Cape Peninsula University of Technology

Effects of pH and Phosphorus concentrations on the cultivation of *Salvia chamelaeagnea* grown in hydroponics.

by

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DECLARATION

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

Signed **Date** 04-12-13

ABSTRACT

This study evaluated the effects of different pH levels and supplementary phosphorous concentrations on *Salvia chamelaeagnea* grown in hydroponics. The treatments consisted of 12 treatments of 4 differing nutrient solutions offering: low concentration of supplementary P (control), balanced concentration of supplementary P, moderate concentration of supplementary P and a high concentration of supplementary P at 3 differing pH levels. Each treatment was replicated 10 times. The objectives of this study were to asses the effect of supplementary phosphorous concentrations and 3 different pH levels on the growth, development and chlorophyll responses of *Salvia chamelaeagnea* grown hydroponically. Growth and development was recorded by measuring weekly heights, numbers of basal shoots, stem diameters and the number of branches, while root length and wet and dry weights of roots and shoots were measured post harvest. Chlorophyll responses were recorded by measuring weekly SPAD-502 measurements while post harvest DMSO analysis of chlorophyll A, B and total chlorophyll were recorded along with nutrient uptake levels of N, P,K, Ca, Mg, Na, Mn, Fe, Cu, Zn and B in the plant leaves.

This study has shown that the use of a hydroponic nutrient system offering a moderate concentration of supplementary P at a pH level of 4 significantly influences the growth and development of *Salvia chamelaeagnea* grown in hydroponics. Plants treated with a pH level of 4 generally produced higher wet and dry shoot weights, root lengths, stem diameters, basal shoot numbers, branch numbers, and plant heights than that of the control and all treatments delivering nutrients at a pH level of 6 and 8.

Although no one treatment offering supplementary P produced consistently high results, in most cases all the plants receiving supplementary P at a pH level of 4 outperformed the pH 6 and pH 8 treatments receiving the same amount of supplementary P. This indicates that at a pH level of 4 the mineral nutrient availability of a nutrient solution is at an adequate level for the growth and development of *Salvia chamelaeagnea*. Furthering studies into the effects of arbuscular mycorrhiza on the uptake of mineral nutrients, root morphology and growth and development are recommended.

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CHAPTER ONE

PROBLEM STATEMENT, AIMS, HYPOTHESIS AND OBJECTIVES

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1.1 Statement of research problem

Salvias have been of great importance to the culinary, medicinal and horticultural industries for many years (Kamatou et al., 2008; Cornara et al., 2009). *Salvia chamelaeagnea* is a medium sized shrub which is propagated for use in the landscape, cut flower, culinary and medicinal industries. While most Salvias grow best in soils with a low pH, the relationships to pH and phosphorus in the cultivation of *S. chamelaeagnea* have never been established (Clebsch, 2003; Richards et al., 1997; Kamatou et al., 2006).

An investigation into the nutritional requirements of *Salvia chamelaeagnea* (*S. chamelaeagnea)* and its responses to pH stresses will give great insight into the development of an effective nutrient solution for commercial production of this South African indigenous plant.

1.2 AIMS

It is expected that the research will indicate whether a particular pH and phosphorous concentration will increase the growth and yield of medicinal *Salvia chamelaeagnea* for crop production. The results will be published in accredited journals.

1.3 HYPOTHESIS

- The growth and development of *Salvia chamelaeagnea* will be influenced by phosphorus concentrations.
- The growth and development of *Salvia chamelaeagnea* will be influenced by different pH levels.

1.4 OBJECTIVES

1.4.1 Main objective

To explore the effects of phosphorous (P) concentrations and pH on the growth and development of *Salvia chamelaeagnea.*

1.4.2 Specific objectives

1) To assess the effects of Hoagland hydroponic nutrient solution with 31 ppm of P at alkaline, base and acidic pH levels on the growth and yield of *Salvia chamelaeagnea.*

2) To assess the effects of Hoagland hydroponic nutrient solution with 90 ppm of P at alkaline, base and acidic pH levels on the growth and yield of *Salvia chamelaeagnea.*

3) To assess the effects of Hoagland hydroponic nutrient solution with 150 ppm of P at alkaline, base and acidic pH levels on the growth and yield of *Salvia chamelaeagnea.*

4) To assess the effects of Hoagland hydroponic nutrient solution with 210 ppm of P at alkaline, base and acidic pH levels on the growth and yield of *Salvia chamelaeagnea.*

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CHAPTER TWO

INTRODUCTION AND LITERATURE REVIEW

INTRODUCTION AND LITERATURE REVIEW

2.1 ABSTRACT

Salvia chamelaeagnea is an attractive water-wise landscape plant from the Western Province of South Africa with remarkable medicinal purposes. This study reviews the cultivation of this species in relation to pH control and phosphorus (P) supplementation. P is a crucial element in plant nutrition, as it is a component for many cellular functions such as respiration, seed development, and the formation of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA). P deficiencies leading to stunted growth can easily be averted with the knowledge of a plant's nutritional requirements. By altering the pH level of a media, particular elements become more readily available to plants than other elements. Without the pH of a media being amended to a favourable level, some plants may experience nutrient deficiencies, whist others may survive or even excel. This review assessed that knowledge on cultivation of *Salvia chamelaeagnea* remains limited. Future studies are likely to provide an understanding of plant nutrient and water requirements which can aid in successful production of this species.

Keywords: medicinal species, commercial cultivation, nutrient requirements

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2.2 INTRODUCTION

Salvia chamelaeagnea (*S. chamelaeagnea*) a member of the Lamiaceae family, is a slow growing evergreen shrub originating from the south western area of the Cape of Good Hope. In its natural habitat, *S. chamelaeagnea* develops into an attractive foliage and flowering landscape plants, with small mid-green egg shaped leaves and masses of bright blue or white flowers which are suitable for cut flowers (Clebsch, 2003; Pienaar, 2000; Ulubelen, 2003). History also documented that extracts from this species exhibit pharmaceutical effects against various microbial and bacterial infections (Kamatou et al., 2007; Kamatou et al., 2008; Nenadis et al., 2003). With these properties this species has the potential of being commercially farmed as a medicinal species. Unfortunately very little information has been documented on the cultivation of this species.

In Africa, and particularly southern Africa, soil degradation and pollution are largely associated with poor resource management. The results of this impact are largely seen during agricultural production (Huttl & Frielinghaus, 1994; Zingore et al., 2006; McGeoch et al., 2008). Adequate soil fertility is a crucial requirement in crop production (Stern, 2006; White & Zasoski, 1999). According to Janssen and de Willigen (2006), the output of nutrients should be replaced by the input of nutrients. This often results in excessive levels of fertilizers being applied to crops which can result in elements (for examples N and P), leaching into neighbouring soils and rivers during periods of high rainfall (Andrews et al., 1997; Saarijärvi et al., 2004; Laird et al., 2010). An alternative to conventional crop production of edible and medicinal species is hydroponic cultivation. With this method a considerable reduction in the application and use of nutrients can be achieved which would reduce wastage and leaching into conventional soils (Montanari et al., 2008).

Nutrient application and concentration play a fundamental role in medicinal and ornamental crop production systems (Stern, 2006; Zahreddine et al., 2007; Hebbar et al., 2004; Fan et al., 2009). This is in particular important and measured in the level and quality of oils being extracted. In some medicinal crops, applications of fertilizers high in P have been used to stimulate the production of nicotinamide adenine dinucleotide phosphate (NADPH), adenosine tri-phosphate (ATP) and secondary metabolites (Bray, 1983; Srivastava et al., 2002; Stern, 2006).

The pH level of a media greatly influences the availability of nutrients, in both soil and hydroponic cultivation. In hydroponics however, the control of pH levels is far easier and improved results in growing can be achieved in much shorter periods of time. It is known that at differing pH levels different amounts of nutrients are available to plants, greatly affecting their functioning and development (Stern, 2006; Follett et al., 1981). In many commercial crops, high pH levels required for growth are relatively standard, while for some medicinal crops, such as some Salvia’s, a lower pH level may be more favourable for the creation of secondary metabolites (Verpoorte & Memelink, 2002; Taarit et al., 2010; Taylor et al., 2001).

This review paper outlines the importance and possibility of growing *S. chamelaeagnea* hydroponically where benefits from P supplementation and regulation of pH levels can improve growth, yield and nutrient uptake.

2.3 Cultivation of Salvias as medicinal and horticultural crops

Dating as far back as 2000 B.C. and before, humans have used plants for food, safety, clothing, health and meditation (Cornara et al., 2009; Craker & Simon, 1986; Gurib-Fakim, 2006). Today, plants continue to be cultivated for basic human needs, but are also valued for culinary, aromatic, nutritive and medicinal uses. Plants are also used for their aesthetic value and soil retention ability (Pienaar, 2000; Bryant & Rodd, 2005; Rittershausen & Rittershausen, 2009). Medicinal plants have been used traditionally for hundreds of years to treat illnesses and disorders. The popularity of many of these species is increasing almost daily as a growing awareness of personal health, well being and the desire to increase life expectancy is leading to greater demands for natural products (Bourgaud et al., 2001; Janicsak et al., 2006). At the same time, the World Health Organization reported that approximately 80% of the people in developing countries continue to rely on traditional medicines to meet their health care necessities (Canter et al., 2005; Gurib-Fakim, 2006; van Wyk, 2008). Whilst many Lamiaceae species have been used in traditional medicines for the treatment of various infections, warts, malaria and cancers (Barros et al., 2010; Marc et al., 2008; Gail-Muhtasib et al., 2000; Guarrera, 1999; Mayer et al., 2009; Lu & Foo, 2002; Kamatou et al., 2006; Kamatou et al., 2008), other species are used in the culinary industry as flavourings and antioxidants (Lee, 2010; Lee & Scagel, 2009; Barros et al., 2010; Gurib-Fakim, 2006; Burt, 2004), in aromatherapy (Jeannot et al., 2003; Sawamura et al., 2004), perfumery (Singh & Rao, 2009) and for their aesthetic properties in the domestic environment (Wester & Claßen-Bockhoff, 2006; Don, 2003).

S. chamelaeagnea has great potential in the medicinal industry as it has shown to have a synergistic effect when combined with *Leonotis leonurus,* in an attempt to defeat the increasing bacterial resistances to antibiotics (Kamatou et al., 2006). *S. chamelaeagnea* also exhibited some inhibitory activity against malarial infection (Kamatou et al., 2008), whilst Kamatou et al., (2007) reported significant results when testing compounds from *S. chamelaeagnea* against the pathogens *Bacillus cerus* and *Staphylococcus aureus.* In addition, this species of Salvia exhibits antioxidant activity due to the presence of the phenolic compounds carnosol, rosmarinic acid and caffeic acid, and may be of benefit to the culinary industry as a preservative (Kamatou et al., 2008; Kamatou et al., 2010; Nenadis et al., 2003). Aside from its medicinal traits, *S. chamelaeagnea* has value in the horticulture industries as a cut flower or a water wise landscaping plant (Clebsch, 2003; Wester & Claßen-Bockhoff, 2006; Pienaar, 2000; Tepe et al., 2006; Flamini et al., 2007). Salvias are a very adaptable species, many being drought hardy including the widely cultivated *S. chamelaeagnea* (Clebsch, 2003; Brickell, 2003). Salvias, with the exception of a few, grow best in well drained moderately fertile soils. Most prefer full sun to dappled light with regular watering in the summer time and a monthly application of a balanced liquid fertilizer (Bird, 2007; Brickell, 2003). Despite *S. chamelaeagnea* marketable values there is a lack of specific knowledge regarding its cultivation and nutrient requirements (Kamatou et al., 2007; Suntar et al., 2011; Barros et al., 2010). Therefore an investigation into its cultivation methods would be beneficial to many crop growers, particular large scale commercial medicinal production.

2.4 Managing soil nutrient levels

A global decrease in soil fertility is stemming from unethical and poorly managed agricultural practices, deforestation and erosion (Huttl & Frielinghaus, 1994; Koning & Smaling, 2005; Zhang et al., 2009; Wang & Frei, 2011). Throughout Africa many crop farmers are affected by soil degradation and infertility directly resulting in decreased quantity and quality of their crop yields (Mowo et al., 2006; Ndakidemi & Semoka, 2006; de Ridder et al., 2004; Scoones & Toulmin, 1998). Furthermore, nutrient deficiencies in soils are common problems that crop farmers encounter, leading to complications in the life cycle of many crops (Mowo et al., 2006; de Ridder et al., 2004; Rengel et al., 1999). Most common of the nutrient deficiencies in crop plants are that of the macronutrients; N, P, K, Mg, Ca and S, mostly are used in large quantities for fundamental processes such as chlorophyll production, respiration and cell development (Stern, 2006; Duffus & Duffus, 1984; Liu et al., 2008; Bray, 1983; Vong et al., 2007). Based on this, it is crucial to amend soils according to crop requirements prior to planting, followed with careful monitoring its status during the growth phases (Gentile et al., 2008; Gilbert et al., 2011; Follett et al., 1981; Stern, 2006).

