.2 Dry shoot weight

In Table 3.1 dry shoot weight showed significance at P≤0.001 in watering frequencies 1, 2 and 3 (WF1, WF1M, WF2, WF2M, WF3 and WF3M treatments. Means for these treatments measured from 2.42 (WF1M), the highest to 1.3 (WF1), the lowest. No differences were observed in treatments with and without the addition of mycorrhiza. This contradicts the findings of a study in 2000 by Pandey *et al*. in which deficit irrigation on maize plants resulted in reduced growth rate, plant height and shoot dry matter.

3.4.3 Wet root weight

Interesting observations in Table 3.1 showed that the wet root weight was not significant in any of the water frequency treatments with or without mycorrhiza additions. Root length at harvest showed that Treatment 4 (WF4) produced the longest roots compared to other treatments. Root growth of a wheat (*Triticum aestivum*) var.'Thatcher', penetrated the growing medium more aggressively and measured longer in water deficit soil in an experiment conducted by Hurd in 1967. This suggests that a lack of water can result in more root growth, possibly a survival mechanism by some species.

3.4.4 Dry root weight

Water frequencies 1, 2, 3 and 5 (WF1M, WF1, WF2M, WF2, WF3M, WF3, WF4M, WF4) were the most significant in contributing to higher dry root weight at P≤ 0.001 significance. The highest weight 0.96 g was measured in the water frequency 3 (WF3) treatment without mycorrhiza and 0.85 g with mycorrhiza (WF3M) followed by water frequency 5 at a mean of 0.94 g. Frequencies 1 and 2 (WF1, WFM1, WF2, WF2M) measured lower but were still significant at P≤ 0.001 (Table 3.1). Root size, weight and surface area has been found to be positively influenced by mycorrhizal associations and sufficient supply of water (Marschener, 1998). This is also confirmed by a study in 1983 by Biermann and Lindeman in which plant growth was measured in examples of *Pelargonium* X *hortorum*. Both shoot and root weights were found to be higher after mycorrhizal inoculation at planting.

3.4.5 Shoot length

The highest shoot length was only significant (P≤ 0.001) in the water frequency without mycorrhiza treatment 5 (WF5) with a shoot length of 31.63 mm. Both the application of mycorrhiza and the water frequency vs mycorrhiza had no statistical significance (See Table 3.2). This finding contradicts that of Bierman and Lindeman (1983) cited in the previous paragraph where inoculation with AM with daily watering enhanced shoot growth. It also contradicts the findings of Mofokeng et al. (2015) whose work on *Pelargonium sidoides* growth responses to different irrigation levels showed that high irrigation resulted in significantly increased plant height and wet root yield.

3.4.6 Total wet weight

Table 3.2 shows the total wet weight measured with the highest significance in watering frequency 3 (WF3, WF3M) with and without mycorrhiza addition. The highest measure was obtained in the no mycorrhiza (WF3) addition at 157.85 g. The addition of mycorrhiza measured 151.09 g in the water frequency treatment 3 (WF3M). Therefore, significance levels occurred at P≤ 0.001 in both these treatments and a significance level to be most effective at P≤ 0.05 in the water frequency vs the mycorrhiza applications. Many studies have been performed regarding the significance of AM in the growth and quality of crops as they have various positive effects on nutrient uptake and changes in plant morphology (Baum et al., 2015). *Pelargonium* plants were found to demonstrate increased leaf areas, leaf weights and root and shoot weights with inoculation with AM in a study by Biermann & Linderman, (1983). Inoculation with *Glomus fasciculatum* at transplant stage resulted in higher growth rates compared to the nonmycorrhizal plants. The ability of AM fungi to prevent the invasion of plant pathogens and therefore improve plant growth has been studied extensively (Azcón-Aguilar & Barea, 1996) while Saleh and Al-Raddad (1987) documented significant increases in root, shoot and total fresh weight in okra plants due to AM inoculation.

3.4.7 Total dry weight

Water frequencies 1 to 4 (WF1, WF1M, WF2, WF2M, WF3, WF3M, WF4, WF4M) with and without mycorrhiza additions showed the highest significance (P≤ 0.001) in the total weight measurements. The means varied between 3.02 and 1.45. The water frequency (WF1) with no mycorrhiza measured the highest at 6.49 grams (Table 3.2). Usually, a higher irrigation

level and frequency will result in better conditions for water availability in the soil and for root uptake (Segal *et al*., 2000) as demonstrated in experimental work on potato tubers. A higher rate of irrigation has been shown to be consistent with more positive responses and in many crop plants (Freeman *et al*., 1976).

3.4.8 Nutrient measurements

The measurements to determine the macronutrients (N, K, P, Ca, Mg and Na) and micronutrients (Cu, Zn, Mn, Fe, Al and B) in plant extracts of *P. reniforme* were successfully determined with the use of an emission spectrophotometer. Results on measuring C, K, Mg, N and P were all significant (P ≤0.001) in the watering frequency treatments without mycorrhiza (Table 3.3). It is however interesting that the watering frequency with mycorrhiza was only but highly significant (P ≤0.05) in Mg availability. Similarly, there was also a higher significant interaction (P ≤0.05) between the watering frequency without and with mycorrhiza in the availability of Mg. These results were measured in watering frequency treatment 3 (WF3, WF3M). (See Table 3.3).

3.4.8.1 The effect of magnesium availability and uptake

Magnesium became more available with less availability of water to plants. A level of significance P ≤0.001 were measured only in treatment 3 with and without mycorrhiza addition (WF3, WF3M). A possible approach to lessen crop loss as a result of drought is the application of Mg which plays many important roles in plant physiology such as chlorophyll formation, enzyme activation, protein synthesis and energy transfer (Thalooth *et al*., 2006). Magnesium is a vital element in the production of chlorophyll in plants, however documented research remains minimal. There is a significant influence of Mg on other ions, for example potassium (Fageria, 1973). Giri and Mukerji (2004) reported that mycorrhiza inoculated seedings displayed a higher concentration of Mg in their tissue. The absorption of Mg and K is interdependent as earlier results (Fageria, 1973), demonstrated. In this relationship, Mg also inhibits the uptake of potassium and calcium. It has been widely shown that magnesium and potassium behave in a competitive manner with regards to uptake in the root zone (Emmert, 1962).

3.4.8.2 The effect of calcium availability and uptake

Calcium was more significant in both the watering frequency with and without mycorrhiza in treatments 1 to 2 (WF1, WF1M, WF2, WF2M) at level P ≤0.001 (Table 3.3). The measurements all these treatments were very similar in reading at the same significance. The results indicate that more watering in treatments 1 and 2 compared to treatments 3 to 5 resulted in higher Ca availability. Ca plays a vital role in many areas of plant physiology and is involved in plant reactions to stresses, as well as controlling numerous important processes (Cabañero *et al.*, 2004). Disorders in plants as a result of Ca deficiency are becoming increasingly important and numerous soil and plant nutrient studies have found that even when the soil contains enough Ca, there can still be deficiencies (Armstrong & Kirkby, 1979; Kirkby, 1979). The availability and uptake of Ca to the plant remains an important area of research. In 1953, Hylmö reported that 75% of the total uptake of Ca by pea plants occurred with high irrigation rates (Hylmö, 1953), whilst the uptake of Ca is limited by lack of availability of water (Ehret & Ho, 1986).

3.4.8.3 The effect of nitrogen availability and uptake

It was expected to see higher readings of N with increased watering frequencies. These results were measured in treatments 1 and 2 (WF1, WF1M, WF2, WF2). The results show that more water availability in the root zone will increase the availability of N levels for plant growth. Water and nutrients are highly important factors that affect biomass and growth of plants (Mofokeng *et al*., 2015). Water deficit conditions reduce N availability in the soil and have a direct negative impact on tissue growth (Lemaire *et al*., 1996). N is necessary to increase the leaf surface area which determines a plants rate of photosynthesis and the manufacture of chlorophyll by the leaves which are the most contributing organs in the determination of yield (Monyo & Whittington, 1973). In N deficient plants, there is a lack of green colour in the leaves, a decrease in leaf surface area and a reduction in photosynthesis. Plants absorb inorganic nitrogen from the water in the root zone, therefore the destination of the nitrogen is linked to water being in the soil root zone. (Alva *et al*., 2006). A study in 2015 by Mofokeng *et. al*. revealed that water stressed *Pelargoniums* along with the different applications of N had an effect on leaf number at harvest but not on the leaf area. This study presented the first findings regarding the response of medicinal Pelargoniums to N and water stress and should prove to be important in the establishment of cultivation practices in the future. With regards to mycorrhiza and its effect on nitrogen uptake, a study by Tobar *et al*. (1994), the mycelia of arbuscular mycorrhiza are critical in the

transportation of dissolved nutrients from the soil water to the root zone thus increasing the efficiency of plant growth. In the findings of this study, N levels in the plant tissue were raised in the AM inoculated plants and the non-inoculated plants with increased irrigation, i.e. WF 1, WF 1M, WF2, WF2M.

3.4.8.4 The effect of potassium availability and uptake

In Table 3.3 Potassium (K) was also increased significantly at P ≤0.001 in treatment 2 (WF2) without mycorrhiza addition. As watering frequency was decreased K become less available to plants in the experiment. Potassium is an essential plant nutrient and is absorbed in much larger amounts than Mg or Ca (Anonymous, 1999). One of the most important stress factors which affect plants adversely is lack of water and is a major cause of crop failure across the world (Gueta-Dahan *et al*., 1997; Wang *et al*., 2003). Drought stress impedes nutrient uptake by the roots and the movement of nutrients to the shoots due to impaired transportation and membrane permeability (Alam, 1999). While drought stress limits plant growth, K is just as important in maintaining turgor pressure in plant cells. Soil moisture content has an effect on the amount and availability of K in the soil (Van der Paauw, 1958). Availability of K to the plant decreases under water stress conditions. Kuchenbuch *et al*. (1986) demonstrated that low soil moisture levels adversely affected root growth and consequently reduced the rate of K into the plant. On the contrary, the uptake of K and the plant growth increased with increased supply of water and a lack of water availability decreased root growth.

3.4.8.5 The effect of phosphorous availability and uptake

Table 3.3 also demonstrates that P became more significantly (P ≤0.001) available at lower watering frequencies of treatments 3 to 5 (WF3, WF4, WF4M, WF5, WF5M). It is well known that the requirement of P is mainly in root development during plant growth. A great deal of inorganic P applied to soils as nutrient supplementation are quickly changed to unavailable forms of P which do not dissolve readily in water and consequently do not move easily to the root zones of plants (Sanyal & De Datta, 1991). The ability of plants to access limited resources in the soil, such as P as a result of adapted root growth has been well documented. In a study by Ho *et al*., (2005), it was concluded that drought conditions in plants, beans in this case, were associated with greater depth of roots for the acquisition of immobile nutrients such as P and water.

3.4.9 Chlorophyll content

The chlorophyll content of the foliage of individual plants was measured weekly utilizing non-destructive fluorometer analysis (Manetas *et al.*, 1998). Two readings were obtained from separate, fully developed leaves of each plant, averaged and used to create a set of data. The CCM-300 chlorophyll meter produced by OPTI-SCIENCES®, Inc. (8 Winn Ave, Hudson, NH 03051, USA) uses a chlorophyll fluorescence ratio (CFR) to measure the chlorophyll content within plants' leaves. The SPAD values obtained were proportional to the leaf chlorophyll content, after calibration against accurate chlorophyll measurements calculated using the standard spectrophotometric method (Manetas *et al.,* 1998).

3.4.10 Chlorophyll production in *P. reniforme*

3.4.10.1 Water frequency: The lowest water frequency (WF5) without mycorrhiza resulted in a significant (P ≤0.001) chlorophyll reading for *P. reniforme* at week 10. With these results plants were able to produce sufficient chlorophyll without the support of mycorrhiza in the root zone area. Week 10 of the growing period showed the first statistical significance in the lowest water frequency level (Table 3.4). This finding contradicts those of Allen *et al.*, (1981), in which increased water uptake led to higher photosynthetic rates in *Bouteloua gracilis*. Significance (P ≤0.001) was continued in week 12 of the experiment but more dominant in the higher water frequencies (WF1, WF2) in plant growth of *P. reniforme* (Table 3.4). Increased chlorophyll production at high water availability is supported by *Lotter et al*. (2014) whose work on *Aspalathus linearis* demonstrated that there was a significant drop in photosynthetic rate in the plants subjected to water deficit. Interestingly, the plants also demonstrated lower rates of transpiration under drought stress as well as an increase in water-use efficiency, clearly a survival method for lack of water availability.

3.4.10.2 Mycorrhiza: By week 14 the significant level (P ≤0.05) was more dominant in the availability of mycorrhiza only in the lowest water frequency with mycorrhiza (WF5M). The amount of water available to *P. reniforme* appeared to not be critical as it was clear that the presence of mycorrhiza provided optimum levels of chlorophyll production (Table 3.4). Allen *et al*. (1981) found that mycorrhizal plants had significantly increased CO^2 uptake therefore increased photosynthetic activity when compared with non-mycorrhizal plants.