As a result of poor soil management practices and the lack of knowledge of many plant species nutrient requirements, macronutrient deficiencies may lead to farmers broadcasting large amounts of fertilizers, some of which are potentially un-needed (Barrios et al., 2006; Saarijärvi et al., 2004). These fertilizers can build up toxic levels in soils and leach into neighbouring soils and water sources altering pH levels and cause toxicity in many plants (Mills & Fey, 2003; Zheljazkov et al., 2008; Janssen & de Willigen, 2006). Furthermore, excess fertilizers levels also threatens wild life and diminishing food resources (Yamaguchi & Blumwald, 2005; Reddy et al., 2003). As soil fertility in crop production is highly important, an investigation into the cultivation requirements of *S. chamelaeagnea* would be beneficial to ensure correct dosage of nutrient applications during crop production.

2.5 Crops responses to plant nutrients

It is evident that plants require nutrients to carry out their growth and development (Diederichs, 2006; Stern, 2006). For many decades farmers have been regulating plant nutrient applications based on the nutrient requirements of their crops at particular stages of growth, so as to attain healthy high yielding crops (Bourgaud et al., 2001; Ashley et al., 2011). Plant nutrients like N, P and K are known as the basic elements responsible for cell development, enzyme activity and respiration (Diederichs, 2006; Stern, 2006; Craker & Simon, 1986). The most common deficiencies in crop plants are of such macronutrients as the fore mentioned as well as of Mg, Ca and S, which are used by plants in larger amounts than micronutrients (Ndakidemi & Semoka, 2006; Stern, 2006; Liu et al., 2008; Vong et al., 2007). As more and more medicinal species are entering the commercial market a continued need exists for experimentation to determine which concentrations of a particular nutrient are essential to increase medicinal, aesthetic, cosmetic or culinary values of a given species in order to apply the correct amounts of fertilizer for healthy plant growth whilst limiting degradation and contamination of soils through deficient or excessive nutrient applications (Janssen & de Willigen, 2006; Huttl & Frielinghaus, 1994; Street et al., 2008).

The effects of nutrients and nutrient ratios are known for many food and medicinal crop plants, such as soybean, thyme, wheat cultivars, barley, spinach and pelargoniums (Ashraf et al., 2009; Craker & Simon, 1986; Ram et al., 2003). In many plant nutrient experiments positive growth results have been recorded with the addition of increased quantities of some macronutrients such as N, P, K, Mg or Ca (Gan et al., 2003; Khazaie et al., 2008; Ichir et al., 2003; Maidl et al., 1996; Stagnari et al., 2007; Manzanares et al., 1997; Liu et al., 2011; Bo et al., 2009; Ram et al., 2003; Zhu et al., 2009). Research carried out on members of the Lamiaceae family has yielded significant results pertaining to the nutrient uptake, use and efficiency of specific nutritional elements (Sotiropoulou & Karamanos, 2010; Patra et al., 2000). Dordas (2009) found that Mg and Ca applications greatly affected *Origanum vulgare ssp* Hitrum, increasing plant mass by 23% with the Mg and Ca applications, and increasing the chlorophyll content by 38% with the Mg treatments alone. Azizi et al., (2009*),* reported that dry weight of *O. vulgare* increased with an increase in N fertilizer application. Knowledge like this may be of a benefit to farmers in reaching their crops genetic potential (Janssen & de Willigen, 2006; Zingore et al., 2006), and in selling their crops to the landscape, cut flower, culinary and medicinal industries, whilst reducing nutrient wastes (Hebbar et al., 2004; Taylor et al., 2001; Zahreddine et al., 2007). Despite the importance of this information, no data regarding the nutritional requirements of *S. chamelaeagnea* has been documented. An investigation into the nutritional requirements of *S. chamelaeagnea* would therefore gain further insight into commercial cultivation of this species.

2.6 Utilization of P in cultivated plants

It has been documented that P is an important element in the synthesis of ATP, respiration, development of plant mass and essential oils, energy transfer and storage, formation of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) and in the germination of seeds (Liu & Zhong, 1998; Kapoor et al., 2004; Stern, 2006; Follett et al., 1981; Resh, 1995). Symptoms of a P deficiency in plants are characterised by a build up of sucrose and starch, a decrease in photosynthesis, stunted growth, purpling of the lower leaves and hindered seed development (Duffus & Duffus, 1984; Stern, 2006; Follett et al., 1981).

Some members of the Lamiaceae family, such as *Dracocephalum, Thymus, Origanum* and *Salvia* species have been tested with varying fertilizer applications in attempts to manipulate their growth and yields (Dordas, 2009; Sotiropoulou &

Karamanos, 2010; Hussein et al., 2006; Khazaie et al., 2008; Hendawy & Khalid, 2005). For example, favourable results have been recorded in the antioxidant capacity and phenolic concentration of *Ocimum basilicum* L. under increased levels of potassium (Nguyen et al., 2010). Naeem et al., (2010) recorded an increase in growth, cell functioning and secondary metabolites of medicinal *Lablab purpureus* L. when supplemented with additional P while Zhu et al., (2009), reported a significant effect on growth and saikosaponin production of *Bupleurum chinense* under P enriched conditions. Similar responses to P have been recorded in crop plants such as Cassava, Rice, Millet and others (Vander Zaag et al., 1979; Shen et al., 2004; Wang et al., 2010; Rebafka et al., 1994). It can be said that research pertaining to the effects of P to improve plant growth and yields could be seen as a very important aspect of crop production and soil fertility management (Ashley et al., 2011; Buerkert et al., 2001; Naeem et al., 2010). The commercial production of *S. chamelaeagnea* would benefit significantly from an investigation into the effects of P concentrations on the growth and yield of this species.

2.7 pH and its effects on nutrient availability

The pH level of a soil or nutrient solution is indicative of the growing medium's acidity or alkalinity, ranging from a scale of 0 to 14, and is used as a guide to indicate the ratio of H+ ions to OH- ions, thus affecting the availability of elements to plants (Follett et al., 1981; Resh, 1995). Many micro nutrients such as Fe, Zn and Mn are required in small quantities for biological processes such as enzyme activity, chlorophyll development and respiration. In fertile soils with a pH level of below 5 these nutrients are abundant, which could lead to toxicity in plants (White & Zasoski, 1999; Smith, 1994; Marschner, 1995). Deficiencies of some macro nutrients such as K, Ca and Mg also occur at low pH levels resulting in death of terminal buds, yellowing and curling of leaf margins and reduced root development and even plant death is possible (Stern, 2006; Follett et al., 1981; Leopold & Kriedemann, 1975; Glass, 1989).

On the opposite end of the scale at pH levels of 8 and above macro nutrients K, Ca and Mg are more readily available, whilst N, Fe and Mn become more deficient. This variation may result in chlorosis, stunted growth and necrotic spots forming on leaves (Stern, 2006; Marschner, 1995; Resh, 1995). Due to the importance of pH and nutrient availability it is crucial that crop farmers maintain soil pH at the required levels so as to attain higher quality yields and maintain healthy soil conditions (Mowo et al., 2006; de Ridder et al., 2004). Golez and Kyumah (1997), clearly demonstrated that highly acidic soils limit the solubility of some nutrients resulting in deficiencies of some micro nutrients like Cu, Zn and Ca. Wei et al., (2006), too found that farming practices and pH levels can affect the interactions of different forms of available P with other soil nutrients, thereby influencing micro nutrient availability and affecting quality and yield of crops. This highlights the matter that knowledge of the correct pH level for a species is paramount for efficient plant nutrient uptake and soil management practices (Follett et al., 1981). Cultivators and researchers therefore need to gain a greater understanding of the nutritional requirements of a chosen plant for cultivation, so as to be able to manipulate the pH levels and nutrient availability accordingly, to effectively produce a healthy and high yielding crop (Gurib-Fakim, 2006).

Whilst sufficient literature is available to highlight the importance of soil pH and availability of nutrients (Golez & Kyuma, 1997; Yeh et al., 2000; Devau et al., 2009), little is known of *S. chamelaeagnea* growth responses to differing pH levels. Understanding the dynamics of nutrient activities on plant development is crucial in predicting their interactions with each other and their activities in nutrient uptake and plant growth. It is therefore important that further investigations into the effects of pH and nutrient supplementation be conducted on the growth of *S. chamelaeagnea* which could benefit the commercial production of this species.

2.8 Hydroponic cultivation systems

The history of hydroponics dates back thousands of years. It has been documented that primitive examples of hydroponic culture were used by the ancient Aztec's, Chinese and Egyptians (Resh, 1995). During World War 2 the United States army implemented hydroponic growth practices to produce fresh fruit and vegetables for soldiers stationed on infertile islands (Harris, 1976). In the more recent past the use of hydroponics systems has become widespread in the plant cultivation and research industries (Mason, 1990; Hanan & Holley, 1970 Caruso et al., 2011).

The increasing awareness of hydroponic techniques has led to many different forms of hydroponic systems being tailored to accommodate the growth requirements of the particular crop to be cultivated (Mason, 1990). Several different types of hydroponic systems have been established such as Deep Water Culture (DWC), Wick absorption, Ebb and Flow, Nutrient Film Technique (NFT), Top Feed and Aeroponics. These various methods are paired with one or a series of substrates such as perlite, vermiculite, coarse sand, expanded clay, coco coir or gravel so as to support the root zone and to provide sufficient water retention effective drainage and plenty of oxygen (Harris, 1976; Resh, 1995). The type of hydroponic system chosen for use is crucial and is based around the crops physiological characteristics and its valued traits, for example swelling root tubers in potatoes needs sufficient substrate in which to develop and the trailing growth habit of butternut will need lots of space to spread out. Thus the hydroponic system needs to be adapted to accommodate and encourage the development of the valued crops attributes (Resh, 1995; Correa et al., 2008; Samartzidis et al., 2005).

The use of hydroponic systems could arguably be of great benefit to our population and to our environment if managed correctly. Plants in the hydroponic systems are not in contact with the earth and as a result leaching of nutrients is diminished and crop diseases due to soil borne pathogens are eliminated (Gontier et al., 2002; Resh, 1995). Nutrients, pH, and treatments can easily be regulated to suit the crops needs and when managed correctly labour, diseases, pesticides, space, water use and costs are reduced considerably compared to in soil cultivation systems (Harris, 1976; Mason, 1990; Sgherri et al., 2010).

Since the 1940's food crops like lettuce, tomatoes and beans have successfully utilised hydroponic systems, resulting in reduced costs labour and pesticide applications whilst significantly improving growth and development (Harris, 1976; Mason, 1990, Sawas et al., 2007; Rouphael & Colla, 2005). In the floriculture industry, crops like, Carnations, Chrysanthemums, Roses, Sunflowers and Gerberas have too been cultivated hydroponically, resulting in considerable increases in their growth and yields (Sawas & Gizas, 2002; Samartzidis et al., 2005: Karras et al., 2007). Livestock farmers have also utilised hydroponic systems for fodder production as less space is required and climatic conditions are easily maintained to promote growth all year long (Harris, 1976). Experiments on many spices herbs and medicinal plants have been successfully conducted using hydroponic systems instead of soil cultivation in order to eliminate possible erroneous results that may be brought about in soil cultivation due to unmanageable variables (Sgherri et al., 2010; Maggini et al., 2012; Soudek et al., 2006).

As global soil fertility is on the decline, the use of hydroponic systems in plant research communities is becoming more wide spread. Thus the use of hydroponic systems in future large scale growth platforms for crop production is more probable (Resh, 1995; Gomez-Lopez et al., 2006; Meric et al., 2011). The effective use of waste water as a nutrient solution in hydroponics systems described by Wallace et al, (1978) can further save on costs of fertilizers in some crops, and floating hydroponic rafts can filter out contaminants from agricultural runoff (Yang et al., 2008). Further uses may branch out to hydroponic vertical gardens, and container gardens onboard space and ocean going vessels (Kitaya et al., 2008; Moraru et al., 2004).

2.9 CONCLUSION

This paper highlights the importance of plant nutrition on the growth and development of plants. It also shows that a lack of research is evident in the cultivation of *S. chamelaeagnea.* This species is valued for its medicinal properties and investigation into nutrient applications and pH levels will greatly contribute to its successful cultivation as a commercial species. Medicinal plants can benefit from hydroponic cultivation however research on most species remains limited. A study on evaluating hydroponic growing for *S. chamelaeagnea* will enhance its future growing potential in possibly increase quantity and quality in medicinal oil production. This could also aid in limiting nutrient wastage, run-off into soils as well as saving water in dry climate countries where *S. chamelaeagnea* is cultivated.

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CHAPTER THREE

EFFECTS OF PH AND PHOSPHORUS CONCENTRATIONS ON THE GROWTH PARAMETERS OF *SALVIA CHAMELAEAGNEA* GROWN IN HYDROPONICS.

EFFECTS OF PH AND PHOSPHORUS CONCENTRATIONS ON THE GROWTH PARAMETERS OF *SALVIA CHAMELAEAGNEA* GROWN IN HYDROPONICS.

3.1 ABSTRACT

Salvia chamelaeagnea is a bushy shrub with attractive foliage and flowers, it has small mid-green ovulate shaped leaves and masses of bright blue flowers borne in midsummer and has value in the landscape and medicinal plant industries. This study investigates the effects of supplementary phosphorus and different pH levels on the growth and development of *Salvia chamelaeagnea* by assessing the height, stem diameter, number of branches, number of basal shoots, wet and dry weights and the root lengths over an eight week period at the Cape Peninsula University of Technology. The treatments of ph 4, pH 6 and pH 8 at 31 ppm, 90 ppm, 150 ppm and 210 ppm of phosphorus were given to twelve groups of plants. This study showed that the use of a nutrient solution with a pH level of 4 supplemented with 90 ppm of phosphorus had a significant effect on the growth and development of *Salvia chamelaeagnea* grown in hydroponics. Furthering studies into higher N and K ratios on the development of this plant would be advantageous.

Key words: pH level, phosphorus, hydroponically grown, supplementary

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3.2 INTRODUCTION

Salvia chamelaeagnea (*S. chamelaeagnea*) is a slow growing evergreen shrub originating from the south western part of the Cape of Good Hope. This species has been found to exhibit favourable results against various microbial and bacterial infections. As Kamatou et al. (2007) found when testing compounds from *S. chamelaeagnea* against the pathogens *Bacillus cerus* and *Staphylococcus aureus, S. chamelaeagnea* also exhibits antioxidant activity due to the presence of the phenolic compounds carnosol, rosmarinic acid and caffeic acid (Kamatou et al., 2008; Kamatou et al., 2010; Nenadis et al., 2003), and has shown a synergy when combined with *Leonotis leonurus,* in an attempt to defeat the increasing bacterial resistances to antibiotics. *S. chamelaeagnea* even shows promising activity against malarial infection (Kamatou et al., 2008).

Given the nature of soil pH and nutrient availability it is essential that crop farmers maintain soil pH at the needed levels so as to achieve higher quality crops and to maintain a healthy soil environment (Mowo et al., 2006; de Ridder et al., 2004). It has been clearly documented that highly acidic soils limit the solubility of some nutrients resulting in inadequacies of some micro nutrients such as Cu, Zn and Ca (Golez & Kyumah, 1997). Poor farming practices and pH levels can impede the interactions of different forms of available P on other soil nutrients, thereby influencing micro nutrient availability and affecting quality and yield of crops (Wei et al., 2006).

The macronutrient phosphorus (P) has long been acknowledged as an essential component in plant nutrition for its roles in; the production of adenosine tri-phosphate (ATP), development of plant mass and essential oils, respiration, the germination of seeds, formation of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) and in energy transfer and storage (Liu & Zhong, 1998; Kapoor et al., 2004; Stern, 2006; Follett et al., 1981). P deficiencies in plants greatly affect the growth and development of a plant and are indicated by a decrease in photosynthesis, a build up of sucrose and starch, purpling of the lower leaves, stunted growth and hampered seed development (Duffus & Duffus, 1984; Stern, 2006; Follett et al., 1981).

As *S. chamelaeagnea* holds value in the domestic environment as a water-wise landscape plant, in the cut flower industry in floral arrangements and as a candidate in the medicinal plant trade. Testing of plant growth responses to nutrient P concentrations and pH levels will determine which P concentration and pH level will produce a healthy plant which will grow to the best of its genetic potential. This in turn will benefit the medicinal plant industries in furthering research into the chemical properties of *S. chamelaeagnea*, and guide for nursery men and the landscape industries in cultivation of this species.

3.3 MATERIALS AND METHODS

3.3.1 Experimental process

The experiment took place in the research glass house at CPUT Cape Town campus, South Africa, latitude and longitude S33°55’ 58 E18°25’ 57, from June 2012 until August 2012. Inside the greenhouse was a 40 % Alunet shade cloth, raised 2 m above the floor resulting in light intensities ranging from 10 Kilolux to 13 Kilolux, determined by using a Toptronic T630 light meter. The climate was controlled to range between 16 – 28 °C in the day while being between 10 – 20 °C during the night, with an average relative humidity of 42 %.

The experiment was laid out in a randomised block design with plants being spaced 30 cm apart, and consisted of 12 treatments of 4 differing nutrient solutions offering: low concentration of supplementary P (control), balanced concentration of supplementary P, moderate concentration of supplementary P and a high concentration of supplementary P at 3 differing pH levels. The control treatment of 31 ppm was chosen due to the nature of fynbos soils being low in available P (Hawkins et al., 2008; Rebelo, 2001; Witkowski & Mitchell, 1987)

Hoagland solution a well known hydroponic nutrient solution modified by Hershey, (1994) and Hershey, (1995), offering all the necessary macro and micro nutrients for healthy plant growth was used as a base nutrient and supplemented with P.

The plants for the experiment were rooted tip cuttings sourced from healthy mother stock plants at the CPUT Glass House Nursery. The rooted cuttings were gently rinsed in de-ionized water to remove any rooting media from the root zone. They were then weighed and planted into 25 cm plastic pots filled with leca clay, placed into a recirculating closed hydroponics system at a spacing of 30 cm apart, where their heights were recorded.

The plants were irrigated with the treatments 15 times per day at equal timed intervals for the duration of the experiment. For each treatment there were 10 plants. The treatments were as follows:

1) Hoagland hydroponic nutrient solution with 31 ppm of P at a pH of 4.
   - Hoagland hydroponic nutrient solution with 31 ppm of P at a pH of 6. (Control)
   - Hoagland hydroponic nutrient solution with 31 ppm of P at a pH of 8.

2) Hoagland hydroponic nutrient solution supplemented with 90 ppm of P at a pH of 4.

- Hoagland hydroponic nutrient solution supplemented with 90 ppm of P at a pH of 6.
- Hoagland hydroponic nutrient solution supplemented with 90 ppm of P at a pH of 8.

3) Hoagland hydroponic nutrient solution supplemented with 150 ppm of P at a pH of 4.

- Hoagland hydroponic nutrient solution supplemented with 150 ppm of P at a pH of 6.
- Hoagland hydroponic nutrient solution supplemented with 150 ppm of P at a pH of 8.

4) Hoagland hydroponic nutrient solution supplemented with 210 ppm of P at a pH of 4.

- Hoagland hydroponic nutrient solution supplemented with 210 ppm of P at a pH of 6.
- Hoagland hydroponic nutrient solution supplemented with 210 ppm of P at a pH of 8.

pH Level

The pH levels of the nutrient solutions were monitored using a Martini Instruments PH55 pH probe and were adjusted accordingly using either hydrochloric acid (HCL) to lower the pH, or sodium hydroxide (NaOH) to raise the pH.

Irrigation

The treatments were set to irrigate every 15 times daily for a duration of 15 minutes using a 1350 L/h hour Boyu submersible pump and a Tedelex analogue timer to regulate irrigation frequencies.

3.3.2 Data collection

Measurement of plant growth

Height

The height of the plants was measured at weekly intervals using a measuring tape. The measurement was recorded from leca clay media level to the tip of the tallest shoot.

Basal Shoots

The number of basal shoots was recorded at weekly intervals. All basal shoots were measured from the point at which the stem emerged from the media. Only once basal shoots were longer than 1 cm from tip to base were they counted.

Stem Diameter

The stem diameter was recorded at the point at which the stem emerged from the media, at weekly intervals using a Vernier calliper.

Root Length

After harvesting the plants, the roots were gently rinsed in deionised water to remove any growing medium and then the lengths were measured from the points at which roots emerge from the stem to the tip of the root mass, using a measuring tape.

Branches

The number of branches was recorded at biweekly intervals. All buds were longer than 1 cm from tip to stem or branch before being counted.

Measurement of fresh root and fresh shoot weights

The plants were harvested after a 2 month growth period. Directly after they were then individually weighed using a RADWAG® WPS 4000/C/2 laboratory scale to establish their fresh weights. The roots and shoots were then separated and individually weighed.

Measurement of dry root and dry shoot weights

After the fresh roots and shoots of the plants were weighed they were dried in a specimen oven at 55 °C for 48 hours and then weighed again using a RADWAG® WPS 4000/C/2 laboratory scale to attain their dry weights.

3.3.3 Statistical analysis

Data collected was analyzed for statistical significance using the One–way analysis of variance (ANOVA), with the computations being done using the software program STATISTICA. The Fisher least significance (L.S.D.) was used to compare significant treatment means *at P* ≤ 0.05 level of significance (Steel & Torrie, 1980).

3.4 RESULTS

3.4.1 Effects of pH and Phosphorus concentrations on the height of *Salvia chamelaeagnea* grown in hydroponics.

Plant heights (See Table 3.1) shows that in weeks 1 to 4 plant heights were not significantly affected by the treatments. At weeks 5 and 6, the pH 8 treatment at 31 ppm P had significantly ($P \leq 0.05$) and ($P \leq 0.001$) respectively higher values than that of the control and those plants which had received additional P. In week 7 the height of plants receiving a pH of 4 at 90 ppm P were significantly ($P \leq 0.01$) greater than that of the control and all the other treatments recorded heights. At week 8 the treatments did not differ significantly, however plants receiving a pH of 4 at 90 ppm P continued to have the highest recorded value when compared to the control and all other treatments.

3.4.2 Effects of pH and Phosphorus concentrations on the branch numbers of *Salvia chamelaeagnea* grown in hydroponics.

Table 3.2 shows the branch development of *S. chamelaeagnea* over a 8 week growth period. During the first 5 weeks there was no statistical significance between treatments however in week 2 pH 8 treatment at 31 ppm P yielded the highest branch numbers closely followed by the pH of 4 at 90 ppm P treatment both of which yielded higher branch numbers than that of the control and all other treatments. In week 4 there was no significant difference among the treatments, however treatments offering pH 6 at 210 ppm P; pH 4 at 90 ppm P; pH 4 at 31 ppm P; pH 4 at 150 ppm P all outperformed the control treatment. During week 6 a pH level of 4 at 90 ppm P and a pH level of 4 at 31 ppm P significantly ($P \leq 0.01$) outperformed the control treatment and all other treatments. In week 8 a pH of 4 at 150 ppm P yielded significantly ($P \leq 0.001$) higher branch numbers than the control and all other treatments.

3.4.3 Effects of pH and Phosphorus concentrations on the root length of *Salvia chamelaeagnea* grown in hydroponics.

The root length shown in Table 3.3 was significantly ($P \leq 0.01$) affected by the treatments, pH 4 at 150 ppm P treatment produced the longest roots followed by pH 4 at 210 ppm P; pH 8 at 31 ppm P; pH 4 at 31 ppm P and pH 4 at 90 ppm P respectively, all of which outperformed the control and all other pH and phosphorus treatments. A trend in that all of the pH 4 treatments which received additional P had significantly ($P \leq 0.01$) longer root lengths that the control and all other treatments was noticeable.

3.4.4 Effects of pH and Phosphorus concentrations on the wet and dry shoot weights of *Salvia chamelaeagnea* grown in hydroponics.

Wet and dry shoot weights were significantly ($P \leq 0.001$) affected by the treatments (See Table 3.3). Results of a pH of 4 at 90 ppm P and a pH of 4 at 150 ppm P respectively produced the highest wet and dry shoot weights, differing significantly from the control and all other treatments. There was a noticeable trend of all pH 4 treatments yielding the highest wet and dry shoot weights when compared to the pH 6 and 8 treatments, with the exception of the treatments delivering 210 ppm of P.

3.4.5 Effects of pH and Phosphorus concentrations on the wet and dry root weights of *Salvia chamelaeagnea* grown in hydroponics.

Wet root weights in Table 3.3 were significantly ($P \leq 0.01$) affected by the treatments. The pH 8 treatments offering lower concentrations of P yielded significantly higher wet root weights when compared to the control and all other treatments. This was followed by treatments delivering a pH of 8 at 90 ppm P, pH 4 at 150 ppm P and pH 4 at 90 ppm P respectively, all of which out performed the control and all other treatments.

The dry root weight shown in Table 3.3 was not significantly affected by the treatments. A pH of 8 at 90 ppm P yielded the highest dry root weight value, with the treatment delivering pH 4 at 90 ppm P yielding the second highest dry root weight, both of which yielded higher dry root weights than the control and all other treatments. Treatments with a pH value of 6 had noticeably lower dry root weights that of those with a pH of 4 or 8, with the exception of at a pH of 8 at 210 ppm P which had a considerably low result on dry root weights. Treatments delivering 210 ppm of P yielded poor results at all pH levels.

3.4.6 Effects of pH and Phosphorus concentrations on the basal shoot development of *Salvia chamelaeagnea* grown in hydroponics.