3.4.10.3 Water frequency interaction with mycorrhiza: At week 12, the results in Table 3.4 show that the interaction between water frequency and the addition of mycorrhiza

became more significant (P ≤0.05) at this growth stage with the application of a higher water frequency (WF1M, WF2M) supply. Mycorrhiza proved to benefit the production of chlorophyll production. At week 13 the results changed where an increase in chlorophyll production showed significance (P ≤0.05) in the interaction of a higher and lower frequency WF1M, WF5M, WF5) and mycorrhiza. Birhane *et al*. (2012) demonstrated the positive effect of mycorrhizal inoculation interaction with high water availability on traits such as root biomass, nutrient uptake, transpiration rates, photosynthesis and water assimilation. These results demonstrate that more mature plants of *P. reniforme* are more adaptable to varying water availability, coping with water stress with mycorrhiza availability in continuing significant levels of chlorophyll production.

Table 3.1 Mean squares from the analysis of variance for the effect of five varied watering treatment frequencies with and without mycorrhiza on the wet shoot weight, dry shoot weight, wet root weight and dry root weight of *Pelargonium reniforme* C. grown in an inert soilless medium.

Treatment		Wet shoot weight		Dry shoot weight		Wet root weight		Dry root weight	
Watering Frequency	**Mycorrhiza**								
WF1M	Yes	34.10	±13.77a	5.20	±2.42a	5.03	±2.91ab	0.71	±0.5a
WF1	No	34.40	±9.a	5.84	±1.3a	8.78	±14.78b	0.65	±0.2a
WF2M	Yes	31.79	±10.03a	5.56	±1.77a	4.67	±4.25ab	0.63	±0.65a
WF2	No	31.07	±11.31a	5.34	±1.87a	3.70	±2.1a	0.54	±0.32a
WF3M	Yes	27.99	±6.5ad	5.46	±1.48a	4.43	±0.95ab	0.85	±0.42a
WF3	No	21.83	±9.21bd	4.74	±2.36ab	5.03	±2.82ab	0.96	±0.82a
WF4M	Yes	17.12	±2.47b	3.82	±0.72b	6.16	±1.91ab	2.00	±1.12b
WF4	No	15.96	±2.51b	3.52	±0.92b	7.03	±2.9ab	1.93	±1.34b
WF5M	Yes	4.90	±1.13c	1.37	±0.48c	3.80	±2.25a	0.94	±0.4a
WF5	No	5.41	±0.78c	1.30	±0.18c	3.55	±0.82a	0.81	±0.18a
Two-way ANOVA F-Statistics									
Watering Frequency		43.7 ***		26.7 ***		1.5 ns		12.6 ***	
Mycorrhiza		0.8 ns		0.2 ns		0.6 ns		0.1 ns	
Watering Frequency * Mycorrhiza		0.6 ns		0.5 ns		0.6 ns		0.1 ns	

Mean values ±SD are shown in columns. The mean values followed by different letters are significantly different at P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference.

Table 3.2 Mean squares from the analysis of variance for the effect of five varied watering treatment frequencies with and without Mychorrhiza on the shoot length, total wet weight and total dry weight of *Pelargonium reniforme* C. grown in an inert, soilless medium..

Treatment		Shoot length		Total wet weight		Total dry weight	
Watering Frequency	**Mycorrhiza**						
WF1M	Yes	54.18	±10.2b	39.19	±16.53f	5.92	±2.88a
WF1	No	60.71	±10.53b	101.67	±34.73d	6.49	±1.45a
WF2M	Yes	62.56	±15.23be	101.71	±40.71d	6.19	±1.9a
WF2	No	60.06	±8.46ce	135.52	±23.37b	5.84	±2.17a
WF3M	Yes	50.45	±9.7c	151.09	±11.57ab	6.31	±1.85a
WF3	No	43.22	±16.91cd	157.85	±20.95ab	5.70	±3.02a
WF4M	Yes	41.74	±9.06ad	168.41	±7.32ac	5.81	±1.38a
WF4	No	33.13	±11.1ad	186.44	±7.31ce	5.45	±1.85a
WF5M	Yes	30.09	±9.79a	162.79	±54.04ac	2.36	±0.86b
WF5	No	31.63	±3.63a	200.85	±3.69e	2.18	±0.47b
Two-way ANOVA F-Statistics							
Watering Frequency		27.4 ***		59.3 ***		14.7 ***	
Mycorrhiza		0.9 ns		34.7 ***		0.2 ns	
Watering Frequency * Mycorrhiza		1.6 ns		3.1 *		0.3 ns	

Mean values ±SD are shown in columns. The mean values followed by different letters are significantly different at P ≤0.05 (*), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference.

Table 3.3 Mean squares from the analysis of variance for the effect of five varied watering treatment frequencies with and without Mychorrhiza on the nutrient (Ca, K, Mg, N, P) uptake determined from dried material of *Pelargonium reniforme* Curtis.

Treatment		Ca		K		Mg		N		P	
Watering Frequency	**Mycorrhiza**										
WF1M	Yes	1.75	±0.05a	3.24	±0.03abcd	0.33	±0.01d	2.64	±0.13a	0.70	±0.07cd
WF1	No	1.78	±0.06a	3.04	±0.07ad	0.32	±0.02bd	2.64	±0.09a	0.70	±0.04c
WF2M	Yes	1.75	±0.18a	3.29	±0.28abce	0.31	±0.03abd	2.64	±0.08a	0.78	±0.08e
WF2	No	1.71	±0.06a	3.27	±0.09abc	0.30	±0.02ab	2.64	±0.08a	0.77	±0.03de
WF3M	Yes	1.19	±0.18c	3.57	±0.1cef	0.29	±0.01a	2.51	±0.04ad	0.29	±0.02b
WF3	No	1.40	±0.16d	3.49	±0.24bce	0.29	±0.02a	2.42	±0.09d	0.30	±0.04b
WF4M	Yes	0.96	±0.08b	3.90	±0.28f	0.23	±0.02c	2.01	±0.09c	0.21	±0.01a
WF4	No	1.16	±0.1c	3.67	±0.36ef	0.30	±0.02ab	2.12	±0.06c	0.20	±0.01a
WF5M	Yes	0.88	±0.11b	2.87	±0.21d	0.22	±0.02c	1.63	±0.21b	0.15	±0.01a
WF5	No	0.89	±0.05b	3.15	±0.29abd	0.24	±0.02c	1.67	±0.09b	0.17	±0.02a

Two-way ANOVA F-Statistics

Watering Frequency	74.1 ***	11.5 ***	30.3 ***	101.4 ***	311.7 ***
Mycorrhiza	4.1 ns	0.4 ns	4.9 *	0.1 ns	0. ns
Watering Frequency * Mycorrhiza	1.7 ns	1.3 ns	6.1 *	0.8 ns	0.2 ns

Mean values ±SD are shown in columns. The mean values followed by different letters are significantly different at P ≤0.05 (*), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference.

Table 3.4 Mean squares from the analysis of variance for the effect of five varied watering treatment frequencies with and without mycorrhiza on the chlorophyll reading of *Pelargonium reniforme* cuttings grown in a hydroponic culture system.

Treatment Watering Frequency	Mycorrhiza	Week 10		Week 11		Week 12		Week 13		Week 14	
WF1M	Yes	46.96	±3.50cd	47.12	±7.09ab	48.03	±2.96abc	48.77	±5.66a	51.94	±4.31abc
WF1	No	46.52	±4.93c	50.90	±4.15abc	50.80	±2.81abc	54.83	±9.13abc	52.94	±6.42abc
WF2M	Yes	49.09	±4.15abcd	47.22	±6.10ab	49.04	±3.94abc	55.42	±10.56abc	48.36	±6.99ab
WF2	No	50.80	±4.76abd	49.98	±4.17abc	51.14	±6.00abc	54.77	±5.59abc	53.49	±4.13abc
WF3M	Yes	52.98	±3.07ae	53.45	±6.52ac	55.60	±10.74c	60.71	±13.52c	54.91	±5.87bc
WF3	No	52.98	±3.07ae	45.98	±18.98b	44.95	±17.46abd	50.22	±19.01ab	53.40	±19.81abc
WF4M	Yes	52.52	±4.86abe	53.59	±5.14ac	44.60	±17.50ad	52.10	±7.06ab	48.77	±5.62abc
WF4	No	55.31	±5.52e	56.79	±6.57c	54.22	±11.68bc	58.39	±4.98bc	56.00	±6.19c
WF5M	Yes	48.93	±5.86bcd	51.67	±2.76abc	36.58	±5.25de	48.02	±4.39a	46.78	±6.04a
WF5	No	51.16	±3.72ab	52.50	±3.47abc	29.70	±13.81e	49.18	±3.93a	52.63	±6.46abc
Two-way ANOVA F-Statistics											
Watering Frequency		8.2 ***		2.4 ns		9.7 ***		2.0 ns		0.8 ns	
Mycorrhiza		2.0 ns		0.2 ns		0.1 ns		0.1 ns		4.5 *	
Watering Frequency * Mycorrhiza		0.5 ns		1.8 ns		2.9 *		2.6 *		1.0 ns	

Mean values ±SD are shown in columns. The mean values followed by different letters are significantly different at P ≤0.05 (*), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference.

3.5 CONCLUSION AND RECOMMENDATIONS

The medicinal importance of *Pelargonium* species such as *P. reniforme* has been well documented and it traditional medicinal value has been firmly established. Research on cultivation of this species remains imperative to ensure its economic viability in the production of sustainable quantities to supply a continual growing medicinal demand. The effects of irrigation frequency, with and without the addition to arbuscular mycorrhiza showed notable increases in the growth variables and nutrients in cultivating *P. reniforme*. The watering frequency had a significant impact in all measurements except the wet root weight. The addition of arbuscular mycorrhiza inoculation resulted in a significance level in the total wet weight measurements of the species, but a more significant observation was recorded in the water frequency and mycorrhiza treatment in the wet root weight.

In the nutrient measurements, several nutrients such as C, K, Mg, N and P resulted in a significant improvement in growth in the watering frequency treatments without mycorrhiza. The only significance in results with mycorrhiza was seen in the Mg availability. Similarly, there was also a higher significant interaction between the watering frequency without and with mycorrhiza in the availability of Mg. The results of this study present valuable information regarding changes in water availability i.e. where less water is available, less potassium becomes available to plants. The opposite was seen in more phosphorous availability at lower water requirements. As water increased, nitrogen availability also increased and more water also resulted in higher Ca availability with a mycorrhiza addition. In conclusion the availability of water to plants has a significant impact on their growth. Farmers can therefore adapt their irrigation regimes to the desired growth required with the aim being conserving water in drought-stricken countries. The study proved that water levels can be reduced during cultivation while the presence of AM had limited impact on the growth parameters of *P. reniforme* Curtis over the eight week period. This is significant due to the high cost of commercially available AM. There is evidence that use of AM in a commercial setting can be of little value unless it is used in an organic system where there is a lack of biocidal inputs and artificial fertilizers. Further studies over extended times are required to determine further relevance of AM at specific growth stages.

3.6 ACKNOWLEDGMENTS

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CHAPTER FOUR

EFFECT OF DIFFERENT WATERING FREQUENCIES AND ARBUSCULAR MYCORRHIZA ON THE GROWTH, LEAF CHLOROPHYLL AND NUTRIENT UPTAKE OF *PELARGONIUM SIDOIDES*

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4.1 ABSTRACT

Pelargonium sidoides DC is an herbaceous black-flowering, perennial sub-shrub with a wide distribution. It is found throughout the Eastern Cape, Lesotho, Free State and southern Gauteng in the Republic of South Africa. The plant carries economic importance as a traditional medicinal species. This study was carried out to measure the effects of different levels of water stress on plant growth of *Pelargonium sidoides* DC were investigated, as well as the effects on plant growth with and without the addition of arbuscular mycorrhiza to the growing medium. The experiment was conducted over a period of four months when a total of ten treatments were applied to ten replicates. Watering frequencies comprised of 320 mls of water from once a day to every twenty- four days. Half of the treatments were inoculated with 30 g of arbuscular mycorrhiza (AM) and the other half were not. Growth parameters such as leaf numbers were increased by moderate watering frequencies, therefore demonstrating the advantage of sufficient irrigation for this species. No significant difference in leaf number was found with the addition of AM to any of the treatments. Uptake of nutrients Ca, K, N, and P was enhanced in all the watering treatments in the experiment, but no significance was seen in the uptake of Mg. Inoculation with AM demonstrated an increase in the uptake of Ca while less water was required to increase the uptake of K. N absorption was increased in higher availability of water, likewise P was absorbed in lower water availability. Potassium uptake was increased with the addition of AM in the high water frequency and uptake of K decreased as water stress increased. No significance was found to make any difference in the uptake of nutrients as a result of the interaction between water availability and AM inoculation. Medium to low water availability resulted in higher chlorophyll

content in *P. sidoides* while the addition of AM increased the production of chlorophyll mainly during the early growth period. In general, there was also increased chlorophyll production due to the interaction between water availability and mycorrhizal inoculation especially towards the end of the experimental period, as the plants matured.