The basal shoot development showed presented no statistically significant differences during the 8 week growth period. There was however a trend of all pH 4 treatments yielding higher basal shoot numbers than that of the pH 6 and pH 8 treatments.

3.4.7 Effects of pH and Phosphorus concentrations on the stem diameter of *Salvia chamelaeagnea* grown in hydroponics.

Stem diameter showed no statistically significant differences over the 8 week growth period. However for the most part, treatments delivering a pH of 8 at 31 ppm P and a pH of 4 at 90 ppm P had more consistent growth than the treatments receiving higher concentrations of P.

3.5 Discussion

In this study the treatments of pH and phosphorus had a great affect on the growth and development of *S. chamelaeagnea.* Similar plant heights at the pH levels of 4 and 8 during the first weeks are likely due to the nature that micro nutrient availability of Mn, Cu, Zn and B which are responsible for enzyme activity, starch formation, respiration and electron transport, are similarly available at these pH levels, thus promoting favourable plant height development. Overall, pH 4 treatments with the exception of pH 4 at 31 ppm P, had greater recordings that of their counterparts receiving a pH 6 and pH 8 (Zhao et al., 2013, Resh, 1995). These results are contrary to other pH studies which yielded favourable results at a pH range of between 5.5 and 7 (Zhao et al., 2013, Resh, 1995; Koehorst et al., 2010). Thus this shows that *S. chamelaeagnea* has a low demand for macro nutrients like N and K which are less readily available at this ph level of 4, and stress the requirement for specie specific cultivation methods and practices. The greatest plant heights over the 8 week growth period were recorded in plants receiving a pH of 4 at 90 ppm P, this concurs with studies by (Naeem et al., 2010; Naeem & Khan, 2009) in that a moderate concentration of P can better the absorption of nutrients in plants at this low pH level of 4 (Graciano et al., 2006).

Branch numbers of the treatments with a pH level of 4 were significantly higher than that of the control and all other treatments with the exception of the pH 6 at 210 ppm P treatment. Phosphorus concentrations too had a strong influence on branch numbers, with the highest over all branch numbers at 150 ppm P. This shows that at a low pH supplementary phosphorus has a positive effect on the plants utilization of available mineral nutrients and productivity (Naeem & Khan, 2009; Khan et al., 2000), and as a result of sufficient nutrition prompts the regulation of auxins and cytokinins responsible for genetic expression within *S. chamelaeagnea* leading to its bushy form (Krouk et al., 2011; Rubio et al., 2009).

Root lengths of treatment offering a pH of 4 at 150 ppm P, were significantly ($P \leq 0.01$) higher than that of the control and all other treatments. With the exception of treatments receiving 31 ppm P, all pH 4 treatments yielded the highest result when compared to their counterparts receiving the same concentration of P and when compared to the control. Phosphorus supplementation at a pH level of 4 too had positive effects on root length development in *S. chamelaeagnea*. This shows that despite the healthy development of aerial parts of the plant a pH level of 4, some nutrient demands are not being met, thus prompting root elongation in order to meet these nutrient demands. This is in agreement with Wang et al., (2009) and Fan and Yang (2007) in which fertilization significantly reduced root length.

The total wet and dry shoot weights of the plants receiving a pH of 4 at 90 ppm P were significantly ($P \leq 0.001$) higher than that of the control and all other treatments. Poor shoot yields in treatments receiving a pH of 6 and 8 are likely as a result of an abundance of phosphorus in the nutrient solution, thus reducing the uptake of Fe, Cu and Zn and hindering respiration, development of plant growth hormones, formation of lignin in cell walls, energy transfers and chlorophyll development in the affected plants (Bhatti & Loneragen, 1970; Hawkins et al., 2008; Marschner, 1995). These improved results in the pH of 4 at 90 ppm P treatment mimics that of its influence on height and branch development yields in *S. chamelaeagnea*, and so confirms that a nutrient solution with a pH of 4 at 90 ppm P significantly improves the growth and development of the aerial parts of *S. chamelaeagnea*.

Wet root weights of plants receiving a pH of 8 were significantly ($P \leq 0.001$) higher than that of the control treatment. Phosphorus concentrations too affected the weight of wet roots in that all pH 8 treatments receiving supplementary P outperformed the control with the best results being recorded under no supplementary P, closely followed by at 90 ppm P, 150 ppm P and 210 ppm P respectively. Such high wet root weights at a pH of 8 suggests that the level of fertility and readily available P prompts the proliferation of roots in *S. chamelaeagnea* (Li et al., 2012; Gupta et al., 2008; Weligama et al., 2008).

3.6 CONCLUSION

In conclusion this study confirms that *S. chamelaeagnea*, like most of the Western Cape fynbos species, grows well in soils with a lower pH level, and shows that the use of a hydroponic nutrient system offering a moderate concentration of supplementary phosphorus at a pH level of 4 significantly influences the growth and development of *S. chamelaeagnea* grown in hydroponics. Further more, these findings could lead to reduced production costs of *S. chamelaeagnea* cultivated in poor soils as fewer amendments will be needed to remedy the soils pH status.

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3.9 TABLES

Table 3.1: The effects of pH and Phosphorus concentrations on the height of *Salvia chamelaeagnea* grown in hydroponics.

Treatments	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8
pH 4, P 31ppm	6.967±1.4ab	7.800±1.9ab	10.633±2.5ab	14.233±3.2abc	16.922±3.7abc	20.789±3.7abc	21.633±2.8cd	27.989±2.4abc
pH 6, P 31ppm (Control)	6.889±1.6ab	7.800±1.9ab	11.133±2.6ab	14.278±3.2abc	16.111±2.7abcd	21.378±4.2abc	24.611±4.6abc	29.989±6.4abc
pH 8, P 31ppm	7.067±1.6a	8.811±1.7a	11.889±1.9a	15.867±2.9a	18.500±3.5a	23.244±5.2a	26.289±4.0ab	30.322±4.9ab
pH4, P 90ppm	6.789±1.4ab	8.033±1.1ab	11.278±1.7ab	13.500±1.8abc	15.400±2.8bcd	22.278±3.5ab	27.200±4.3a	31.578±6.6a
pH 6, P 90ppm	6.378±1.0ab	7.389±1.5ab	10.933±1.7ab	15.022±2.1ab	18.078±2.4ab	23.033±2.4a	24.578±4.5abc	27.078±6.9abc
pH 8, P 90ppm	7.111±1.9a	8.011±2.1ab	10.411±2.3ab	13.111±2.7bc	14.789±2.5cd	18.711±2.3cde	22.644±2.4bcd	25.822±3.6bc
pH 4, P 150ppm	6.667±1.0ab	7.600±1.3ab	9.844±1.5b	13.089±2.1bc	15.222±2.6cd	20.256±3.0abcd	24.111±5.0abc	27.433±6.5abc
pH 6, P 150ppm	5.767±1.9b	6.878±2.2b	9.967±2.8ab	13.167±3.3bc	14.567±3.2cd	18.956±4.4bcde	22.644±5.3bcd	28.022±6.6abc
pH 8, P 150ppm	6.578±0.7ab	7.467±0.8ab	10.389±1.5ab	12.511±2.9c	13.744±3.4d	16.278±4.3e	20.000±5.7d	24.933±5.6a
pH 4, P 210ppm	6.744±1.2ab	8.133±1.5ab	10.733±1.5ab	13.089±1.4bc	14.200±2.9cd	18.544±4.4cde	20.922±5.8cd	25.544±7.3bc
pH 6, P 210ppm	6.778±1.3ab	7.722±1.6ab	10.956±2.3ab	14.567±2.8abc	14.956±3.1abcd	20.544±2.5abc	21.111±2.2cd	25.322±2.2bc
pH 8, P 210ppm	6.733±0.8ab	7.811±1.0ab	10.456±1.8ab	12.433±2.3c	16.32222±2.6cd	17.067±3.0de	21.678±4.2cd	24.822±4.6c
One - Way ANOVA (F-Statistic)	0.603NS	0.773NS	0.675NS	1.456NS	2.266*	3.339***	2.347**	1.497NS

NS Represents no statistical significance, * Represents a statistical significance of (P ≤ 0.05), ** Represents a statistical significance of (P ≤ 0.01) and *** Represents a statistical significance of (P ≤ 0.001) according to Fishers least significant difference.

Table 3.2: The effects of pH and Phosphorus concentrations on branch numbers of *Salvia chamelaeagnea* grown in hydroponics.

Treatments	Wk2	Wk4	Wk6	Wk8
pH 4, P 31ppm	6.444±1.7ab	8.889±1.7a	13.111±4.0a	26.889±9.1abcd
pH 6, P 31ppm (Control)	6.111±2.9ab	7.333±2.4ab	11.111±3.7abc	21.222±8.5bcde
pH 8, P 31ppm	7.778±3.7a	8.667±4.2ab	12.111±4.7ab	24.222±6.7abcde
pH4, P 90ppm	7.444±3.4a	9.444±3.9a	13.333±5.2a	28.667±11.0abc
pH 6, P 90ppm	4.889±1.5b	8.333±2.4ab	10.222±3.6abcd	20.333±6.2cde
pH 8, P 90ppm	4.667±1.7b	6.111±2.1b	8.111±4.0cd	24.444±11.7abcd
pH 4, P 150ppm	6.667±2.9ab	9.111±3.2a	11.111±3.0abc	32.111±10.0a
pH 6, P 150ppm	5.333±1.5ab	8.333±2.3ab	9.667±3.9bcd	18.778±10.4de
pH 8, P 150ppm	5.556±2.2ab	7.333±3.4ab	7.556±2.6d	18.556±7.7de
pH 4, P 210ppm	6.111±3.1ab	8.111±2.5ab	10.444±2.4abcd	25.000±10.2abcd
pH 6, P 210ppm	6.111±1.8ab	9.778±2.8a	10.667±3.0abcd	29.000±10.9ab
pH 8, P 210ppm	6.556±3.6ab	7.889±3.1ab	8.333±2.1cd	15.667±4.8e
One - Way ANOVA (F-Statistic)	1.1528NS	1.1061NS	2.3754**	2.6141***

NS Represents no statistical significance, * Represents a statistical significance of ($P \leq 0.05$), ** Represents a statistical significance of ($P \leq 0.01$) and *** Represents a statistical significance of ($P \leq 0.001$) according to Fishers least significant difference.

Table 3.3: The effects of pH and Phosphorus concentrations on post harvest root lengths and weights of *Salvia chamelaeagnea* grown in hydroponics.

Treatments	Root Length	Wet Roots	Wet Shoots	Dry Roots	Dry Shoots
pH 4, P 31ppm	34.211±10.3abc	16.167±3.3ab	50.007±10.7a	1.514±0.3abcd	7.519±1.8abc
pH 6, P 31ppm (Control)	29.233±6.1cd	13.448±4.6abcd	47.013±13.2a	1.461±0.8abcd	6.332±1.9bc
pH 8, P 31ppm	36.144±11.5abc	17.981±6.4a	49.106±16.0a	1.780±0.7abc	7.032±2.5abc
pH4, P 90ppm	33.933±6.8abc	16.473±5.1ab	56.278±21.8a	1.787±0.7ab	8.668±3.7a
pH 6, P 90ppm	29.044±3.4cd	12.757±4.5bcd	42.728±12.1ab	1.301±0.4bcd	6.129±1.8c
pH 8, P 90ppm	30.533±7.3bcd	17.606±7.7ab	42.456±18.3ab	1.851±0.8a	6.190±2.7c
pH 4, P 150ppm	39.489±17.3a	16.726±6.1ab	54.374±16.2a	1.699±0.4abc	8.437±2.7ab
pH 6, P 150ppm	32.444±6.5abcd	8.567±2.6d	31.392±15.9bc	1.104±0.4d	5.352±2.2cd
pH 8, P 150ppm	24.822±7.0d	16.000±3.3abc	25.587±6.9c	1.268±0.4cd	3.502±1.0d
pH 4, P 210ppm	37.889±8.9ab	13.414±6.5abcd	43.822±16.4ab	1.387±0.5abcd	6.973±2.8abc
pH 6, P 210ppm	32.167±5.0abcd	11.240±5.4cd	46.963±16.8a	1.378±0.4abcd	7.056±2.4abc
pH 8, P 210ppm	25.756±4.1d	14.279±5.0abc	26.683±8.7c	1.358±0.5abcd	3.667±1.2d
One - Way ANOVA (F-Statistic)	2.465**	2.5871**	4.1245***	1.6980NS	4.3024***

NS Represents no statistical significance, * Represents a statistical significance of ($P \leq 0.05$), ** Represents a statistical significance of ($P \leq 0.01$) and *** Represents a statistical significance of ($P \leq 0.001$) according to Fishers least significant difference.

CHAPTER FOUR

EFFECTS OF PH AND PHOSPHORUS CONCENTRATIONS ON THE CHLOROPHYLL RESPONSES OF *SALVIA CHAMELAEAGNEA* GROWN IN HYDROPONICS.