Key Words: growth parameters, EPs 7630®, chlorophyll, nutrients, irrigation
Clarification of terms: The terms 'fresh' and 'wet' are used interchangeably when referring to plant weight.

4.2 INTRODUCTION

P. sidoides is a valuable medicinal plant which is used traditionally in South Africa to treat a number of ailments including respiratory tract infections and gastrointestinal problems (Moyo & van Staden, 2014). Most plants which are used for traditional medicine are harvested from wild populations (Fennell *et al*., 2004) thus, unfortunately, the demand for this valuable plant has resulted in overharvesting of the species and populations in Lesotho and South Africa continue to decline (De Castro *et al*, 2012). Plant derived healing has been used since the beginning of time and is now regarded as preferable treatment by many people (Hoareau & Da Silva, 1999.) There has been a widespread global resurgence of the use of natural medication which has resulted in a high demand for it in Western countries (Makunga *et al.*, 2008). The shift to the use of natural medicines in the West could be attributed to the rising costs of modern health care and the general move towards natural health care.

4.2.1. Medicinal value of *P. sidoides*

Several South African plant species have demonstrated scientific potential. Pelargoniums are an important group of plants which have been proven to possess a wide range of medicinal properties (Watt & Breyer-Brandwijk, 1962). Two species of *Pelargonium* in particular, *P. reniforme* and *P. sidoides*, contain complex phenolic compounds and others with anti-oxidant abilities (Kolodziej, 2007). A randomised, double-blind trial conducted in 2003 by Mattys *et al*. confirmed that the commercial extract of the root of *P. sidoides* (Eps 7630) could successfully treat acute bronchitis. Ethanolic extracts of *P. sidoides* have been shown to inhibit the growth of fungal organisms such as *Aspergillus niger* and *Fusarium oxysporum* (Mantivandlela *et al*., 2006). A root extract of *P. sidoides* has been commercialised and is used by physicians in the west to treat rhinosinusitis and acute tonsillopharyngitis (Bachert *et al*., 2009). Due to the medicinal value and importance of this

species, new cultivation methods and practices are necessary in order to improve crop quality and support the demands of future markets. There is very little documentation of cultivation of cultivation practices and many commercial growers work in isolation where growing methods are not shared.

4.2.2 Watering requirements of *P. sidoides* in cultivation

A study by Henson *et al.*, (2006), demonstrated the many ornamental bedding plants, including *Pelargoniums*, indicated moderately healthy growth with minimal visual signs of water deficit under medium to low irrigation levels. *P. sidoides* is able to survive long dry periods by becoming dormant and then resprouting from its tuberous roots (Lawrence, 2001). This is a useful attribute for commercial cultivation of this plant due to the increasing drought conditions in South Africa.

4.2.3 Nutrient requirements of *P. sidoides* in cultivation

Pelargoniums respond to normal fertilizer regimes, however the pH of the medium is critical for nutrient uptake, especially iron and manganese which can be taken up too quickly at a low pH and result in toxicity (Argo & Fisher, 2009.) These species require moderate amounts of macronutrients, in particularly calcium (Ca) and iron (Fe).

4.2.4 Potential of Arbuscular Mycorrhiza in Cultivation

Arbuscular mycorrhiza (AM) has been well documented in playing a part in alleviating water stress in plants (Heidari & Karami, 2012). Water stress is one of the major reasons for crop failure worldwide and can reduce yields by more than 50% (Wang *et al.*, 2003). Results from many studies have shown that colonization by AM helps the host plant deal with reasonable drought stress with rapid recovery after irrigation was started (Subramanian *et al.*, 1997). The fungi colonise the root cortex and form a mycelium which helps the plant to take up more nutrients and to form more stable soil aggregates which in turn improve soil quality (Jeffries & Barea, 1994; Bethlenfalvay & Schüepp, 1994). Valuable horticultural crops are produced with the addition of AM and often display mycotrophy, and their optimal growth and development is often seen as dependant on early AM establishment (Gianinazzi-Pearson, V. 1996). AM represents a mutually beneficial relationship between host plants and various fungi species which enables the host plant to take up some nutrients and water more efficiently (Parniske, 2008).

It has been well documented that plants require both macro and micro nutrients for optimal growth. Most commercial crop plants use only 50% of applied fertilisers and therefore the remainder is wasted. Mobile nutrients for example nitrates and sulphates are easily leached out below the root zones of the plants. Immobile nutrients such as phosphorus, potassium and zinc, can also be leached or eroded out when bound to organic matter. These nutrient losses can impact negatively on crop production. (Cavagnero *et al*., 2015). Many vegetable species are able to host mycorrhizal fungi and therefore become more efficient at maintaining water and nutrients and also able to improve their ability to withstand environmental stress factors such as nematode damage and root disease (Baum *et al*., 2015). This study therefore aimed to investigate the effect of water stress in cultivation and AM on the nutrient uptake parameters of the medicinal species *P. sidoides* DC.

4.3 MATERIALS AND METHODS

4.3.1 Greenhouse experiment

The experiment was conducted from June to September 2014. It was located in the research greenhouse at the Cape Peninsula University of Technology, Cape Town, South Africa with the GPS co-ordinates - 33° 55ˈ 58.27S, 18° 25ˈ 57.04E. This facility ensured that the environment in which the experiment was conducted was controlled. Minimum and maximum temperatures and relative humidity were recorded 3 times daily. Temperatures within the greenhouse ranged between 14 - 34°C during daytime and 10 - 19°C at night. Relative humidity of the facility averaged 37%. The water used in the experiment was municipal water filtered through a Hager IP 65 Water Filtration Plant de-ioniser.

4.3.2 Plant preparation

The plant sample consisted of *P. sidoides* were obtained from Pico Gro growers, 60 Milner Road, Glen Austin, Midrand, South Africa 1685. One hundred and fifty uniform cuttings were made using the stock material and placed in cutting trays containing washed and sterilized coarse river sand (Lawrence, 2001). The cutting trays were then placed in the main greenhouse on the Cape Town campus of the Cape Peninsula University of Technology on heated propagation beds. Once rooted, one hundred *P. sidoides* plants of uniform size were then planted into individual 12.5cm pots containing an inert, sterilized medium (2:1:1:1) consisting of two parts coco peat, one part foamalite, one part Consol® silica sand grade 6/17 and one part perlite. The plants were then placed under 40% shade cloth for one week

to harden off before being planted out into the experimental site. The plants were placed onto galvanized steel tables covered in black plastic sheeting with ten replicates of each treatment.

4.3.3 Experimental treatments

The experiment consisted of five watering regimes which were applied to fifty plants of the species *P. sidoides* without inoculation with arbuscular mycorrhiza and to fifty plants of *P. sidoides* in conjunction with inoculation with arbuscular mycorrhiza. There were therefore a total of one hundred plants of species *P. sidoides* with fifty receiving mycorrhiza and fifty not receiving mycorrhiza upon planting out into the experiment. A randomized block design, made up of 10 replicates each with 10 individual plants was used to study the effects of various irrigation frequencies with and without mycorrhiza inoculation on *P. sidoides.* Drip irrigation was used with the following irrigation frequencies based on experiments conducted by Wang *et al.*, (2003) on the effect of different drip irrigation frequencies on potato growth and yield:

1) WF1 (320 mls once every day to PC). Control treatment (Kirnak *et al.*, 2001).
2) WF2 (320 mls once every three days to PC
3) WF3 (320 mls once every six days to PC)
4) WF4 (320 mls once every twelve days to PC)
5) WF5 (320 mls once every twenty-four days to PC)

The experiment consisted of the following treatments:

Group A: *Pelargonium sidoides* without mycorrhiza receiving WF1, WF2, WF3, WF4 and WF5.

Group B: *Pelargonium sidoides* with mycorrhiza receiving WF1M, WF2M, WF3M, WF4M and WF5M.

Plants which received mycorrhiza were inoculated with 30 grams of AM (commercially available product Mycoroot™ obtained from the Horti Shop, 125 Belvedere Road, Claremont, Cape Town) on planting. Each individual pot was irrigated with half-strength Hoaglands solution (Recipe for Hoaglands Solution : nitrogen 210 ppm, potassium 235 ppm, calcium 200 ppm, phosphorus 31 ppm, sodium 64 ppm, magnesium 48 ppm, boron 0.5 ppm, iron 1-5 ppm, manganese 0.5 ppm, zinc 0.05 ppm, copper 0.02 ppm and molybdenum 0.01 ppm) (Anonymous, 2012). A half-strength nutrient solution was used due to the reduced nutrient requirements of South African fynbos species in the wild (Midgley *et al.*, 1995). Pot capacity was determined at 320 millilitres.

4.3.4 Acclimatization of experimental plants

Rooted cuttings from *P. sidoides* gradually acclimatized in the sterilized growing medium (2:1:1:1) of two parts coco peat, one part foamalite, one part Consol® silica sand, grade 6/17 sand and one part perlite under 40% shade cloth for one week to harden off before being moved to the experimental site.

4.3.5 Hardening-off and growing period

The plants were kept for one week adapting to the greenhouse situation after hardening off. The experiment was conducted from the second of June 2014 until the eighteenth of September 2014. After this period all plants were harvested and various postharvest measurements were taken. Parameters measured included chlorophyll content (SPAD), root and shoot wet weights and root and shoot dry weights.

4.3.6 Determination of plant growth

4.3.6.1 Plant weight

The weight of plants was measured using a standard laboratory scale before planting out on 2 June 2014 to ensure homogeneity within the sample. Post-harvest, shoot and root systems were separated and individual samples' fresh/ wet weights were recorded. The plant material was then oven dried at 55 ^{o}C in a LABTECH™ model LDO 150F (Daihan Labtech India. Pty. Ltd. 3269 Ranjit Nagar, New Dehli, 110008) oven until all water was removed from the material and a constant weight was reached; the dry weights were measured and recorded. The difference between the wet and dry weights correlates with the amount of water held within the plants' tissues.

4.3.6.2 Chlorophyll content of leaves

The chlorophyll content was measured weekly using a SPAD-502 meter supplied by Konica-Minolta. This device measures transmission of red light at 650 nm (the frequency at which chlorophyll absorbs light) and transmission of infrared light at 940 nm (at which no absorption occurs). Using these two transmission values the instrument calculates a SPAD (Soil Plant Analysis Development) level which is indicative of chlorophyll content. The readings of two fully formed leaves were taken from each plant and the figures were

averaged out by the SPAD-502 meter to produce a final number. The readings were taken between 11 am and midday from weeks 11 to 15 of the experiment with average daylight levels of 10 kLux (light intensity).

4.3.6.3 Leaf Count

Each newly formed leaf was manually counted on every plant in the experiment on a weekly basis. Dead and senescent leaves were not counted. Unopened buds were likewise not counted. The results were recorded on a data sheet.

4.3.6.4. Nutrient uptake

In order to determine the nutrient uptake of each set of replicates in the experiment, three plants were randomly selected from each set at the end of week 16, the vegetative material was removed and labelled and sent to Bemlab Laboratory, 16 van der Berg Crescent, Gant's Centre, Strand, Cape Town. The methodology to determine macronutrients (N, K, P, Ca, Mg and Na) and micronutrients (Cu, Zn, Mn, Fe, Al and B) was conducted by ashing 1g ground sample of plant material in a porcelain crucible at 500°C overnight. This was followed by dissolving the ash in 5mL of HCI and placing it in an oven at 50°C for 30 min. Thirty-five millilitres of deionised water was then added and the extract filtered through Whatman no, 1 filter paper. Nutrient concentrations in plant extracts were determined using an inductively-coupled plasma (ICP) emission spectrophotometer (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusettes, USA), (Giron, 1973).

4.3.6.5 Statistical analysis

All data was analysed using two-way analysis of variance (ANOVA), using the computing software program STATISTICA 13.2. Occurrence of statistical difference was determined by using the Fisher Least Significance Difference (L.S.D.) at values of $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$ levels of significance (Steel & Torrie, 1980).

4.4 RESULTS AND DISCUSSION

4.4.1 Leaf number in *P. sidoides*

4.4.1.1 Watering frequency: Water frequency applications played a major role in leaf number formation from weeks 3 to 5. In Table 4.1 the water frequency treatments (WF1M, WF3M, WF3, WF4) on *P. sidoides* were highly significant at (P ≤0.05). The results show that lower and medium water frequencies were successful in the increase of leaf numbers in the earlier growth stages of plant development. From weeks 7 to 15 the leaf number count remained significant at P ≤0.001 (***) in water frequency treatments (WF1M, WF3M from weeks 7 to 9), (WF2 at week 11) and (WF3 from weeks 13 to 15). These results indicated that during later growth stages of *P. sidoides* a higher water frequency was required to support leaf production. What is most significant from the results in Table 4.1 is that the water frequency treatments (WF3M, WF3) were significant throughout the growing period from weeks 3 to 15 with the exception of the treatment WF3 in week 11. The water frequency WF3 was thus most beneficial to growth improvement and deduced that Pelargonium growth will neither benefit from a too low or too high a watering frequency during a growing period. A moderately high watering frequency was found to result in a higher leaf area, plant height and fresh root weight in a study by Mofokeng *et al*. in 2015. The production of leaf numbers is highly relevant for improving plant health and growth to support root development. Although traditionally only the tuberous root material is used to make the remedies, it has been found that the shoots of *P. sidoides* are of equal value. In a study by Lewu *et al.* (2006), it was discovered that an extract of the shoot material was equally as effective against most Gram-positive bacteria. In fact, the values were slightly higher in the acetone extracts of the shoots. This would be an effective approach in commercial cultivation of *P. sidoides* to protect the species in the wild as well. Therefore, this study shows that water frequency can also play a critical role in commercial cultivation of *P. sidoides.* The results from this current study also show that watering frequency can play a critical role in increasing leaf density during production of *P. sidoides*.