EFFECTS OF PH AND PHOSPHORUS CONCENTRATIONS ON THE CHLOROPHYLL RESPONSES OF *SALVIA CHAMELAEAGNEA* GROWN IN HYDROPONICS.

4.1 ABSTRACT

Salvia chamelaeagnea is a slow growing water wise evergreen shrub originating from the Western Province of South Africa, which holds value as an attractive landscape and medicinal plant. This study investigates the effects of 3 supplementary phosphorus concentrations and 3 pH levels of supplied irrigation water on the production of chlorophyll A, chlorophyll B, total chlorophyll, leaf colour and the nutrient uptake of *Salvia chamelaeagnea* grown in hydroponics over an 8 week period at Cape Peninsula University of Technology. The treatments of pH 4, pH 6 and pH 8 at 31 ppm, 90 ppm, 150 ppm and 210 ppm of phosphorus were received by twelve groups of plants and were replicated 10 times. The results indicated that a pH level of 4 had a significant effect on the chlorophyll responses of *S. chamelaeagnea* grown in hydroponics.

Key words: Hydroponically grown, pH level, chlorophyll production

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4.2 INTRODUCTION

Salvia chamelaeagnea (*S. chamelaeagnea*) is a member of the Lamiaceae family. This family contains many culinary and medicinal herbs like *S. officinalis, S. verbenacea* and *S. libanotica* which have been used for many years against diarrhoea, indigestion, colic, abdominal trouble, influenza, bacterial infections, tuberculosis, cough, colds and many other ailments (Kamatou et al., 2008; Gali-Muhtasib et al., 2000; Cornara et al., 2009). Some of these uses are dating back to medieval times (Clebsch, 2003). Many of the Lamiaceae secondary metabolites are of a commercial interest to the food industry as sources of natural preservatives, flavourants, and antioxidants (Thorsen & Hildebrandt, 2003; Kamatou et al., 2008), as well as to the pharmaceutical industry as sources of anti-oxidants, antiinfammatories (Kamatou et al., 2010), anti-bacterials, and anti-mycobacterials (Kamatou et al., 2007).

Salvias are renown for their wonderful variety, their many uses around the home and garden, attracting birds to it (Wester & Claßen-Bockhoff, 2006), In its natural habitat *S. chamelaeagnea* will develop into attractive foliage and flowering landscape plants, with small mid-green egg shaped leaves and masses of bright blue or white flowers borne at the tops of each stem, which are suitable for the cut flower trade (Clebsch, 2003; Pienaar, 2000; Ulubelen, 2003). *S. chamelaeagnea* also has value in the medicinal plant trade as it contains the phenolic compounds carnosol, rosmarinic acid and caffeic acid which exhibits antioxidant and anti bacterial activity (Kamatou et al., 2008; Kamatou et al., 2010; Nenadis et al., 2003). Unfortunately very little information has been documented on the cultivation of this species.

As chlorophyll is a fundamental part of the light dependant reactions of the photosynthesis process, capturing light rays from the sun and producing energy storing adenosine tri-phosphate (ATP) molecules is essential for the functioning of a healthy plant (Glass, 1989; Stern, 2006). Effects of nutrients and nutrient ratios are known in many food and medicinal crop plants, as in; soybean, thyme, wheat cultivars, barley, spinaches, pelargoniums and many others. In most cases a positive result in growth is noticed with the addition of some macronutrients such as N, P, K, Mg or Ca (Gan et al., 2003; Khazaie et al, 2008; Ichir et al, 2003; Maidl, et al, 1996; Stagnari et al., 2007; Manzanares et al., 1997; Liu et al, 2011; Bo et al, 2009; Ram et al, 2003; Zhu et al, 2009). It is therefore crucial that adequate plant nutrition and soil pH levels are met for any given plant so that cell functioning is not impeded. The effects of poor nutrition, be it through infertile soils or incorrect soil pH level, directly affects the production of chlorophyll molecules resulting in chlorosis of leaves and a reduced photosynthetic rate, thus inhibiting some biological processes and decreasing the general health of the plants. (Leopold & Kriedemann, 1975; Marschner, 1995; Stern, 2006).

This study aims to investigate the effects phosphorus (P) and pH on the chlorophyll production, leaf colour and nutrient uptake of medicinal *Salvia chamelaeagnea* in hydroponics, in order to determine a fertilizer regime which will promote the development *S. chamelaeagnea* without degrading soils and leaching nutrients into the water table.

4.3 MATERIALS AND METHODS

4.3.1 Experimental process

The experiment took place in the research glass house at CPUT Cape Town campus, South Africa, latitude and longitude S33°55' 58 E18°25' 57, from June 2012 until August 2012. Inside the greenhouse was a 40 % Alunet shade cloth, raised 2 m above the floor resulting in light intensities ranging from 10 Kilolux to 13 Kilolux, determined by using a Toptronic T630 light meter. The climate was controlled to range between 16 – 28 °C in the day while being between 10 – 20 °C during the night, with an average relative humidity of 42 %.

The experiment was laid out in a randomised block design with plants being spaced 30 cm apart, and consisted of 12 treatments of 4 differing nutrient solutions offering: low concentration of supplementary P (control), balanced concentration of supplementary P, moderate concentration of supplementary P and a high concentration of supplementary P at 3 differing pH levels. The control treatment of 31 ppm was chosen due to the nature of fynbos soils being low in available P (Hawkins et al., 2008; Rebelo, 2001; Witkowski & Mitchell, 1987)

Hoagland solution a well known hydroponic nutrient solution modified by Hershey, (1994) and Hershey (1995), offering all the necessary macro and micro nutrients for healthy plant growth was used as a base nutrient and supplemented with P.

The plants for the experiment were rooted tip cuttings sourced from healthy mother stock plants at the CPUT Glass House Nursery. The rooted cuttings were gently rinsed in de-ionized water to remove any rooting media from the root zone. They were then weighed and planted into 25 cm plastic pots filled with leca clay, placed into a recirculating closed hydroponics system at a spacing of 30 cm apart, where their heights were recorded.

The plants were irrigated with the treatments 15 times per day at equal timed intervals for the duration of the experiment. For each treatment there were 10 plants. The treatments were as follows:

1) Hoagland hydroponic nutrient solution with 31 ppm of P at a pH of 4.
- Hoagland hydroponic nutrient solution with 31 ppm of P at a pH of 6. (Control)
- Hoagland hydroponic nutrient solution with 31 ppm of P at a pH of 8.

2) Hoagland hydroponic nutrient solution supplemented with 90 ppm of P at a pH of 4.
- Hoagland hydroponic nutrient solution supplemented with 90 ppm of P at a pH of 6.
- Hoagland hydroponic nutrient solution supplemented with 90 ppm of P at a pH of 8.

3) Hoagland hydroponic nutrient solution supplemented with 150 ppm of P at a pH of 4.
- Hoagland hydroponic nutrient solution supplemented with 150 ppm of P at a pH of 6.
- Hoagland hydroponic nutrient solution supplemented with 150 ppm of P at a pH of 8.

4) Hoagland hydroponic nutrient solution supplemented with 210 ppm of P at a pH of 4.
- Hoagland hydroponic nutrient solution supplemented with 210 ppm of P at a pH of 6.
- Hoagland hydroponic nutrient solution supplemented with 210 ppm of P at a pH of 8.

pH Level

The pH levels of the nutrient solutions were monitored using a Martini Instruments PH55 pH probe and were adjusted accordingly using either hydrochloric acid (HCL) to lower the pH, or sodium hydroxide (NaOH) to raise the pH.

Irrigation

The treatments were set to irrigate every 15 times daily for a duration of 15 minutes using a 1350 L/h hour Boyu submersible pump and a Tedelex analogue timer to regulate irrigation frequencies.

4.3.2 Data collection

Measurement of leaf colour

Chlorophyll meter readings were taken from the top 3 fully developed leaves of each plant with a hand-held, dual-wavelength meter (SPAD 502, chlorophyll meter, Minolta Camera Co., Ltd., Japan). For each treatment, 30 fully developed leaves were used weekly. The SPAD meter stored and automatically averaged the recordings to generate one reading per plant.

Measurement of chlorophyll content in leaves

The extraction of leaf chlorophyll concentrations by dimethylsulphoxide (DMSO) were carried out as described in Hiscox and Israelstam, (1979). A third of plants leaves from the tip were collected from each plant. A hundred (100) mg of the middle portion of fresh leaf slices were placed in a 15 mL vial containing 7 mL DMSO and incubated at 4°C for 72 h. After the incubation, the extract was diluted to 10 mL with DMSO. A 3 mL sample of chlorophyll extract was then transferred into curvets for absorbance determination. A spectrophotometer (UV/Visible Spectrophotometer, Pharmacia LKB. Ultrospec II E) was used to determine absorbance values at 645 and 663 nm, which was then used in the equation proposed by Arnon, (1949) to determine total leaf chlorophyll contents against DMSO blank, expressed as mg L^{-1} as follows:

$$\text{Chl } a = 12.7\text{D}663 - 2.69\text{D}645$$

$$\text{Chl } b = 22.9\text{D}645 - 4.68\text{D}663$$

Total Chl = 20.2D645 + 8.02D663

Measurement of levels of macro- and micro-nutrients in dry plant material

The measurements of macronutrients (N, P, K, Ca, Mg, and Na) and micronutrients (Cu, Zn, Mn, Fe, and B) were determined by ashing a 1 g ground sample in a porcelain crucible at 500 °C overnight. This was followed by dissolving the ash in 5 mL of 6 M HCl and putting it in an oven at 50 °C for 30 min; 35 mL of deionised water was added and 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).

4.3.3 Statistical analysis

Data collected was analyzed for statistical significance using the One –way analysis of variance (ANOVA), with the computations being done using the software program STATISTICA. The Fisher least significance (L.S.D.) was used to compare significant treatment means *at* $P \leq 0.05$ level of significance (Steel & Torrie, 1980).

4.4 RESULTS

4.4.1 Effects of pH and Phosphorus concentrations on the chlorophyll content of *Salvia chamelaeagnea* grown in hydroponics.

Treatments seen in Table 4.1 significantly ($P \leq 0.001$) effected the chlorophyll A, chlorophyll B and total chlorophyll development of *S. chamelaeagnea* grown hydroponically. The chlorophyll A and total chlorophyll values of the plants all followed the same pattern, in that all pH 4 treatments yielded significantly ($P \leq 0.001$) higher values than that of the control, pH 6 and pH 8 treatments. A pH of 4 at 31 ppm P yielded highest chlorophyll A and total chlorophyll contents, this result was closely followed by pH 4 at 90 ppm P; pH 4 at 150 ppm P and pH 4 at 210 ppm P respectively. All of which yielded significantly ($P \leq 0.001$) higher chlorophyll A and total chlorophyll values than the control and all other treatments. The chlorophyll B content of the plants followed a similar trend, that being that all plants receiving a pH of 4 produced significantly higher chlorophyll B values than that of the control and all other treatments. The highest chlorophyll B contents were recorded in the treatment delivering pH 4 at 31 ppm P followed by, pH 4 at 210 ppm P and pH 4 at 150 ppm P respectively.

4.4.2 Effects of pH and Phosphorus concentrations on the leaf colour of *Salvia chamelaeagnea* grown in hydroponics.

During week 1, shown in Table 4.2, there was no statistical significance between the treatments. In the following weeks (weeks 2-8), the plants were significantly affected ($P \leq 0.001$) by the treatments. During the growth period there was a noticeable trend in that all pH 4 treatments yielded higher results than that of their counterparts under the same P enrich conditions and the lowest values were presented in pH 8 treatments. While treatment 1 offering a pH level of 4 at 31 ppm P generally yielded the highest leaf colour values over the 8 week growth period these values did not differ significantly from that of the other pH 4 treatments receiving supplementary P. Of these treatments receiving supplementary P the highest results were recorded at a pH of 4 receiving 210 ppm P closely followed by pH 4 at 90 ppm P and pH 4 at 150 ppm P treatments respectively, all which differed from the control treatment.

4.4.3 Effects of pH and Phosphorus concentrations on the uptake of macro nutrients in *Salvia chamelaeagnea* grown in hydroponics.

Macro nutrient uptake of P , K, and Mg shown in Table 4.3 was significantly ($P \leq 0.001$) affected by the treatments. There was a noticeable trend of the pH 8 treatments receiving supplementary P yielding higher uptake values than that of the control, pH 4 and pH 6 treatments. The highest uptake values of N were recorded at a pH of 6 at 90 ppm P which differed significantly from the control and all other treatments. Ca uptake value of treatments delivering pH 4 at 31 ppm P, pH 6 at 31 ppm P (control) and pH 8 at 90 ppm P did not differ significantly from each other or the control but the did differ significantly from all other treatments. Highest uptake of Ca was recorded at a pH of 8 at 90 ppm P.