4.4.1.2 Mycorrhiza: Table 4.1 shows that the application of mycorrhiza treatments to the growth medium of *P. sidoides* made no significant difference to leaf numbers from weeks 3 to 15. These results showed that added mycorrhiza had no effect on the growth of the plants and that *P. sidoides* can be cultivated efficiently without the addition of mycorrhiza. For a commercial grower, not having the added costs of AM would be a benefit financially. However, this finding does contradict those of Csima *et al. (*2012), whose study on

inoculation of AM fungi on *Pelargonium hortorum* showed that the use of AM in the early growth phase of plants played a large role in aiding the uptake of nutrients in the rhizosphere.

4.4.1.3 Water frequency interaction with mycorrhiza: Similar observations as with evaluating mycorrhiza additions were seen in the water frequency interaction with mycorrhiza. No significance levels were observed (Table 4.1). From the experiment it is clear that the addition of mycorrhiza and the interaction thereof with the water frequency application had no positive effect on the growth of *P. sidoides*. The amount of water alone as stated earlier made the only significant difference in production of leaf numbers. This finding is supported by that of dos Santos *et al.* (2010), where non-mycorrhizal specimens of *Zingiber officinale* were found to have significantly lower leaf numbers than the mycorrhizal treatments.

4.4.2 Nutrient uptake in *P. sidoides*

The measurements to determine the macronutrients and micronutrients (N, P, K Ca and Mg) uptake in plant extracts of *P. sidoides* were successfully determined with the use of an emission spectrophotometer. Results on measuring Ca, K, N and P were all significant (P ≤0.05) and P ≤0.001) in the watering frequency treatments throughout the experiment (see table 4.2). No significance was observed in Mg of any of the treatments. All minerals important for life processes access the biosphere and animal and human food chains via the rhizosphere of higher plants. Water availability is critical for the movement of nutrient solutes through the root zone by mass flow or molecular diffusion (Ŝimůnek & Hopmans, 2009). Therefore, movement of all macro and micro nutrients can only occur efficiently in the presence of sufficient water.

4.4.2.1 Watering frequency: The lower water frequency treatment (WF4M) produced the highest Ca reading being the most significant (P ≤0.05) of all nutrient uptakes in the experiment. The addition of mycorrhiza did show an increased uptake of Ca at this level. The results show that Ca uptake was improved at lower water frequencies similar to P which was absorbed during the lower water frequency (WF4M) treatment with mycorrhiza at a significance level of (P ≤0.001) (See Table 4.2). The results proved that less water was required to enhance P absorption. Mg absorption was not significant in the watering frequency of the experiment (see Table 4.2). The higher water frequency treatments (WF1M, WF1, WF2M, and WF2) in *P. sidoides* showed significance levels (P ≤0.001) of N absorption

during the experimental growth period of fifteen weeks. These results in Table 4.2 show that higher water amounts were successful with N absorption to facilitate the most desired growth in *P. sidoides.* Table 4.2 also shows that K was absorbed at lower water levels in treatment WF4M at a significant level of P ≤0.001. This is in contrast to Seiffert *et al.* (1994), who reported that K uptake decrease as water was decreased. Interestingly, however Mg were more readily absorbed at lower and higher water frequencies respectively. This finding supports the case for successful cultivation of *P. sidoides* with more P absorption for root tuber growth with less watering frequency. This way irrigation schedules can be adapted to ensure water savings and optimum production levels of *P. sidoides.*

4.4.2.2 Mycorrhiza: The addition of mycorrhiza was not significant in the uptake of N, P, Ca and Mg during the experiment in growing *P. sidoides.* Phosphorus uptake however benefitted from the addition of mycorrhiza with significance level at P ≤0.05 and the lowest water frequency WF4M (See Table 4.2). As watering frequency increased with mycorrhiza present, K was less absorbed in the growing of *P. sidoides*. This is an interesting finding as it concurs with earlier studies in particular a study in 1994 by Seiffert *et al.* where it was found that K uptake decreased as water availability decreased, in fact K uptake was reduced by 50% at low water availability.

4.4.2.3. Water frequency interaction with mycorrhiza: The interaction between water frequency and addition of mycorrhiza was not significant in benefitting the absorption of nutrients N, P, K, Ca and Mg. These results thus indicate that the amount of water with mycorrhiza available had no impact on the nutrient uptake in the *P. sidoides* plants and that the two selective treatments reacted independently from each other (see Table 4.2). A study in 1984 by Sweatt & Davies however showed an interaction between water availability and AM. *Pelargonium* X *hortorum* seedlings were grown under high and low soil water potential and were inoculated with AM or left as uninoculated controls. The inoculated plants under high moisture regimes had a high P uptake, while plants that received low watering regimes showed a higher N uptake than the non-inoculated plants. These findings appear to demonstrate an interaction between water frequency and mycorrhizal presence in a similar species.

4.3 Chlorophyll production in *P. sidoides*

4.4.3.1 Effect of water frequency: In Table 4.3 water frequency treatments (WF3M, WF3, WF4M, WF4) on *P. sidoides* showed significance levels in chlorophyll production in weeks

10 (P ≤0.05) and 11 to 13 (P ≤0.001). The results show that a medium to lower water frequency in the earlier growth stages of *P. sidoides* has resulted in higher chlorophyll readings of the experiment. These findings contradict those in a study on *Pelargonium graveolens* by Amiri *et al.,* 2017 where it was found that leaf chlorophyll content decreased as the level of water stress increased. It is known that chlorophyll production is reliant on not only water availability, but also the presence of certain nutrients such as Mg which is necessary for the formation of the chlorophyll molecule.

4.4.3.2 Effect of mycorrhiza: Between week 10 and 12 the mycorrhiza treatments delivered the highest significance (P ≤0.05) level of chlorophyll. These treatments were WF4M (week 10), WF1M, WF2M (week 11) and WF4M, WF5M (week 12) (See Table 4.3). It is therefore clear that growth of *P. sidoides* benefited in chlorophyll increases with the addition of mycorrhiza over the range of watering schedules. Mycorrhiza benefited chlorophyll production mainly in the earlier growth stages of *P. sidoides* plant growth. *Pelargonium graveolens* plants grown in an outdoor pot experiment by Amiri *et al.* in 2017 demonstrated increased chlorophyll content in the leaves of plants inoculated with AM with a decrease in leaf chlorophyll in plants under water stress.

4.4.3.3 Effect of water frequency interaction with mycorrhiza: Similar significance levels (P≤0.05) were obtained in the interaction of water frequency treatments (WF5) and mycorrhiza additions (WF1M, WF2M) in week 11 and (WF1M, WF2, WF3M, WF3, WF4M and WF5M) in week 15. These results in Table 4.3 show that there was a significant level of chlorophyll production during the interaction of water amounts and mycorrhiza present in plant growth of *P. sidoides*. An increase in chlorophyll benefited from both higher and lower water frequency treatments with the addition of mycorrhiza. A decrease in leaf chlorophyll has been demonstrated under water deficit conditions (Manoharan *et al.*, 2010) and many similar results have been reported, for example by Asrar *et al.*, 2012, where the combination of mycorrhizal inoculation and moderate irrigation levels can increase the rate of photosynthetic activity and therefore chlorophyll content of the leaves.

Table 4.1 Mean squares from the analysis of variance for the effect of five varied watering treatment frequencies with and without mychorrhiza on the leaf number (n) of *Pelargonium sidoides* grown from week 3 to 15 in an inert, soilless medium.

Treatment Watering Freq	Myc	Week 3		Week 5		Week 7		Week 9		Week 11		Week 13		Week 15	
WF1M	Yes	19.80	±4.13a	24.20	±4.26a	24.80	±7.61a	29.70	±8.73a	42.50	±14.07bc	48.30	±18.43de	50.90	±18.86cg
WF1	No	17.00	±4.08ab	22.50	±4.25ac	26.40	±4.03a	30.90	±5.70a	43.50	±9.52c	50.60	±13.15e	59.90	±14.29g
WF2M	Yes	18.60	±5.80ab	20.60	±6.26abc	23.20	±9.26ab	28.00	±11.69ac	34.80	±14.55abc	41.40	±16.36bde	42.00	±14.63abc
WF2	No	18.20	±7.02ab	21.10	±9.06abc	24.30	±10.66ab	25.10	±12.06abc	34.10	±16.83ab	38.00	±13.46ab	41.20	±15.13abc
WF3M	Yes	20.70	±6.06a	24.60	±7.18a	26.70	±10.91a	31.80	±12.68a	36.60	±9.52abc	39.20	±9.62abd	44.90	±10.00bc
WF3	No	20.80	±2.25a	24.00	±3.53a	22.50	±5.76abc	26.20	±8.05abc	35.00	±7.72abc	36.60	±7.92ab	40.80	±7.66ab
WF4M	Yes	18.20	±1.93ab	20.20	±2.04abc	18.20	±1.62bcd	21.80	±2.30bcd	24.20	±1.75de	23.90	±3.00cf	26.70	±3.06ef
WF4	No	20.30	±3.43a	23.90	±4.43a	22.90	±5.02abc	25.50	±5.99abc	29.20	±6.80ae	29.60	±6.55af	33.50	±7.00af
WF5M	Yes	15.30	±3.06b	18.00	±3.71bc	16.70	±4.74cd	19.90	±5.30bd	18.40	±5.97d	15.70	±3.71c	15.20	±2.97d
WF5	No	14.80	±2.70b	17.80	±3.71b	15.50	±4.43d	17.20	±3.79d	16.80	±4.34d	15.80	±3.85c	19.70	±6.46de
Two-way ANOVA F-Statistics															
Watering Frequency		4.6 *		4.5 *		6.0 ***		6.3 ***		18.1 ***		28.1 ***		32.4 ***	
Mycorrhiza		0.1 ns		0.1 ns		0.1 ns		0.6 ns		0.0 ns		0.0 ns		1.9 ns	
Watering Frequency * Mycorrhiza		0.8 ns		0.8 ns		1.1 ns		1.0 ns		0.4 ns		0.6 ns		1.2 ns	

Mean values ±SD are shown in columns. The mean values followed by different letters are significantly different at P ≤0.05 (*), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference

Table 4.2 Mean squares from the analysis of variance for the effect of five varied watering treatment frequencies with and without mycorrhiza on the nutrient uptake of *Pelargonium sidoides* grown in an inert, soilless medium.

Treatment Watering Frequency	Mycorrhiza	Ca		K		Mg		N		P	
WF1M	Yes	1.40	±0.07cd	4.29	±0.11ab	0.34	±0.01abc	3.33	±0.07a	0.48	±0.01c
WF1	No	1.32	±0.1abc	4.18	±0.13abc	0.32	±0.02abc	3.26	±0.11a	0.48	±0.05c
WF2M	Yes	1.38	±0.26bcd	4.74	±0.35d	0.32	±0.04abc	3.27	±0.02a	0.60	±0.04d
WF2	No	1.63	±0.21d	4.21	±0.13abc	0.35	±0.03ab	3.25	±0.02a	0.54	±0.05d
WF3M	Yes	1.19	±0.22abc	4.33	±0.14abd	0.29	±0.04ac	2.97	±0.16d	0.27	±0.01b
WF3	No	1.18	±0.09abc	4.05	±0.44ac	0.28	±0.04c	2.77	±0.18b	0.26	±0.03ab
WF4M	Yes	1.12	±0.16a	4.51	±0.18bd	0.34	±0.02abc	2.70	±0.11b	0.22	±0.02a
WF4	No	1.15	±0.06abc	3.82	±0.4c	0.36	±0.05b	2.71	±0.16b	0.25	±0.02ab
WF5M	Yes	1.14	±0.06ab	3.32	±0.19e	0.30	±0.03abc	2.45	±0.09c	0.25	±0.02ab
WF5	No	1.17	±0.11abc	3.21	±0.19e	0.34	±0.05ab	2.49	±0.06c	0.25	±0.01ab
Two-way ANOVA F-Statistics											
Watering Frequency		6.9 *		20.2 ***		2.5 ns		60.6 ***		168.4 ***	
Mycorrhiza		0.6 ns		13.9 *		0.8 ns		1.5 ns		0.7 ns	
Watering Frequency * Mycorrhiza		1.1 ns		1.6 ns		0.8 ns		1.2 ns		1.9 ns	

Mean values ±SD are shown in columns. The mean values followed by different letters are significantly different at P ≤0.05 (*), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference.

Table 4.3 Mean squares from the analysis of variance for the effect of five varied watering treatment frequencies with and without mycorrhiza on the chlorophyll reading (SPAD) of *Pelargonium sidoides* grown in an inert, soilless medium.

Treatment Watering Frequency	Mycorrhiza	Week 10		Week 11		Week 12		Week 13		Week 14		Week 15	
WF1M	Yes	43.74	±6.76b	41.99	±5.01a	43.18	±6.69bd	47.22	±9.52ab	45.39	±7.32a	49.01	±5.98ab
WF1	No	45.86	±5.74ab	44.77	±6.59ab	47.17	±4.64ad	47.94	±4.37ab	48.83	±4.53ab	55.51	±5.54b
WF2M	Yes	41.64	±8.75b	42.49	±5.34a	43.19	±6.61bd	50.20	±13.71a	48.09	±5.44ab	49.85	±6.33abb
WF2	No	49.67	±6.34a	49.72	±6.17bb	50.04	±7.40ab	51.43	±6.80a	48.81	±5.50ab	54.14	±5.73ab
WF3M	Yes	45.85	±7.49ab	46.95	±8.29ab	51.59	±9.89ab	49.96	±11.32ab	46.87	±5.36ab	53.68	±6.91ab
WF3	No	49.71	±6.27a	53.45	±8.31b	53.34	±6.22b	51.15	±7.19a	50.34	±6.59ab	52.46	±7.54ab
WF4M	Yes	49.52	±4.23a	52.39	±4.66b	51.86	±4.14ab	53.41	±7.28a	52.37	±8.13b	53.75	±6.06ab
WF4	No	49.91	±5.19a	53.45	±5.50b	52.85	±5.47ab	46.34	±4.11ab	48.10	±7.95ab	51.91	±6.28abb
WF5M	Yes	45.26	±4.67ab	46.63	±3.90ab	39.08	±5.01b	42.67	±8.07bb	48.92	±6.17ab	52.88	±7.38ab
WF5	No	43.22	±4.55b	43.30	±5.49a	38.84	±7.38b	37.57	±6.84b	45.67	±4.97a	46.35	±6.02b
Two-way ANOVA F-Statistics													
Watering Frequency		2.7 *		8.4 ***		14.8 ***		5.7 ***		0.8 ns		0.9 ns	
Mycorrhiza		4.0 *		5.5 *		4.2 *		1.2 ns		0.0 ns		0.0 ns	
Watering Frequency * Mycorrhiza		1.9 ns		2.5 *		0.9 ns		1.1 ns		1.7 ns		3.3 *	

Mean values ±SD are shown in columns. The mean values followed by different letters are significantly different at P ≤0.05 (*), P ≤0.001 (***) and ns = not significant as calculated by Fisher's least significant difference.

4.5 CONCLUSION AND RECOMMENDATIONS

Water plays and important role in the functioning and growth of plants. Without sufficient water, vital processes at a cellular level such as photosynthesis and respiration cannot take place. The availability of higher amounts of water had a key role in increased leaf numbers in the early stages of the experiment. High to moderate watering frequencies in remained significant throughout the experimental period from 3 to 15 weeks. Optimal water availability of water increases plant growth, especially when other growth requirements such as nutrient availability are met. Additional water can increase growth and quality for commercial production. This is especially important where the leaves of *P. sidoides* can be used for medicinal purposes. In this study a half-strength Hoaglands solution provided the experimental plants with nutrients while the exposure to a controlled greenhouse environment provided a conducive growing environment for the experimental plants. Although the water frequency treatments included the addition of mycorrhiza, the effect of AM did have a significant impact on the plant growth as far as leaf number was concerned. The study showed no significance between watering frequencies and the addition of AM in the production of new leaves in *P. sidoides*. It is clear that the significance of leaf growth was not affected by the addition of AM and any application to improve volume in leaf weight would be an unneccessary expense for a commercial grower. Significant results were recorded in the uptake of all macronutrients with the exception of Mg. High Ca uptake was found more significant at a lower watering frequency. K absorption was most effective at the highest watering frequency. The addition of mycorrhiza also showed significance in the uptake of K. It is clear that K uptake was enhanced at greater water availability. N was absorbed at a significantly higher rate during the experiment in the higher water frequencies. This finding correlates with a higher leaf number counted with higher availability of water as N is necessary for increased vegetative growth. P was significantly absorbed at lower water availability which agrees with most studies where P moves less readily through the soil where there is a water deficit. Inoculation with AM did not make any significant difference to the uptake of N, P, Ca or Mg during the experimental phase. Significant results were shown in the uptake of K at the highest watering frequency along with the addition of AM. At the lower water availability, K was less readily absorbed while Mg uptake was not significant at any treatment. No significance was found regarding the interaction between water availability and addition of AM in benefitting the uptake of N, P, Ca, K and Mg. The study showed that the nutrient absorption varied with different amounts of water available for different nutrients. The study also confirmed various other studies that the addition of AM did enhance nutrient uptake. The commercial production of medicinal species *P. sidoides* can be improved if

amounts of water frequencies and applications of AM are correctly calculated to control limited available nutrients to enhance plant growth. This will limit wastage of excess and limited available nutrients. Chlorophyll production was most significant at medium to lower water availability. Medium to lower water availability, both with and without AM inoculation resulted in significantly higher chlorophyll production. AM inoculation clearly enhanced the production of chlorophyll across the range of water treatments in the experiment but notably over the 10 to 12 week period. These results drop with no significance in the remaining weeks. The interaction between watering frequencies and mycorrhiza addition were erratic with significant levels of chlorophyll production at weeks 11 and 15 at medium watering frequencies. These results indicate that mycorrizal action to support chlorophyll production was assisted with water availability. This study indicated that moderate levels of irrigation are necessary for optimal root and shoot growth and chlorophyll production of *P. sidoides.* The benefit of the addition of AM was limited to the growth of the plants. Repeating this experiment using a field study over a longer period of time in order to further establish an optimal fertigation program for the commercial production of this valuable medicinal *Pelargonium* species is recommended.

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CHAPTER 5

COMPARATIVE ANTIOXIDANT-CAPACITY OF *PELARGONIUM RENIFORME* CURTIS AND *PELARGONIUM SIDOIDES* DC UNDER DIFFERENT WATERING FREQUENCIES AND ARBUSCULAR MYCORRHIZA APPLICATIONS.

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5.1 ABSTRACT

Pelargonium reniforme Curtis and *Pelargonium sidoides* DC are medicinally important plants of the large family Geraniaceae and indigenous to the southern areas of Africa. Both species are small shrublets or sub-shrubs whose tuberous roots contain essential metabolites used for the treatment of a number of disorders. The efficacy of these two species has been well documented, in particular their antioxidant potential. In this study, the antioxidant activity and accumulation of metabolites within the root tubers (dry weight) were assessed using assays for total polyphenols, ferric reducing antioxidant power (FRAP) and oxygen radical absorbance capacity (ORAC). The total polyphenols in the water frequency treatments were found to be significantly higher ($P \leq 0.05$) in *P. reniforme* compared to that of *P. sidoides* at $P \leq 0.001$. FRAP values of *P. reniforme* were found to be significantly ($P \leq 0.05$) higher in the mid water frequency with mycorrhiza addition (WF3M) compared to *P. sidoides* with no significance. The results show that the addition of mycorrhiza improved the FRAP content (613.31±96.34abcd) in the dry root tuber material of *P. reniforme.* The water frequency, mycorrhiza and interaction between the two treatments showed no significant difference in the ORAC values of the species. A strong correlation was evident in the lower water frequency treatments in both species between the total polyphenols and FRAP content.

Key Words: Antioxidant, Ferric reducing antioxidant power (FRAP), Oxygen Radical Absorbance Capacity (ORAC), Polypehnolics, Reactive oxygen species (ROS).

5.2 INTRODUCTION

The plant genus Pelargonium belongs to the family Geraniaceae. There are four other genera in the family, namely Geranium, Erodium, Monsonia and Sarcocaulon (Lawrence, 2001). Eighty per cent of Pelargonium species are indigenous to Southern Africa with most of them endemic to the south-western Cape province (Lalli *et al*., 2008). Historically, Pelargoniums have been commonly used as medicinal plants, with many of the species possessing valuable healing compounds, with studies having already proved the presence of phenolics, proanthocyanins, flavonoids, tannins and coumarins (Makunga, 2015). The tuberous roots of *P. sidoides* are traditionally used as traditional medicine for a wide range of ailments, in particular colds and influenza, gastrointestinal disorders and chest pain (Schnitzler *et al.*, 2008). Both *P. reniforme* and *P. sidoides* are widely used by traditional healers in certain parts of South Africa and Lesotho (Latté & Kolodziej, 2004).

5.2.1 Medicinal Value of Plants

Many South African indigenous plants, including the genus Geraniaceae, are used and exported for the production of natural herbal medicines which are marketed internationally (Van Wyk *et al*., 2000). An estimated 27 million South Africans alone make use of traditional remedies for their basic health care (Mander,1998), and therefore uncontrolled harvesting practices and storage methods have contributed to the negative attitude generally held by the international market with respect to African natural products (Tadmor *et al*., 2002). There has been a significant increase in interest in the therapeutic potential of medicinal plants, especially with regards to the reduction of cellular damage due to the presence of free radicals (Schuler, 1990). The medicinal value of some plant species has been recognised in the last thirty years mainly due to the discovery of a range of secondary metabolites that possess powerful antioxidant abilities (Akinmoladun *et al*., 2007). Phenolic compounds (antioxidants) often occur in plants, both edible and non-edible, and are known to possess a number of beneficial qualities, in particular antioxidant ability. Antioxidants are important in the maintenance of optimal health and are involved in the prevention of diseases such as cancer and coronary heart disease (Kähkönen *et al*., 1999).

5.2.2 Reactive oxygen species (ROS)

Various pathogenic agents such as bacteria, fungi and oxidative stress are instrumental in the progression of many diseases in mankind (Adewusi & Afolayan, 2009). Oxidative stress, caused by free oxygen radicals, has been proved to be a leading factor in degenerative disease as well as normal processes of aging in human beings and animals (Halliwell *et al*., 1992). There is much evidence that indicates the vital role that free radicals play in cancer, coronary heart disease, arthritis, dementia and many more harmful conditions (Latte & Kolodziej, 2004). There has been a significant increase in interest in the therapeutic potential of medicinal plants, especially with regards to the reduction of cellular damage due to the presence of free radicals (Schuler, 1990). Cell damage due to free radicals, otherwise known as reactive oxygen species (ROS), is known to be the primary mechanism that is responsible for many human neurological disorders. Free radicals, or oxidative stress, has now been accepted as the main cause underlying many diseases (Atawodi, 2004). Common examples of these are disorders of the digestive tract, viral infections, diabetes mellitus and general inflammatory disease (Atawodi, 2004). Free radicals known as electrons, move around the nucleus of an atom in pairs, singularly or unpaired. The unpaired electrons change the chemical reactivity of an atom or molecule, thereby making it more reactive than the corresponding non-radical (Halliwell,1994).

These free radicals have been found to contribute to a wide number of negative and potentially dangerous conditions in people such as arthritis, ischaemia, atherosclerosis, gastritis, AIDS and cancer (Cook and Samman, 1996; Kumpulainen and Salonen, 1999). They can also be associated with the depletion of antioxidants after exposure to pollutants in the atmosphere, radiation, chemicals and ingested toxins (Halliwell, 1994; Kuhnan, 1976; Kumpulainen & Salonen, 1999; Younes, 1981). There has been an increase in interest recently in the ability of some medicinal plants to reduce the damage of free radicals (Schuler, 1990), and plant-based antioxidants are becoming more popular than artificial ones due to the general swing to natural products (Akinmoladun *et al*., 2007).

5.2.3 Phenolic compounds with antioxidant values

Phenolic compounds (antioxidants) often occur in plants, both edible and nonedible and are known to possess a number of beneficial qualities, in particular antioxidant ability.

Antioxidants are important in the maintenance of optimal health and are involved in the prevention of diseases such as cancer and coronary heart disease (Kähkönen *et al*., 1999). There are a great number of medicinal plants which contain high levels of antioxidants, for example polyphenols. These play an important part in neutralizing free radicals (Djeridane *et al*., 2006).

Fruits and vegetables contain high levels of polyphenolic compounds with antioxidant activity and can reduce the risk of chronic inflammation in humans (Finley, 2004). Plant-based antioxidants are now preferred to the synthesized ones because of safety concerns (Akinmoladun *et al*., 2007). These factors have inspired the widespread screening of plants for possible medicinal and antioxidant properties, and the development and utilization of antioxidants of natural origin (Jayaprakasha *et al*., 2001).