4.4.4 Effects of pH and Phosphorus concentrations on the uptake of micro nutrients in *Salvia chamelaeagnea* grown in hydroponics.

The micro nutrient uptake of Na, Mn, Fe, Cu, Zn, and B seen in Table 4.4 was significantly (P ≤ 0.001) affected by the treatments. The highest nutrient uptake values of Na and Zn were recorded in the pH 8 at 210 ppm P treatment, these results were significantly higher than that of the control and all other treatments. Mn uptake values in pH 4 at 31 ppm P were significantly higher than that of the control and all other treatments, however these results did not differ significantly from recordings in pH 8 at 90 ppm P. Fe uptake values in the treatment delivering pH of 4 at 210 ppm recorded the highest values, this differed significantly from the control and all other treatments. Highest recorded uptake values of Cu were recorded in plants receiving a pH of 4 at 31 ppm P closely followed by the plants receiving a pH of 4 at 210 ppm, these results did not differ significantly from each other however they did differ significantly from the control and all other treatments. Improved B uptake values were recorded at a pH of 4 at 90 ppm P and at pH 6 at 31 ppm P respectively, these results did not differ significantly from each other however, both significantly outperformed the control and all other treatments.

4.5 DISCUSSION

In this study significantly (P ≤ 0.001) higher chlorophyll values recorded in the treatments receiving a pH of 4 with no supplementary P and at 90 ppm P respectively shows that a supplied irrigation that is acidic and low in available N, K and Ca will largely increase chlorophyll production of *S. chamelaeagnea* in hydroponic production. These findings are in agreement with Eriksen and Iversen, (1995) and Lafarga-De la Cruz et al., (2006), in which higher chlorophyll concentrations were recorded in soils with a lower availability of nutrients. It is not surprising that these conditions mimic that of the soils in the fynbos biome, in which *S. chamelaeagnea* naturally occurs (Richards et al., 1997).

The high leaf colour values in treatments receiving a pH of 4 when compared to that of treatments receiving a pH of 6 and 8 as well as the control, shows that a pH level of 4 is highly influential on mineral nutrient availability and development of leaf colour in *S. chamelaeagnea* grown hydroponically. Notably these findings concur to that of Minotta and Pinzauti, (1996) in which chlorophyll concentrations and SPAD recordings increased at a low soil fertility level. While it seems that supplementary P has had a minimal effect on leaf colour furthering studies into supplementary N, P and K are recommended to acquire more knowledge on the effect of supplementary P on leaf colour development at this low ph level in *S. chamelaeagnea*.

Despite of the high nutrient uptake values in plants receiving a nutrient solution with a pH 8, chlorosis of their leaves was apparent during the growth period. This suggesting that the uptake of some essential nutrients responsible for chlorophyll development were affected at this pH level, namely the mineral nutrients Cu, B, N and Fe which are directly involved in photosynthesis, respiration, cell division and protein formation (Msilini et al., 2013, Molassiotis et al., 2006; Stern, 2006). Highest nutrient uptake values were recorded in 9 of the treatments receiving supplementary P, with only Cu and Mn yielding the highest values in treatments receiving no supplementary P. Thus it is evident that phosphorus treatments had a significant effect on nutrient uptake in *Salvia chamelaeagnea* grown hydroponically (Naeem et al., 2010). Future research may benefit from further investigating the effects of supplementary N and Fe in conjunction with supplementary P in attempts to improve leaf chlorophyll development in *Salvia chamelaeagnea* (Pestana et al., 2012).

4.6 CONCLUSION

In conclusion this study give insight into the unknown cultivation requirements of the leaf chlorophyll development of *S. chamelaeagnea* and shows that the use of a hydroponic nutrient system offering little to no supplementary phosphorus at a pH level of 4 significantly influences the chlorophyll development of *Salvia chamelaeagnea* grown in hydroponics.

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4.9 TABLES

Table 4.1: The effects of pH and Phosphorus concentrations on chlorophyll content of *Salvia chamelaeagnea* grown in hydroponics.

Treatments	Chlorophyll A	Chlorophyll B	Total Chlorophyll
pH 4, P 31ppm	12.242±1.7a	3.446±0.5a	15.684±2.2a
pH 6, P 31ppm (Control)	10.384±1.0cd	2.848±0.3cde	13.229±1.3cd
pH 8, P 31ppm	10.173±1.1cde	2.784±0.3ef	12.954±1.5cde
pH4, P 90ppm	11.419±0.5ab	3.233±0.2ab	14.649±0.6ab
pH 6, P 90ppm	8.348±1.1g	2.227±0.3hi	10.574±1.4g
pH 8, P 90ppm	9.327±1.3ef	2.600±0.4g	11.924±1.7ef
pH 4, P 150ppm	10.929±0.7bc	3.014±0.3bcd	13.941±0.9bc
pH 6, P 150ppm	8.463±1.4fg	2.282±0.4h	10.744±1.8fg
pH 8, P 150ppm	7.063±0.6h	1.988±0.2i	9.049±0.7h
pH 4, P 210ppm	10.900±0.7bc	3.108±0.3bc	14.005±0.9bc
pH 6, P 210ppm	9.817±1.0de	2.650±0.3g	12.465±1.3de
pH 8, P 210ppm	3.547±0.5i	0.910±0.2j	4.456±0.7i
One - Way ANOVA (F-Statistic)	46.757***	43.425***	46.388***

NS Represents no statistical significance, * Represents a statistical significance of ($P \leq 0.05$), ** Represents a statistical significance of ($P \leq 0.01$) and *** Represents a statistical significance of ($P \leq 0.001$) according to Fishers least significant difference.

Table 4.2: The effects of pH and Phosphorus concentrations on leaf colour of *Salvia chamelaeagnea* grown in hydroponics.

Treatments	Wk1	Wk2	Wk3	Wk4	Wk5	Wk6	Wk7	Wk8
pH 4, P 31ppm	30.156±3.6ab	31.667±4.1a	31.433±3.0a	30.878±1.3a	30.189±3.1ab	30.011±2.2a	31.078±1.8a	28.467±1.9ab
pH 6, P 31ppm (Control)	32.800±2.5a	31.644±4.0a	30.533±1.3ab	29.933±2.0a	30.122±2.4ab	28.822±2.6ab	28.644±1.6bcd	28.922±1.4a
pH 8, P 31ppm	30.033±4.5ab	30.567±3.8ab	28.933±1.4b	29.944±2.1a	29.189±1.6ab	30.111±2.4a	29.589±1.7abc	28.278±2.3ab
pH4, P 90ppm	29.689±3.8ab	29.911±5.3ab	30.867±2.2ab	31.111±1.5a	30.711±2.1ab	29.656±2.4a	30.567±2.1a	28.122±2.0ab
pH 6, P 90ppm	31.789±3.6ab	31.156±4.6ab	31.078±2.8ab	30.578±2.0a	29.444±1.6ab	27.000±1.4b	28.067±2.2cde	27.056±1.0bc
pH 8, P 90ppm	29.411±3.3b	27.356±3.0bc	22.756±3.0c	20.067±2.3b	20.278±1.2c	24.489±1.6c	27.000±1.9e	27.800±1.7abc
pH 4, P 150ppm	30.289±3.6ab	30.044±3.7ab	31.233±2.7ab	30.622±1.5a	29.189±2.6ab	29.989±4.0a	30.178±1.8ab	27.956±1.1ab
pH 6, P 150ppm	29.333±3.5b	30.944±3.6ab	29.933±2.1ab	29.356±2.7a	28.633±2.3b	28.233±2.2ab	26.456±1.5e	25.100±2.1de
pH 8, P 150ppm	29.267±3.0b	22.422±4.9d	15.978±2.8d	15.756±3.5c	15.156±2.7d	15.267±3.1e	21.756±1.6f	24.278±2.3e
pH 4, P 210ppm	30.944±2.8ab	30.456±3.1ab	28.956±2.4b	31.033±2.0a	30.911±3.2a	28.767±2.0ab	29.533±0.7abc	29.278±1.4a
pH 6, P 210ppm	31.756±3.9ab	28.489±3.2abc	29.478±2.4ab	29.444±1.2a	29.722±1.0ab	28.622±1.7ab	27.756±2.0de	26.278±1.7cd
pH 8, P 210ppm	31.411±2.6ab	24.922±5.7cd	16.167±3.6d	13.900±2.7c	14.511±1.8d	18.044±1.7d	18.567±1.6g	16.067±1.8f
One - Way ANOVA (F-Statistic)	1.013NS	4.333***	45.53***	78.77***	66.25***	38.79***	41.52***	37.33***

NS Represents no statistical significance, * Represents a statistical significance of (P ≤ 0.05), ** Represents a statistical significance of (P ≤ 0.01) and *** Represents a statistical significance of (P ≤ 0.001) according to Fishers least significant difference.

Table 4.3: The effects of pH and Phosphorus concentrations on the uptake of macro nutrients in *Salvia chamelaeagnea* grown in hydroponics.

Treatments	N %	P %	K %	Ca %	Mg %
pH 4, P 31ppm	4.18±0,29bcd	0.64±0,06g	4.23±0,29g	1.13±0,07a	0.28±0,01h
pH 6, P 31ppm (Control)	4.24±0,55abc	0.73±0,06f	4.41±0,23fg	1.12±0,10a	0.36±0,03e
pH 8, P 31ppm	4.19±0,18abcd	0.62±0,08g	4.47±0,26efg	1.10±0,11ab	0.43±0,04c
pH4, P 90ppm	4.27±0,26abc	0.77±0,08ef	4.64±0,36cdef	1.10±0,10ab	0.31±0,02g
pH 6, P 90ppm	4.41±0,20a	0.82±0,08cde	4.53±0,13defg	1.08±0,07ab	0.38±0,03d
pH 8, P 90ppm	4.20±0,12abcd	0.80±0,07def	4.45±0,24efg	1.14±0,05a	0.48±0,03b
pH 4, P 150ppm	4.37±0,19ab	0.82±0,04cde	4.79±0,58cd	1.07±0,06abc	0.32±0,03fg
pH 6, P 150ppm	4.09±0,22cd	0.88±0,07bc	4.87±0,19c	1.01±0,07cd	0.36±0,02de
pH 8, P 150ppm	4.00±0,08de	1.07±0,08a	6.29±0,39a	0.77±0,03e	0.55±0,02a
pH 4, P 210ppm	4.13±0,18cd	0.84±0,06bcd	4.73±0,32cde	1.05±0,06bc	0.31±0,02g
pH 6, P 210ppm	4.05±0,18cd	0.87±0,08bcd	4.23±0,35g	0.97±0,06d	0.35±0,02ef
pH 8, P 210ppm	3.77±0,16e	0.91±0,13b	5.71±0,38b	0.46±0,03f	0.48±0,03b
One - Way ANOVA (F-Statistic)	4.35***	21.34***	31.67***	68.64***	89.74***

NS Represents no statistical significance, * Represents a statistical significance of (P ≤ 0.05), ** Represents a statistical significance of (P ≤ 0.01) and *** Represents a statistical significance of (P ≤ 0.001) according to Fishers least significant difference.

Table 4.4: The effects of pH and Phosphorus concentrations on the uptake of micro nutrients in *Salvia chamelaeagnea* grown in hydroponics.

Treatments	Na mg/kg	Mn mg/kg	Fe mg/kg	Cu mg/kg	Zn mg/kg	B mg/kg
pH 4, P 31ppm	477.89±36,27fg	84.67±7,48efg	151.56±7,32cde	5.22±1,99a	39.56±2,88bc	37.78±3,63ab
pH 6, P 31ppm (Control)	479.78±57,99fg	105.89±11,40c	139.11±10,17def	2.89±0,60d	38.00±3,20c	38.56±3,09a
pH 8, P 31ppm	472.89±58,58g	156.78±9,11a	137.11±8,25ef	2.89±0,33d	37.89±3,44c	37.33±2,29ab
pH4, P 90ppm	548.44±74,72ef	84.00±8,19fg	144.11±10,59def	4.11±0,60bc	40.11±4,31bc	38.67±3,67a
pH 6, P 90ppm	505.78±39,02fg	101.00±4,69cd	153.33±13,87bcd	2.56±0,53de	41.33±6,12bc	37.44±2,40ab
pH 8, P 90ppm	532.56±70,06efg	150.33±12,56a	167.33±13,27abc	3.22±0,44cd	39.78±6,28bc	36.56±1,24abc
pH 4, P 150ppm	604.22±102,07de	82.67±9,84g	168.56±23,51ab	4.67±1ab	41.33±6,24bc	37.67±2,29ab
pH 6, P 150ppm	680.33±55,08bc	94.00±19,68de	151.00±34.86de	3.11±1,90d	38.00±6,75c	35.22±3,03bcd
pH 8, P 150ppm	716.00±117,06b	131.44±7,32b	152.78±16,20bcde	4.33±0,5ab	39.44±4,48bc	31.67±3,35e
pH 4, P 210ppm	696.78±69,99bc	82.11±4,43g	175.00±14,42a	5.11±0,60a	44.00±2,92ab	34.56±2,51cd
pH 6, P 210ppm	640.89±36,55cd	90.78±9,38efg	145.11±14,16def	2.44±0,53de	43.89±5,69ab	35.33±2,45bcd
pH 8, P 210ppm	867.67±131,72a	93.44±8,14def	129.33±21,17f	1.89±0,60e	46.78±7,31a	33.33±2,40de
One - Way ANOVA (F-Statistic)	22.746***	62.30***	5.590***	11.975***	2.573***	5.56***

NS Represents no statistical significance, * Represents a statistical significance of (P ≤ 0.05), ** Represents a statistical significance of (P ≤ 0.01) and *** Represents a statistical significance of (P ≤ 0.001) according to Fishers least significant difference.