5.2.4 *Pelargonium reniforme* Curtis and *Pelargonium sidoides* DC

Pelargonium reniforme Curtis and *Pelargonium sidoides* DC have been studied and researched at great length due to their high antioxidant capacity. *P. reniforme* is a shrublet or sub-shrub with pink to purple flowers, small, cordate, velvety leaves and tuberous roots (Jones & Adams, 2011). It is found in the grasslands and dry flats between Knysna and Umtata in the Eastern Cape Province of South Africa (Van Der Walt, 1997). It is normally found in short grassland areas in stony soil in sand to clay-loam (Mativandlela *et al*., 2006). This species reaches a height of 300 – 400 mm but can attain a height of 1 meter. The velvet-textured leaves are grey-green in colour with distinct venation on the upper surface. *P. reniforme* flowers throughout the year with pink to magenta flowers (Jones & Adams, 2011). It is highly adapted to a variety of altitudes and is widely used by indigenous people in traditional cures for a variety of illnesses and diseases (Newton *et al*., 2013; Brendler & van Wyk, 2008). It is traditionally utilised for treating dysentery, liver problems in calves and sheep and for the treatment of diarrhoea, colic and fever in people (Watt & Breyer-Brandwijk, 1962).

Pelargonium sidoides DC is a perennial geophytic shrublet indigenous to South Africa and endemic mainly to the highlands of Lesotho and the Eastern Cape Province of South Africa. The species grows in a rosette-like formation with its leaves close together and has a system of thickened, underground tuberous roots which are traditionally used as a treatment for ailments such as bronchitis, throat infections and tuberculosis (Lewu *et al*., 2006). It is distinguishable from *P. reniforme* by its dark purple, almost black flowers which are present

throughout the year (Lawrence, 2001). This species is traditionally used for treating dysentery, liver problems in calves and sheep and for the treatment of diarrhoea, colic and fever in people (Watt & Breyer-Brandwijk, 1962).

5.2.5 Water regulation

Humans use up to 80% of fresh water across the world, mainly in agriculture and crop production. Unfortunately, in many areas of the earth this use is not sustainable and water supplies are under constant pressure from other sources and overpopulation, as well as climate change and diminishing rainfall (Morison *et al*., 2008). A nutrient deficient state exists where rainfall is minimal and evaporation rate is high, and these factors contribute toward an unfavourable environment for plant growth (Manoharan *et al*., 2010). Drought stress is widely believed to be the most common factor limiting crop survival (Kramer, P.J. & Boyer, J.S., 1995). Water stress has been found to alter the production of secondary metabolites in medicinal plants (Petropoulos *et al*., 2008). A number of studies on water deficit and metabolites have shown that less water can have a positive effect on the accumulation of valuable compounds such as phenolics (Bettaieb *et al*., 2010). As limited water results in drought, and the inevitable economic and sociological problems and follow, it is essential that methods are found to produce as many food and medicinal crops as possible (Pereira *et al*., 2007). This study was undertaken in order to establish the optimal irrigation levels necessary when growing *P. reniforme* and *P. sidoides* commercially whilst achieving optimal production of antioxidant products.

5.2.6 Arbuscular mycorrhiza

Arbuscular mycorrhiza (AM) has been well researched as it has been shown to be instrumental in alleviating water stress in plants (Heidari & Karami, 2012). Drought is one of the major reasons for crop failure worldwide and can reduce yields by more than 50% (Wang *et al*., 2003). Many studies have demonstrated that root colonization by AM assists the host plant deal with reasonable drought stress with a higher recovery rate after irrigation was started (Subramanian *et al*., 1997). The fungi colonise the root cortex and form a mycelium which helps the plant to take up more nutrients and to form more stable soil aggregates which in turn improve soil quality (Jeffries & Barea, 1994; Bethlenfalvay & Schüepp, 1994). Valuable horticultural crops are produced with the addition of AM and often display mycotrophy, and their optimal growth and development is often seen as dependant on early AM establishment (Gianinazzi-Pearson, V. 1996).

AM represents a mutually beneficial relationship between host plants and various fungi species which enables the host plant to take up some nutrients and water more efficiently (Parniske, 2008). An AM relationship or association with plant roots were shown to improve plant growth and nutrient uptake and enhance water uptake (Al-Karaki *et al*., 2004).

Plants need macro- and micro- nutrients for optimal growth; crops use up to 50% of inorganic fertilisers which means that the remaining fertiliser is wasted while mobile nutrients such as nitrates and sulphates are lost by leaching out beyond the root zone. Nutrients which are immobile, such as phosphates, zinc and potassium, can be eroded out and lost. This loss of nutrition will have a negative impact on crop production (Cavagnero *et al*., 2015). A study by Essahibi *et al*. (2017), demonstrated that AM relationships assisted with the ability of *Ceratonia siliqua* (Carob) to cope with water deficit by increasing cell wall rigidity and osmolyte accumulation. Under severe water stress AM plants maintained high cell membrane function and high osmotic potential. Many vegetable species are able to host mycorrhizal fungi and therefore become more efficient at maintaining water and nutrients and also able to improve their ability to withstand environmental stress factors such as nematode damage and root disease (Baum *et al*., 2015). The aim of this study was to assess the comparative antioxidant activity and accumulation of metabolites using assays for total polyphenols, oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) in *Pelargonium reniforme* Curtis and *Pelargonium sidoides* DC under different watering schedules and arbuscular mycorrhiza applications.

5.3 MATERIALS AND METHODS

5.3.1 Greenhouse experiment

The experiment was conducted from June to September 2014. It was located in the research greenhouse at the Cape Peninsula University of Technology, Cape Town, South Africa with the GPS co-ordinates - 33° 55' 58.27S, 18° 25' 57.04E. This facility ensured that the environment in which the experiment was conducted was controlled. Minimum and maximum temperatures and relative humidity were recorded 3 times daily. Temperatures within the greenhouse ranged between 14 - 34°C during daytime and 10 - 19°C at night. Relative humidity of the facility averaged 37%. The water used in the experiment was municipal water filtered through a Hager IP 65 Water Filtration Plant de-ioniser.

5.3.2 Plant preparation

The plant sample consisted of *P. reniforme* cuttings which were obtained from Trevor Adams, horticulturist at Kirstenbosch Botanical Gardens, Cape Town, South Africa and plant material of *P. sidoides* which were obtained from Pico Gro growers, 60 Milner Road, Glen Austin, Midrand, South Africa 1685. One hundred and fifty uniform cuttings were made using the stock material and placed in cutting trays containing washed and sterilized coarse river sand (Lawrence, 2001). The cutting trays were then placed in the main greenhouse on the Cape Town campus of the Cape Peninsula University of Technology on heated propagation beds. Once rooted, one hundred *P. reniforme* plants and one hundred *P. sidoides* plants of uniform size were then planted into individual 12.5cm pots containing an inert, sterilized medium (2:1:1:1) consisting of two parts coco peat, one part foamalite, one part Consol® silica sand grade 6/17 and one part perlite. The plants were then placed under 40% shade cloth for one week to harden off before being planted out into the experimental site. The plants were placed onto galvanized steel tables covered in black plastic sheeting with ten replicates of each treatment.

5.3.3 Experimental treatments

The experiment consisted of five watering regimes which were applied to fifty plants of each species *P. reniforme* and *P. sidoides* without inoculation with arbuscular mycorrhiza and to fifty plants of each species of *P. reniforme* and *P. sidoides* in conjunction with inoculation with arbuscular mycorrhiza. A total of one hundred plants of each species of *P. reniforme* and *P. sidoides* with fifty each receiving mycorrhiza and fifty each not receiving mycorrhiza upon planting out into the experiment. A randomized block design, made up of 10 replicates each with 10 individual plants was used to study the effects of various irrigation frequencies with and without mycorrhiza inoculation on both species. Drip irrigation was used with the following irrigation frequencies based on experiments conducted by Wang *et al*., (2003) on the effect of different drip irrigation frequencies on potato growth and yield:

1) WF1 (320 mls once every day to PC). Control treatment (Kirnak et al., 2001).
2) WF2 (320 mls once every three days to PC
3) WF3 (320 mls once every six days to PC)
4) WF4 (320 mls once every twelve days to PC)
5) WF5 (320 mls once every twenty-four days to PC)

The experiment consisted of the following treatments:

Group A: *P. reniforme* and *P. sidoides* without mycorrhiza receiving WF1, WF2, WF3, WF4 and WF5.
Group B: *P. reniforme* and *P. sidoides* with mycorrhiza receiving WF1M, WF2M, WF3M, WF4M and WF5M.
Plants which received mycorrhiza were inoculated with 30 grams of AM (commercially available product Mycoroot™ obtained from the Horti Shop, 125 Belvedere Road, Claremont, Cape Town) on planting. Each individual pot was irrigated with half-strength Hoaglands solution due to the reduced nutrient requirements of South African fynbos species in the wild (Midgley *et al*., 1995). Hoaglands solution consisted of nitrogen 210 ppm, potassium 235 ppm, calcium 200 ppm, phosphorus 31 ppm, sodium 64 ppm, magnesium 48 ppm, boron 0.5 ppm, iron 1-5 ppm, manganese 0.5 ppm, zinc 0.05 ppm, copper 0.02 ppm and molybdenum 0.01 ppm (Anonymous, 2017). Pot capacity was determined at 320 millilitres.

5.3.4 Acclimatization of experimental plants

Rooted cuttings from *P. reniforme* and *P. sidoides* gradually acclimatized in the sterilized growing medium (2:1:1:1) of two parts coco peat, one part foamalite, one part Consol® silica sand, grade 6/17 sand and one part perlite under 40% shade cloth for one week to harden off before being moved to the experimental site.

5.3.5 Hardening-off and growing period

The plants were kept for one week adapting to the greenhouse situation after hardening off. The experiment was conducted from the second of June 2014 until the eighteenth of September 2014. After this period all plants were harvested and various postharvest measurements were measured. Parameters measured included chlorophyll content (SPAD), root and shoot wet weights and root and shoot dry weights.

5.3.6 Sample preparation

Root material of both species was extracted by mixing 100mg of the dried powdered material with 25 ml of 80% (v/v) ethanol (EtOH) (Merck, South Africa) for 1 hour. It was centrifuged at 4000 rpm for 5 min and the supernatants were used for all analyses.

5.4. Determination of antioxidant capacity and content

Antioxidant activity and accumulation of metabolites within the roots were assessed using assays for total polyphenols, oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP).

It was recommended that three methods be used for the standardization of antioxidant capacity and determination of total polyphenols at the 'First International Congress on Antioxidant Methods in 2004 (Prior *et al*., 2005). The ORAC assay – oxygen radical absorbance capacity, the TEAC assay – trolox equivalent antioxidant capacity and the FRAP assay – ferric reducing ability of plasma were the three assay methods in question.

5.4.1. Oxygen radical absorbance capacity (ORAC) assay

The ORAC assay was performed according to the method as described by Prior *et al.* (2005). 2,2′-azobis (2-amidino-propane) dihydrochloride (AAPH, peroxyl radical) (Sigma-Aldrich, South Africa) was prepared by mixing 150 mg with 6 mL of Phosphate buffer (75mM, pH 7.4). A 48nM Fluorescein sodium salt (Sigma, South Africa) was also prepared in Phosphate buffer. 12 µl of the crude extract was then mixed with 138 µl of the fluorescein and 50 µl AAPH in a black 96-well plate. Fluorescence conditions was set at 485 nm excitation and 530 nm emissions and the plate was read every minute for two hours using a Fluorskan Ascent plate reader (Thermo Electron Corporation, USA). Trolox (Sigma, South Africa) was used as the standard with concentrations between 0 and 25 µM Trolox. The results were expressed as µM trolox equivalent (TE) per g dry weight (µM TE/g DW).

5.4.2. Polyphenol assay

The total polyphenols assay (Folin assay) was performed as described by Ainsworth and Gillespie (2007). Folin & Ciocalteu's phenol reagent (2N, Sigma South Africa) was diluted 10 times with distilled water and a 7.5% sodium carbonate (Sigma, South Africa) solution was prepared. In a 96-well plate, 25µl of the crude extract was mixed with 125µl Folin & Ciocalteu's phenol reagent and 100µl sodium carbonate. The plate was incubated for 2 hours at room temperature. The absorbance was then measured at 765 nm in a Multiskan spectrum plate reader (Thermo Electron Corporation, USA). The samples polyphenol values were calculated using a gallic acid (Sigma, South Africa) standard curve with concentration varying between 0 and 500 mg/L. The results were expressed as mg gallic acid equivalents (GAE) per g dry weight (mg GAE/g DW).

5.4.3. Ferric reducing antioxidant power (FRAP) assay

The FRAP assay was performed using the method of Benzie and Strain (1999). FRAP reagent was prepared by mixing 30 mL Acetate buffer (0.3M, pH 3.6) (Merck, South Africa) with 3 mL 2,4,6- tripyridyl-s-triazine (10mM in 0.1M Hydrochloric acid) (Sigma, South Africa), 3 mL Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H2O$) (Sigma, South Africa) and 6 mL of distilled water. In a 96-well plate, 10 µL of the crude sample extract was mixed with 300 µL of the FRAP reagent and incubated for 30 min at room temperature. The absorbance was then measured at 593 nm in a Multiskan spectrum plate reader (Thermo Electron Corporation, USA). The samples FRAP values were calculated using an L-Ascorbic acid (Sigma-Aldrich, South Africa) standard curve with concentrations varying between 0 and 1000 µM. The results were expressed as µM ascorbic acid equivalents (AAE) per g dry weight (µM AAE/g DW) (Ainsworth & Gillespie, 2007).

5.4.4. Statistical analysis

All data was analysed using one-way analysis of variance (ANOVA), using the computing software program STATISTICA 13.2. Occurrence of statistical difference was determined by using the Fisher Least Significance Difference (L.S.D.) at values of $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$ levels of significance (Steel & Torrie, 1980). The statistical significance between antioxidant activity values of the various crude plant extracts was determined by an analysis of variance (ANOVA) where $P \leq 0.05$ was considered to be statistically significant. The computer program employed for the statistical analysis was Medcalc version 9.4.2.0 (Medcalc, Belgium).

5.5 RESULTS AND DISCUSSION

5.5.1 Antioxidant capacity

5.5.1.1 Total polyphenol content

Water frequency: The total polyphenols in the water frequency treatments were found significantly higher ($P \leq 0.05$) in *P. reniforme* compared to that of *P. sidoides* at $P \leq 0.001$ (Table 5.1). No significant differences were found in the addition of mycorrhiza in either species. In Table 5.1 the polyphenols showed higher significance ($P \leq 0.05$) in WF2 without

the addition of mycorrhiza in *P. reniforme*, while higher water frequencies (WF1, WF2 and WF2M) were more significant at (P ≤0.001) in *P. sidoides*. The highest significance was therefore seen in WF2 in both species. These plants received water every 3 days, a moderate to high watering regime. There is a strong correlation of polyphenols evident in both *P. reniforme* (65.39±12.66a) and *P. sidoides* (78.60±6.34a) at the WF2 water frequency (Fig. 5.1 and 5.2). These were the lowest values of polyphenols recorded in the experiment. Many studies have demonstrated the relationship between water availability and polyphenol production and in general the highest levels of these compounds are found in plant material of plants exposed to water deficit conditions (Alinian *et al*., 2015; Amiri *et al*., 2017; Bogale *et al*., 2016). In this study, the highest values of polyphenols were measured at WF5 in both species, i.e. at water deficit conditions. Although not statistically significant, these results are supported by numerous other studies. The effects of drought on the production of phenolic compounds has been the subject of many research studies in many different plant species and involving many different plant parts (Aninbon *et al*., 2016). Bogale *et al*. (2016), recorded results in a study of tomato cultivars subjected to various watering regimes which showed that a higher amount of phenolic compounds was measured in plants subjected to water deficit. Further field studies over a longer period would be useful to establish whether water deficit has an effect on polyphenol production in more mature plants and over a longer growing period.

Mycorrhiza: Table 5.1 shows that the application of mycorrhiza treatments to the growth medium made no significant (ns) difference to the polyphenol count of the dry weight of both species of *P. reniforme* and *P. sidoides*. In contrast to the water frequency treatments these results showed that added mycorrhiza had no effect on the improvement of polyphenols during the study. However, the highest value of polyphenols was measured in *P. sidoides* (114.70 ±20) at the lowest watering frequency with the addition of mycorrhiza. The study on mycorrhiza inoculation and its effects on phenolic content of *Pelargonium graveolens* by Amiri *et al*. (2017) demonstrated that the amounts of antioxidant substances, including phenolics, increased with AM inoculation across all watering regimes, in particular low watering regimes. Therefore, the argument exists that the addition of AM to plants at the root zone is likely to enhance their antioxidant capacity in all parts of the plant.

5.5.1.2. Ferric reducing antioxidant power (FRAP) content

Water frequency: In Table 5.2 the water frequencies show no significance in the FRAP value of both species *P. reniforme* and *P. sidoides.* Both species showed a similar trend with

the change in water amounts having no impact in measurements of the FRAP values on the dry weights of the species.

Mycorrhiza: The FRAP values of *P. reniforme* were found to be significantly (P ≤0.05) higher in the mid water frequency with mycorrhiza addition (WF3M) compared to *P. sidoides* with no significance (Fig 5.3). The results show that the addition of mycorrhiza improved the FRAP values (613.31±96.34abcd) in the dry root weight of *P. reniforme* (see Table 5.2). Amiri *et al*. (2017) found similar results in their experiment on *P. graveolens* in which the application of AM resulted in an increased antioxidant level in the form of essential oils.

Water frequency interaction with mycorrhiza: No significance was observed in water frequencies and mycorrhiza interaction between the two species of Pelargonium. These results show that no significance (ns) in the ORAC values of both *P. reniforme* and *P. sidoides* occurred (Table 5.3). From the experiment it is clear that FRAP values showed no significant difference.

Figure 5.1 *Pelargonium reniforme* at harvest showing root development.

Figure 5.2 *Pelargonium sidoides* at harvest showing root development in WF4 treatment with no mycorrhiza.

Figure 5.3 *Pelargonium reniforme* at harvest showing root development in WF1 treatment with mycorrhiza.

5.5.1.3. Oxygen radical absorbance capacity (ORAC) content

Water frequency: Water frequency applications played a major role in plant growth and root tuber development. Statistically the ORAC values of both species *P. reniforme* and *P. sidoides* of the dry weight of root tubers were found to be not significant (ns) (see Table 5.3). The water frequency had no significant impact on the ORAC value of the *Pelargonium* species.

Mycorrhiza: Table 5.1 reveals a similar trend with no significant (ns) ORAC values in the dried root tubers in mycorrhiza treatments for both *P. reniforme* and *P. sidoides.* These results showed that added mycorrhiza had no statistical effect on the ORAC values.

Water frequency interaction with mycorrhiza: A strong correlation with only water frequencies and mycorrhiza treatments were found in the water frequency and mycorrhiza interaction. The addition of AM also showed no significance (ns) in the ORAC values of both *P. reniforme* and *P. sidoides* (see Table 5.3). From the experiment it is clear that ORAC values showed no significant difference.

Table 5.1. The total polyphenols (mg GAE/g dry weight) content of the roots of *P. reniforme* and *P. sidoides* plants. Mean squares from the analysis of variance for the effect of five various watering treatment frequencies with and without mycorrhiza.

Treatment		*P. reniforme*		*P. sidoides*	
Watering Frequency	**Mycorrhiza**				
WF1M	Yes	83.70	±20.27bcd	87.14	±10.96ab
WF1	No	74.69	±7.96abc	81.28	±3.86a
WF2M	Yes	68.20	±8.03abc	76.59	±10.59a
WF2	No	65.39	±12.66a	78.60	±6.34a
WF3M	Yes	66.19	±21.02ab	90.21	±2.84abc
WF3	No	81.73	±8.49abc	104.16	±7.36cde
WF4M	Yes	68.96	±8.6abc	96.20	±6.31bcd
WF4	No	82.08	±7.45abc	98.99	±11.71bcd
WF5M	Yes	84.70	±14.28cd	114.70	±20.14e
WF5	No	101.21	±5.81d	104.35	±2.45de

Two-way ANOVA F-Statistics

Watering Frequency	4.7 *	13.3 ***
Mycorrhiza	2.8 ns	0.0 ns
Watering Frequency * Mycorrhiza	1.8 ns	1.8 ns

Bars represent mean values ±SD (all treatments n=10) are shown in columns. The mean values followed by different letters are significantly different at P ≤0.05 and P ≤0.001 as indicated by an (*) as higher value when comparing with plants part of two species, ns = not significant as calculated by Fisher's least significant difference.

Table 5.2 Mean squares from the analysis of variance for the effect of five varied watering treatment frequencies with and without mycorrhiza on antioxidants of *P. reniforme* and *P. sidoides* cuttings grown from week 3 to 15 in an inert, soilless medium. The ferric reducing antioxidant power (FRAP) (µM AAE/g dry weight of the roots of these species.

Treatment		***P. reniforme***		***P. sidoides***	
Watering Frequency	**Mycorrhiza**				
WF1M	Yes	700.69	±160.4abcd	696.28	±81.78a
WF1	No	575.02	±49.75ad	782.98	±84.96abc
WF2M	Yes	739.70	±212.04abc	760.19	±84.96abc
WF2	No	538.06	±184.84d	699.39	±134.05a
WF3M	Yes	613.31	±96.34abcd	798.06	±282.69abc
WF3	No	581.41	±77.91abd	931.70	±165.55c
WF4M	Yes	781.15	±205.84bc	762.65	±98.72abc
WF4	No	740.72	±116.9abc	721.00	±72.2ab
WF5M	Yes	798.41	±104.93c	854.33	±111.8abc
WF5	No	748.14	±65.17abc	916.32	±142.11bc

Two-way ANOVA F-Statistics

Watering Frequency	2.6 ns	2.4 ns
Mycorrhiza	4.2 *	0.7 ns
Watering Frequency * Mycorrhiza	0.5 ns	0.7 ns

Bars represent mean values ±SD (all treatments n=10) are shown in columns. The mean values followed by different letters are significantly different at P ≤0.05 as indicated by an (*) as higher value when comparing with plants part of two species, ns = not significant as calculated by Fisher's least significant difference.

Table 5.3 Mean squares from the analysis of variance for the effect of five varied watering treatment frequencies with and without mycorrhiza on antioxidants of *P. reniforme* and *P. sidoides* cuttings grown from week 3 to 15 in an inert, soilless medium. The oxygen radical absorbance capacity (ORAC) (µM TE/g dry weight) of the roots of these species.

Treatment		***P. reniforme***		***P. sidoides***	
Watering Frequency	**Mycorrhiza**				
WF1M	Yes	461.78	±96.83a	519.13	±115.16ab
WF1	No	432.02	±77.5a	613.27	±138.35abc
WF2M	Yes	481.95	±134.66a	472.04	±13.74b
WF2	No	422.15	±100.76a	500.98	±116.73ab
WF3M	Yes	421.03	±86.47a	517.43	±77.ab
WF3	No	549.37	±170.71a	632.59	±133.47ac
WF4M	Yes	507.50	±87.64a	499.91	±72.86ab
WF4	No	425.80	±29.97a	575.12	±103.44abc
WF5M	Yes	503.00	±17.97a	678.88	±162.18c
WF5	No	555.33	±64.64a	639.57	±88.67ac
Two-way ANOVA F-Statistics					
Watering Frequency		0.9 ns		2.6 ns	
Mycorrhiza		0.0 ns		2.5 ns	
Watering Frequency * Mycorrhiza		1.6 ns		0.6 ns	

Bars represent mean values ±SD (all treatments n=10) are shown in columns. The mean values followed by different letters are, ns = not significant as calculated by Fisher's least significant difference.

5.6 CONCLUSION AND RECOMMENDATIONS

There is a great deal of research involving plant antioxidant capability, especially nowadays in the age of increasing diagnoses of illnesses such as cancer, heart disease and many life-style related diseases. Oxidative stress is known to be partly responsible for several diseases, as well as the pathogenesis of some of them. Naturally occurring antioxidants are considered preferable to synthesised antioxidants due to the worldwide swing towards natural treatments and medicines. Research into antioxidant activity and its presence in plants is becoming one of the most widely studied area of the natural sciences, as well as the area of food technology. Many plant species have been investigated and these investigations have found there to be thousands of natural compounds which have antioxidant capacity. This experiment revealed that a certain amount of water deprivation (experimental plants watered every 24 days) revealed the highest values of polyphenols statistically. It was also clear from the experiment that the addition of mycorrhiza to the root zone at planting could have a positive impact on the formation of antioxidant compounds.

This study confirmed that *Pelargonium reniforme* and *Pelargonium sidoides*, two medicinally valuable species, possess antioxidant compounds in the form of polyphenols. Although these species have been widely researched, there is a need to establish the growing conditions and farming practices necessary for successful commercial production of these plants. This is especially important in the light of a burgeoning world population which will no doubt lead to a higher demand for their products. As overharvesting in the wild of these species is already considered to be a problem, commercial production is a viable alternative. This is underway at present, but there are at present very few commercial growers. Further research using field studies is indicated to establish their optimal growing conditions.

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CHAPTER 6

RESULTS AND DISCUSSION

In chapter 2 it was concluded that there is a worldwide interest in natural remedies and that this phenomenon has resulted in a threatened status for certain plant species in the wild. As there is a high value placed on the two *Pelargonium* species discussed in this study, both medically and culturally, there is a need to identify the challenges involved in producing these plants commercially in order to minimise the future possibility of overharvesting and eventual extinction.

Chapter 3 focused on different growth parameters of *P. reniforme* and how they responded to different amounts of water as well as the inoculation with AM or the absence of AM. Wet shoot weight of the plants showed significant difference at P≤ 0.001 in the highest water availability, both with and without the presence of AM. The highest mean of wet shoot weight was found in the control (daily watering) without mycorrhizal inoculation. Dry shoot weight revealed no significance across the range of treatments. Wet root weight at harvest was not significant in any of the treatments, although the root length in the plants from treatment 4, i.e. minimal watering, was the longest compared to the other treatments. This result indicates that plants may produce longer roots as a survival mechanism under water deficit conditions. Dry root weights were higher at water frequencies 1,2,3, and 5, with the highest dry root weights measured in WF3 without mycorrhiza and WF3M with mycorrhiza. Shoot length in the treatment WF5 (minimal water with no AM) showed the only significance (P≤ 0.001) and both the application of AM as well the interaction between watering and addition of AM produced no significant differences. Total wet weight of *P. reniforme* showed the highest means measured to be WF3 (moderate watering) with and without AM, with the highest mean measured being WF3 with no addition of AM. There was a high level of significance at P≤ 0.05 in watering frequencies combined with AM additions. Water frequencies 1 to 4, with and without AM addition, were statistically significant with regards to total dry weight of the plants, with WF1 (daily watering) showing the highest means at 6.49 grams. This is consistent with many studies on water deficit where it is found to result in decreased vegetative growth.