CHAPTER FIVE

COMMERCIAL HYDROPONIC CULTIVATION POTENTIAL OF *SALVIA CHAMELAEAGNEA* AS A MEDICINAL PLANT. A REVIEW.

COMMERCIAL HYDROPONIC CULTIVATION POTENTIAL OF *SALVIA CHAMELAEAGNEA* AS A MEDICINAL PLANT. A REVIEW.

5.1 Abstract

Salvia chamelaeagnea is a slow growing evergreen shrub with small mid-green egg shaped leaves and masses of bright blue flowers. This species of Salvia is a favourable choice for landscapers as a water wise landscaping plant which is also suitable candidate for the cut flower industry. Extracts from this species have been found to exhibit pharmaceutical effects against various microbial and bacterial infections. This study investigates the effects of pH levels and phosphorus (P) concentrations on the growth and development of *S. chamelaeagnea* grown in hydroponics over an 11 week growth period. It was found that the treatments of 3 pH levels and 4 differing P concentrations had a significant effect on the growth and development of *S. chamelaeagnea* grown hydroponically. This study includes an analysis of suitable cultivation variables, and also includes a proposed hydroponic production schedule for the commercial cultivation of *S. chamelaeagnea.*

Keywords: water wise, pH levels, production schedule.

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5.2 Introduction

The genus *Salvia* is the largest genus of the family Lamiaceae and includes more than 900 species globally. Many of Lamiaceae secondary metabolites are of a commercial interest to the food industry as sources of natural preservatives, antioxidants and flavourings (Thorsen & Hildebrandt, 2003; Kamatou et al., 2008), as well as to the pharmaceutical industry as sources of anti-inflammatories, antioxidants (Kamatou et al., 2010), antimycobacterials and antibacterials (Kamatou et al., 2007).

Salvias are often found in sub tropical and temperate areas in sunny spots, many having slightly toothed woolly or hairy leaves which are aromatic when crushed (Bryant & Rodd, 2005; Kintzios et al., 1999; Clebsch, 2003; Kamatou et al., 2008).

While some Salvias are known for their interesting variety and their many domestic uses (Wester & Claßen-Bockhoff, 2006), others are valued for their medicinal properties in treating indigestion, colic, diarrhoea, influenza, bacterial infections, tuberculosis, coughs, abdominal trouble, colds and other ailments (Gali-Muhtasib et al., 2000; Cornara et al., 2009; Kokkini et al., 1997; Lu & Foo, 2002; Kamatou et al., 2008). Some are also used in aromatherapy for their valued phenolic compounds (Jeannot et al., 2003; Sawamura et al., 2004) and others such as *S officinalis* and *S elegans* as culinary herbs (Clebsch, 2003).

A growing consciousness of personal health, well being and an increase in life expectancy is leading to greater demands for natural products (Gurib-Fakim, 2006; van Wyk, 2008). The medicinal plant trade in South Africa consists of between 50 and 100 million customers who acquire medicinal plants through two kinds of market system. A formal market system that supplies processed materials in the forms of herbal remedies, essential oils, soaps and plant based medicines, and a more informal market system, which supplies raw plant material such as rhizomes, roots, leaves and fruits which are then processed by the purchaser before being taken as medicine (Canter et al., 2005; Diederichs, 2006).

The history of growing plants in hydroponics dates back thousands of years. Research has shown that primitive examples of hydroponic culture were used by the ancient Aztec's, Chinese and the Egyptians (Resh, 1995). During the years of World War 2 the United States army utilised hydroponic growth systems to grow fresh fruit and vegetables for soldiers posted on infertile islands (Harris, 1976) while in the more recent past the use of hydroponic growth systems has become widespread in the plant cultivation and research industries (Mason, 1990; Hanan & Holley, 1970; Caruso et al., 2011).

Given the values of Salvias as domestic and medicinal plants, a need to maintain the quality of their medicinal and aesthetic characteristics is fundamental so that every plant grown meets prerequisite criteria ensuring the quantity and quality of the given plant to be sold to its respective markets (Street et al., 2008).

5.3 *Salvia chamelaeagnea* as a possible commercial crop.

Salvia chamelaeagnea (*S. chamelaeagnea*) is a slow growing evergreen shrub originating from the western side of the Cape of Good Hope. It can reach 1.5-2m in height and 1.2m in spread and it can be an attractive foliage and flowering plant with small mid-green egg shaped leaves, having slightly toothed margins and masses of small pale violet-blue flowers borne at the tops of each stem (Clebsch, 2003; Pienaar, 2000).

This species of Salvia has been found to exhibit favourable results against various microbial and bacterial infections. As Kamatou et al., (2007) reported when testing compounds from *S chamelaeagnea* against the pathogens *Bacillus cerus* and *Staphylococcus aureus. S. chamelaeagnea* too exhibits some antioxidant activity due to the phenolic compounds carnosol, rosmarinic acid and caffeic acid (Kamatou et al., 2008; Kamatou et al., 2010; Nenadis et al., 2003). Kamatou et al., (2006) recorded a synergistic effect when experimenting with *S. chamelaeagnea* and *Leonotis leonurus,* in an attempt to defeat the increasing bacterial resistances to antibiotics. *S. chamelaeagnea* also exhibited promising activity against malarial infection (Kamatou et al., 2008).

In the South African nursery and landscape industries *S. chamelaeagnea* is utilised for its aesthetic attributes such as its striking light blue flowers, its contrasting leaves and appearance in a planting/bed and for its effectiveness as a water-wise landscaping plant (Clebsch, 2003; Pienaar, 2000). Due to its all round hardiness and ability to grow well in dry sandy soils *S. chamelaeagnea* is often a likely candidate to be used in the many landscaping projects which require little to no intervention after installation.

5.4 Environmental growing requirements

5.4.1 Climate and Temperature

S. chamelaeagnea is adaptable to many cultural situations however it grows best in full sun, with temperature and day length playing a large role in flower development and maturation (Clebsch, 2003). While it can grow in a variation of climates, *S.*

chamelaeagnea grows well in the Western Cape where the climate is relatively temperate with humidity ranging between 40 % and 90 % and temperatures ranging from 18 °C to 34 °C in summer and 12 °C to 22 °C in the cooler short days of the winter months (Clebsch, 2003; Pienaar, 2000; Brickell, 2003; Ulubelen, 2003). While the hardy nature of this particular Salvia can make it a favourable candidate for horticultural production systems, researchers have noticed trends in members belonging to the Lamiaceae family being affected by environmental factors like light, temperature and day length, effecting their essential oil compositions (Circella et al., 1995; Hornok, 1983; Johnson et al., 1999). Thus attention to the quality of light and temperatures needs to be considered when cultivating *S. chamelaeagnea* for its medicinal uses.

5.4.2 Soil preparation, pH, Plant spacing

In southern African soils degradation and pollution, due to poor resource management, results in many soils under agricultural cultivation being impacted adversely (Huttl & Frielinghaus, 1994; Zingore et al., 2006; McGeoch et al., 2008). While *S. chamelaeagnea* grows well in many areas, preparing the soil with grit and some humus rich material assures the roots of a free draining yet fertile medium which aids in the prevention of fungal attacks, a common problem amongst Salvias (Clebsch, 2003; Pienaar, 2000; Brickell, 2003; Ulubelen, 2003).

The pH of a medium greatly influences the availability of nutrients, whether the plants are in soil or hydroponics. It is known that at differing pH levels different amounts of nutrients are available to the plants, greatly affecting functioning and development (Stern, 2006). In many crop plants pH levels are slightly acidic, whilst for some medicinal crops a more acidic pH may be more favourable for the elicitation of secondary metabolite production (Verpoorte & Memelink, 2002; Taylor et al., 2001; Taarit et al., 2010). Given the recorded data on pH and its effects on soil nutrient availability, *S. chamelaeagnea* would likely benefit from a moderate pH level of between 5 and 7, at which the majority of macro and micro nutrients are available for plants roots to take up into the plant.

Intercropping and plant spacing of food, medicinal and domestic plants has yielded great results and is a feasible plan to adopt given the global decrease in arable land

and soil fertility stemming from unethical and poorly managed agricultural practices (Huttl & Frielinghaus, 1994; Zhang et al., 2009; Wang & Frei, 2011). In crops like *Pelargonium graveolens* and *Mentha arvensis* intercropping has proved feasible and that weed biomass can drastically be reduced as a result, thus less fertilizer is going to waist and weeding costs are eliminated (Rao, 2002; Singh et al., 2013). In many food crops like cereal and legume crops intercropping has yielded significant results in their nutritional quality (Reddy et al., 2003). Intercropping *S. chamelaeagnea* in soil cultivation systems with a plant specie which improves soil quality, conditioning and possibly deter pests could therefore be of a great advantage to crop farmers.

5.4.3 Growing, Watering frequency, Fertilizing

While there are a multitude of plant species on earth it can be understood that their growth habits differ from species to specie, from the conical form of *Cupressus bakeri* trees to the low spreading habit of *Soleirolia soleirolii* ground cover (Brickell, 2003; Bryant & Rodd, 2005). The growth habit of *S. chamelaeagnea* is that of an evergreen shrub with many branches stemming from the base of the plant, growing to 1.4 m in height and 1.3 m in width after several growing seasons**.** Attractive light-blue flowers are borne atop the branches in mid summer and through out autumn (Clebsch, 2003; Pienaar, 2000). A light pruning during the year is recommended to prevent a messy appearance, while hard pruning after flowering promotes new growth for the season ahead. Pinching out the highest apical buds early in the growing season prompts an even bushier appearance and would greatly influence the amount of dry leaf material produced for the medicinal markets (Mohamed & Wahba, 1993).

Water and its quality play a vital role in the existence of plants. Considering that plants are comprised mostly of water, it is crucial that their demand for water is met to continue cell to cell functioning and development. On the other hand, as crucial as the roles water plays in plants are, too much water can also have a negative effect on development as it may lead to root rot, damping off of seedlings and cuttings, a change of essential oil composition, reduced hardiness and leeching out of soil nutrients (Stern, 2006; Marschner, 1995). Therefore crop water use efficiency and watering frequency is a very important aspect of plant cultivation systems world wide. Though *S. chamelaeagnea* may be considered a water-wise plant it benefits from weekly deep watering in the warmer months of the year while too much water can lead to fungal attacks, of which many Salvias are prone to.

Effects of nutrients and nutrient application ratios are known for many food and medicinal crop plants, such as; thyme, cereals, spinaches and grains. In most cases positive results are obtained with the addition of macronutrients such as N, P, K, Mg or Ca (Gan et al., 2003; Khazaie et al., 2008; Ichir et al., 2003; Maidl et al., 1996; Stagnari et al., 2007; Manzanares et al., 1997; Liu et al., 2011; Bo et al., 2009; Ram et al., 2003; Zhu et al., 2009). Various experiments on medicinal Lamiaceae species have shown that meeting nutritional requirements for growth and yield remain essential for successful cultivation (Sotiropoulou & Karamanos, 2010; Patra et al., 2000). For example Dordas, (2009) found that Mg and Ca applications greatly affected *Origanum vulgare ssp* Hitrum, increasing plant mass by 23% and in Mg treatments chlorophyll content increased by 38%. Azizi et al., (2009) reported that dry weight of *O. vulgare* increased with an increase in N fertilizer application. Information as fore mentioned can greatly benefit farmers in producing *S. chamelaeagnea* for medicinal plant trades.

While there is little data available regarding *S. chamelaeagneas* nutritional requirements it is however known that the soils of the fynbos belt in which it naturally occurs are poor and acidic, thus giving us a general idea of its basic growth needs (Richards et al., 1997; Kruger et al., 1983).

5.5 Harvesting

Harvesting of medicinal plants found in the wild has devastated many species through over harvesting (Diederichs, 2006; Lubbe & Verpoorte, 2011). It is therefore crucial to establish cultivation systems for these valued plants in attempts to reduce the rate at which some plants may be becoming extinct in the wild.