Results for nutrient uptake in *P. reniforme* revealed significance (P≤0.001) in the availability of C, K, Mg, N and P in all watering frequencies with no mycorrhizal inoculation. However, the only nutrient which showed high significance with mycorrhizal inoculation was Mg. There

was also a highly significant interaction (P ≤0.05) with and without mycorrhiza in the moderate watering frequency 3, i.e. Mg was more available to the plants at lower water availability. It would therefore be recommended that additional Mg be used in a fertigation program under drought conditions. Calcium (C) uptake by the plants was higher in high water availability at a significance level of P≤ 0.001 both with and without mycorrhizal inoculation, this has been reported in numerous studies. Nitrogen uptake was positively affected by higher water availability, i.e. WF1 and WF2, with and without mycorrhiza. More water availability at the root zone therefore increased nitrogen uptake by the plants. This study found that potassium (K) uptake was significantly increased at P ≤0.001 in WF2, with relatively high water availability and without the presence of mycorrhiza. This confirms that water is necessary for the movement of K through the soil to the root zone. Studies on nutrient uptake have found decreases root growth as a result of water deficit situations. This experiment demonstrated that phosphorus availability was more significant (P ≤0.001) with less water available, at water frequencies 3, 4 and 5. The presence of mycorrhiza made no significant difference to the P uptake in this study.

Chlorophyll content of leaves was measured weekly for a duration of six weeks. In the first week, the most significant chlorophyll reading (P ≤0.001) was at the lowest water frequency with no mycorrhiza and this significance persisted through week 12 but was more dominant in the treatments with high water availability i.e. WF1 & WF2. At week 14 there was a clear significance at P ≤0.05 in the lowest water frequency with mycorrhiza. Table 3.4 showed in week 12 that the interaction between water availability and the presence of mycorrhiza became more significant at P ≤0.05 at the highest water frequency. These results show that chlorophyll production benefitted from sufficient levels of irrigation and the action of the mycorrhiza. Interestingly, results changed at week 13 where increased chlorophyll production occurred at a significance of P ≤0.05 in treatments WF1M, WF5M and WF5, i.e. low and high water availability, possibly indicating that more mature plants are able to photosynthesise at different levels of water availability with mycorrhizal support.

In chapter 4 various growth parameters for *P. sidoides* were measured. Leaf numbers of all plants were measured weekly. Water frequency was important for new leaf development. High to medium water availability was significant at P ≤0.05 in weeks 3 to 5 of the experiment. From weeks 7 to 15, the most significant reading was found at P ≤0.001 in treatments WF1M and WF3M, WF2 at week 11 and WF3 at week from weeks 13 to 15. These results demonstrate that a moderate to high amount of water is necessary for consistent leaf production. The most significant results at P ≤0.05 were shown in WF3M and

WF3 from weeks 3 to 15 of the experimental period. It is therefore clear from this result that a medium irrigation frequency is necessary for the successful growth of *P. sidoides*. This is supported by Mofokeng *et al*. (2015), whose study on *P. sidoides* demonstrated increased growth parameters under moderate irrigation levels. The inoculation of mycorrhiza was shown to make no significant difference to leaf numbers throughout the duration of the experiment, and no significant difference was also demonstrated in the interaction between water frequency and the presence of mycorrhiza. This finding is contradictory to that of Csima et al. (2012) who found that the addition of mycorrhiza to the growth medium of *Pelargonium hortorum* plants benefitted nutrient uptake. This finding is supported by dos Santos *et al*. (2010) in experiment on *Zingiber officinale* where leaf numbers in non-mycorrhizal plants were much lower than in mycorrhizal plants. It was clear that water availability is the most important factor in the development of new leaves.

Varying significance ($P \leq 0.05$ and $P \leq 0.001$) was noted in the uptake of nutrients by the *P. sidoides* plants, particularly that of N, P, K and Ca. No significance was shown in the uptake of Mg (see Table 4.2). The uptake of Ca was the most significant at the low water frequency, WF4M, at a significance of $P \leq 0.05$ with the addition of mycorrhiza demonstrating an increase in uptake as well. This showed a similar result to that of P uptake which was more significant at lower water frequencies with mycorrhizal addition. Absorption of Mg was no significant in the watering frequencies. These results suggest that minimal water is necessary for both P and Ca absorption. High water availability resulted in more efficient uptake of N, while K was taken up by the plants more efficiently at $P \leq 0.001$ at a lower water frequency, i.e.WF4M. Inoculation with mycorrhiza showed no significance in the absorption on N, P, Ca or Mg. Potassium absorption was increased at a significance level of $P \leq 0.05$ at the highest watering frequency WF1M (see Table 4.2). Most recent studies have concluded that mycorrhizal inoculation has a positive effect on nutrient uptake (Ŝimůnek & Hopmans, 2009). The interaction between water availability and mycorrhiza showed no significance (ns) in the uptake of N, P, K, Ca or Mg. It was therefore deduced that the two treatments acted independently of each other. This is opposite to the findings of Sweatt & Davies (1984), who also experimented with *Pelargonium hortorum* and found that seedlings of experimental plants under high moisture regimes and with mycorrhizal inoculation demonstrated high phosphorus uptake.

The effect of water availability on chlorophyll production was seen in Table 4.3. Medium to low water availability resulted in higher chlorophyll readings. Treatments WF3M, WF3, WF4M and WF4 all showed significance at $P \leq 0.05$ in week 10 and $P \leq 0.001$ at weeks 11 to

13 in chlorophyll production levels. This result is opposite to the findings of Amiri *et al*., (2017), where leaf chlorophyll content decreased as the water deficit increased. Chlorophyll production of *P. sidoides* was increased with mycorrhizal inoculation between weeks 10 and 12 at a significance level of P ≤0.05 in treatments WF4M, WF1M, WF2M, WF4M and WF5M. This suggests that the presence of mycorrhiza clearly improved the manufacture of chlorophyll even with a decrease in water availability and is consistent with numerous studies. There were also similar significance levels (P ≤0.05) in the interaction between water frequency in treatment WF5 and mycorrhiza in the treatments WF2M in week 11 and WF1M, WF2, WF3M, WF3, WF4M and WF5M in week 15 of the experiment. There was increased chlorophyll production in high and low water frequencies with the presence of mycorrhiza, a finding similar to that of Asrar *et al*. (2012), where the combination of mycorrhizal inoculation and moderate irrigation levels increased the leaf chlorophyll.

In chapter 5 antioxidants in the form of polyphenol content were measured by three standard assays; FRAP, ORAC and Folin assay. The total polyphenol content was found to be significantly higher (P ≤0.05) in the moderate to high watering frequency (WF2) in *P. reniforme* and in WF1, WF2 and WF2M in *P. sidoides*. Both species therefore showed the highest significance in WF2, moderate to high watering frequency. The highest polyphenol values were found at the lowest watering frequency, i.e. water deficit conditions, although these readings were not statistically significant. Water deficit has been found to result in an increase in polyphenols and other antioxidants in many studies (Alinian *et al*., 2015; Amiri *et al*., 2017; Bogale *et al*., 2016). The addition of mycorrhiza showed no significance in the statistics but it is worth noting that the highest values of phenolic content was measured at the lowest watering frequency (WF5M) in *P. sidoides* with the addition of mycorrhiza, further supporting the use of AM to increase antioxidant potential of crop plants.

Water frequencies revealed no significance in the FRAP assay in either species. The different watering frequencies had no effect on measurements of FRAP values of polyphenol content in the dry root material. Interestingly, the FRAP values were found to be significantly higher in *P. reniforme* at WF3M compared to *P. sidoides* which showed no significance. This result shows once again the positive effect of mycorrhiza on plant functions. In their study on *Pelargonium graveolens,* Amiri *et al*. (2017), found similar results, where the essential oil content increased with the addition of AM. There was no significance observed in the FRAP values and the ORAC values in the interaction between watering frequency and mycorrhizal addition in either species. There was also no significance noted in watering

frequency on the ORAC values. No significance was also noted in ORAC values in the mycorrhizal treatments.

CHAPTER 7

CONCLUSION AND RECOMMENDATIONS

7.1 CONCLUSION

The importance of the genus *Pelargonium* has been well-documented and researched over the years and much is now known about their medicinal value. It remains important that more research is carried out using field or other commercial methods of cultivation in order to ensure the availability of its extracts for generations to come and to satisfy an increasing demand worldwide. The effects of water availability, with and without the addition of mycorrhiza demonstrated an increase in the growth parameters and uptake of some nutrients in both species. The experiment showed that, while irrigation can be decreased slightly during cultivation and the growth remains satisfactory, the use of arbuscular mycorrhiza is not necessarily recommended due to its high cost and limited benefit. This is significant due to the high cost of commercially available AM.

Further studies over extended periods are required to determine further relevance of AM at specific growth stages. The results of this study present valuable information regarding water and nutrient availability. Less potassium becomes available to plants as irrigation decreases. The opposite was seen with more phosphorous available at lower water requirements. As water increased, nitrogen availability also increased and more water also resulted in higher Ca uptake with mycorrhiza addition.

The availability of water to plants therefore has a significant impact on their growth. Farmers can therefore adapt their irrigation regimes to the desired growth required with the aim being conserving water in drought-stricken countries. The experiment proved that while water levels can be reduced during cultivation, the presence of AM had limited impact on the growth parameters of both species over the experimental period. The availability of higher amounts of water had a key role in increased leaf numbers in the early stages of the experiment in *P. sidoides.* Again, a higher availability of water resulted in increased plant growth, with high to moderate watering frequencies remaining significant throughout the experimental period from 3 to 15 weeks. Optimal water availability of water increases plant growth, especially when other growth requirements such as nutrient availability are met. Additional water can increase growth and quality for commercial production. This is

especially important where the leaves of *P. sidoides* can be used for medicinal purposes. This study found no significance between watering frequencies and the addition on AM in the production of new leaves in *P. sidoides*.

The study also agreed with various others that the addition of AM did enhance nutrient uptake. The commercial production of medicinal species such as *P. reniforme* and *P. sidoides* can be improved if amounts of water frequencies and applications of AM are correctly calculated to control limited available nutrients to enhance plant growth. The commercial production of these medicinal species can be improved if amounts of water frequencies and applications of AM are correctly calculated to control limited available nutrients to enhance plant growth. This will limit wastage of excess and limited available nutrients. There is a great deal of research involving plant antioxidant capability, especially nowadays in the age of increasing diagnoses of illnesses such as cancers, heart disease and many life-style related diseases. There is a great deal of research involving plant antioxidant capability, especially nowadays in the age of increasing diagnoses of illnesses such as cancer, heart disease and many life-style related diseases. This experiment revealed that a certain amount of water deprivation (experimental plants watered every 24 days) revealed the highest values of polyphenols statistically. It was also clear from the experiment that the addition of mycorrhiza to the root zone at planting could have a positive impact on the formation of antioxidant compounds. This experiment revealed that a certain amount of water deprivation (experimental plants watered every 24 days) revealed the highest values of polyphenols statistically. It was also clear from the experiment that the addition of mycorrhiza to the root zone at planting could have a positive impact on the formation of antioxidant compounds.

Pelargonium reniforme and *Pelargonium sidoides* possess these polyphenolic compounds. Although these species have been widely researched, there is a need to establish the growing conditions and farming practices necessary for successful commercial production of these plants. This is especially important in the light of a burgeoning world population which will no doubt lead to a higher demand for their products. As overharvesting in the wild of these species is already considered to be a problem, commercial production is a viable alternative. This is underway at present, but there are at present very few commercial growers. Further research using field studies is indicated to establish their optimal growing conditions.

7.2 RECOMMENDATIONS

South Africa, like the rest of the world, is currently experiencing climate change, with drought, floods, tornadoes and many other unusual climatic patterns. Water shortage poses an enormous problem for the agricultural sector and, as a result there is an ongoing need for research into producing crops using water saving technology. Vegetable crop varieties are generally water-dependant, however the medicinal Pelargoniums can clearly be successfully produced commercially without complicated irrigation programs. It has been seen from this study that minimal water results can contribute to more marketable root tubers with higher antioxidant compounds. In South Africa a large section of the rural population live below the poverty line and the temptation to harvest plants from the wild for a small income is a reality. Both of the species are potentially threatened in the wild as a result of overharvesting and desperation. There is an obvious need to increase commercial production of these Pelargoniums for the local and international natural medicine market, and therefore the more knowledge that is made available about their cultivation requirements, the better. Our recommendation therefore would be that more research is initiated into large scale field cultivation of these species for commercial purposes. This could potentially create work and employment opportunities for poverty- stricken communities. In addition, this could expand the agricultural economy of the country and also contribute to saving these two valuable Perlargonium species from possible extinction in the wild.

CHAPTER 8

8.1 REFERENCES:

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