As there are many kinds of medicinal plants it is understandable that different plant parts are used depending on type of plant and treatment method, each using different harvesting techniques to prepare them for their intended use (Lubbe & Verpoorte, 2011; Diederichs, 2006). Traditionally harvesting of medicinal plants has been carried out in a number of rudimentary ways, depending on which part of the plant is to be used. For example; above ground parts of a plant are separated from roots and hung to dry, tree bark being peeled off the trunk of a tree and then dried, or roots from a plant unearthed and either hung or laid on mesh to dry out before use (van Andel & Havinga, 2008).

Today these same methods or adaptations of them are used for harvesting purposes. With thanks to the development of some technologies these rudimentary processes have been refined and have become more efficient in retaining the quality of these plant parts. Commonly several methods are practiced in the harvesting of medicinal and culinary plants such as; hot air drying, sun drying, shade drying, vacuum microwave drying and vacuum freeze drying (Lin et al., 2011).

In Salvias and other Lamiaceae leaf crops, the microwave drying method has been most beneficial as it causes little disruption to essential oil makeup and concentrations thereof (Arslan et al., 2010; Lin et al., 2011). It is likely that *S. chamelaeagnea* will benefit from this particular method as well, as it is crucial to maintain the secondary metabolite concentrations when drying this plant for its valued medicinal uses.

5.6 Extracting oil

The World Health Organization stated that approximately 80% of the people in developing countries rely on traditional medicines to meet their health care necessities (Canter et al., 2005; Gurib-Fakim, 2006; van Wyk, 2008). Plants such as; *Artemesia nanschanica* Krasch., *Psidium guajava, Rauwolfia vomitoria, Alchornea cordifolia, Hoslundia opposita* and *Entada africana a*re used for basic antibacterial properties (Magassouba et al., 2007; Pesewu et al., 2008; Shang et al., 2012). *Ficus sur, Heteromorpha trifoliate, Acacia nilotica*, *Agathosma betulinia, Thymus vulgaris* and even *Plectranthus madagascariensis* (Eldeen & Van Staden, 2007; McGaw et al., 2008; Moolla & Viljoen, 2008) are generally used for Tuberculosis, flu and bronchial disorders, while *Ocimum basilicum, Zingiber officinalis, Carum carvi, Rosmarinus officinalis, Cynamonum zeylanicum, Origanum vulgare* and *Pelargonium graveolens a*re used as flavorings and antimicrobial agents in foods and teas (Saxena et al., 2008; Hinneburg et al., 2006; Wojdylo et al., 2007).

Like many Salvia species which poses medicinal or aromatic traits. *S. chamelaeagnea* exhibits antioxidant activity due to the presence of the phenolic compounds carnosol, rosmarinic acid and caffeic acid (Kamatou et al., 2008; Kamatou et al., 2010; Nenadis et al., 2003). For the extraction of these phenolic compounds there are four methods generally used, notably; steam distillation, enzyme aided ensiling, near-critical extraction using CO_2 and the conventional organic solvent extraction process (Wang et al., 1998; Durling et al., 2007). While either method can be used it has been reported that in Salvia species the conventional organic solvent extraction method using ethanol is the most suitable for the essential oils trade. Due to the nature of carnosol, rosmarinic acid and caffeic acid in *S. chamelaeagnea* being highly influenced by the solvents polarity (Durling et al., 2007), and that it is acceptable for human consumption suggests that the conventional organic solvent extraction process is an acceptable method for the extraction of phenolic compounds found in *S. chamelaeagnea.*

5.7 Marketing potential

A large trend in the South African domestic plant trade is that of water-wise plantings. Due to the nature of the climate and regular water use regulations, water-wise plants are a favourable choice in landscape plantings in the home environment (van Kooten et al., 2008; Dehnen-Schmutz et al., 2010), in large landscape projects at shopping centres, in public areas and in land reclamation sites. *S. chamelaeagnea* is often a choice for such projects as it is a hardy species with much aesthetic value in a mass planting or amongst other plants.

The use of traditional medicines dates back centuries and some store bought medicines are still derived from the same plant based compounds. For example *Pelargonium sidoides* which has been used traditionally for cold related ailments, is now being used in a German companies medicine called Umckaloabo® which is made for the treatment of bronchitis in children (Lewu et al., 2007). While *S. chamelaeagnea* has not been used in any commercially available medicines it has value in the medicinal plant trade as it is used in traditional herbal teas to treat colds, colic and coughs among other ailments (Kamatou et al., 2008; Gali-Muhtasib et al., 2000; Cornara et al 2009), however it may be used in the near future against various microbial and bacterial infections and against some antibiotic resistant strains of which its research has yielded significant results (Kamatou et al., 2008).

5.8 Hydroponics

Since the 1940's crop farmers have successfully utilised hydroponic systems to produce the likes of tomatoes, beans and herbs, significantly bettering their growth and development (Harris, 1976; Mason, 1990, Sawas et al., 2007; Rouphael & Colla, 2005). While in the floriculture industry crops like Sunflowers, Roses, Chrysanthemums, Carnations and Gerberas have too been successfully cultivated hydroponically (Sawas & Gizas, 2002; Samartzidis et al., 2005: Karras et al., 2007). Livestock farmers have also utilised hydroponic systems for fodder production as less space is required and climatic conditions are easily maintained to promote growth all year long (Harris, 1976). Experiments on many spices herbs and medicinal plants have been successfully conducted using hydroponic systems rather than soil cultivation systems in order to eliminate possible erroneous results that may be brought about in soil cultivation environments due to many unmanageable variables (Sgherri et al., 2010; Maggini et al., 2012; Soudek et al., 2006).

The use of hydroponic systems could arguably be of great benefit the population and to the environment if managed correctly. Plants in the hydroponic systems are not in contact with the earth and as a result leaching of nutrients is diminished and crop diseases due to soil borne pathogens are eliminated (Gontier et al., 2002; Resh, 1995). Nutrients treatments and pH levels are easily regulated to suit the crops needs at particular growth stages, and as a result crop quality can be drastically improved thus having a direct effect on its market value, and water use, fertilizer costs and labour costs are considerably reduced compared to that of soil cultivation systems (Harris, 1976; Mason, 1990; Sgherri et al., 2010). The effective use of waste water as a nutrient solution in hydroponics systems described by Wallace et al., (1978) could further save on costs of fertilizers in some crops.

The increasing awareness of hydroponic cultivation has led to many different forms of hydroponic systems being created such as Deep Water Culture, Wick absorption, Ebb and Flow, Nutrient Film Technique, Top Feed and Aeroponics (Harris, 1976; Resh, 1995). The type of hydroponic system chosen for use is crucial and is suited to the crops physiological characteristics and its valued traits (Mason, 1990).

Hydroponic media selection can greatly affect the root zone development of a plant. A number of substrates are commercially available such as; perlite, vermiculite, coarse sand, expanded clay, rock wool, coco coir or gravel. Either of which can be combined or used alone to support the plant root zone and to provide sufficient water retention, effective drainage and plenty of oxygen (Harris, 1976; Resh, 1995). While there are a number of commercially available hydroponic media available one is not limited to using just them. Some studies have utilised less conventional media like sawdust (Bowen, 1983).

Choice of hydroponic system is a crucial factor and is based on the crops physiology, for example swelling root tubers of potatoes needs deep substrate in which to develop, and the trailing growth habit of butternut will need lots of space to spread out and may need netting to keep the fruits suspended off of the media to prevent rotting or infection. Thus the hydroponic system needs to be adapted to accommodate and encourage the development of the valued crops attributes (Resh, 1995; Correa et al., 2008; Samartzidis et al., 2005). The benefits of hydroponic cultivation on plant harvests are in most cases an improvement in the quality and quantity of oil yields, dry matter yields, fresh weight, leaf and flower colour and scent as a result of the system being tailor made to suit the crops needs (Ashraf et al., 2009; Bowen, 1983; Gontier et al., 2002; Fan et al., 2009; Nxawe et al., 2011; Sgherri et al., 2010).

Growing *S. chamelaeagnea* in hydroponics could be of benefit to both the medicinal plant and domestic plant industries as the plants have grown to the best of their abilities and are healthy and well developed before being sent to the respective markets.

5.9 PRODUCTION SCHEDULE

5.9.1 Weeks 1-3 - Rooting of Cuttings

The propagation of *S. chamelaeagnea* by method of tip cuttings is favoured over that of propagation by seeds as cuttings remain true to type, unlike that of cultivation by seed, where there is a likelihood of genetic variation brought about by cross pollination (Stern, 2006). In selecting mother stock from which to take cuttings from one must insure that the mother stock is healthy and does not suffer from any diseases, pests, deficiencies or environmental stresses that may hinder the development of the cuttings (Hartmann & Kester, 2002). Tip cuttings should be propagated in plug trays with a medium of 50% coarse sand and 50% perlite with a pH level of 6 to promote root growth (Loach, 1985), using Seradix 2, a semi hardwood rooting hormone (Hartmann & Kester, 2002; Clebsch, 2003). Tip cuttings should be irrigated with a fine mist every 30 minutes for a duration of 2 minutes at a time. At weeks 2 and 3 a light foliar application of CHEMICULT ®, a balanced fertilizer, should be given to the cuttings once a week (Marschner, 1995). In week 3 cuttings should receive a light watering 4 times daily for a duration of 10 minutes at a time. In following the correct measures during the rooting stage, 80% or higher rooting percentages are attainable.

5.9.2 Weeks 4-5 - Juvenile plant growth

Juvenile plants are relatively fragile by nature as they do not have many roots or leaves, as a result stresses can have a significant effect on their development. For this reason care should be taken so as to not damage or impede any development of the plants in this crucial stage of growth. Well rooted cuttings should be gently rinsed in deionised water to remove the rooting media, and then should be transplanted into 25 cm plastic pots filled with moistened Leca clay and placed into the closed hydroponic system at a spacing of 30 cm apart (See Chapter 3). Apical buds should be pinched using sterilized secateurs to prompt a bushy form to develop by week 5 (Hartmann & Kester, 2002) (See Table 5.1). Juvenile plants should be irrigated at intervals of 8 times daily for a duration of 15 minutes with a modified Hoagland solution (Hershey, 1994; Hershey, 1995), with phosphorus (P) increased to 90 ppm and a pH level of 4 (See Chapter 3). It is important to closely monitor the juvenile plants to ensure sufficient irrigation while the plants continue to develop. As a result of the treatments and pinching of the apical buds an increase in the branch numbers at the end of week 5 should be noticeable (See Table 3.2).

5.9.3 Weeks 6-8 - Developing plant growth

The growth requirements of developing plants differs considerably from those of cuttings. As the biomass of developing plants increases constantly, the irrigation frequency to should be increased to meet the crops growth requirements. Developing plants in the closed hydroponic system should be irrigated at intervals of 15 times daily for a duration of 15 minutes with a modified Hoagland solution (Hershey, 1994; Hershey, 1995), with P increased to 90 ppm and a pH level of 4 (Garcia-Navarro et al., 2011) (See Chapter 3). Developing plants at this stage become susceptible to pests and diseases, therefore an organic pesticide should be applied to control pests in week 7 (Gerber, 2007). Over time a salt build up on top of the Leca clay may occur. To remedy this with out diluting the nutrient solution, pour some of the nutrient solution onto the top of the Leca clay so that the salts can be flushed back into the hydroponic system (Hartmann & Kester, 2002; Resh, 1995). As a result of increasing the irrigation frequency, branch numbers and leaf colour at the end of week 8 are expected to yield improved results (See Table 3.2 and Table 4.2).

5.9.4 Weeks 9-10 - Maturing plant growth

In the maturing plant growth phase the development of plant roots and shoots increases dramatically. Maturing plants should be irrigated at intervals of 15 times daily for a duration of 15 minutes with a modified Hoagland solution (Hershey, 1994; Hershey, 1995), with P increased to 90 ppm and a pH level of 4 (Garcia-Navarro et al., 2011) (See Chapter 3). An organic pesticide should again be applied to control pests in week 9. An increase in plant height may call for the plants to be staked so as to attain a desired growth form and prevent any damages (Don, 2003). In following these practices throughout the growth period, it can be expected that by the end of week 10 plant height, branch numbers and leaf colour will yield favourable results (See Table 3.1, Table 3.2 and Table 4.2).

5.9.5 Week 11 - Mature plant growth

The mature plant growth phase is a crucial point in the production of *S. chamelaeagnea,* as this is when the plant is close to being a marketable product or close to flowering, and as a result care should be taken to not cause any irreparable damages that may diminish the plants marketability or hinder future development. For research purposes mature plants should be irrigated at intervals of 15 times daily for a duration of 15 minutes with a modified Hoagland solution (Hershey, 1994; Hershey, 1995), with P increased to 90 ppm and a pH level of 4 (Garcia-Navarro et al., 2011) (See Chapter 3). For use in the landscape and nursery industries hardening off of plants through a decrease in irrigation and increased temperatures should be carried out in week 11 so as to attain a hardy and marketable plant. As a result of the treatments, plant height, branch numbers, root length, wet shoot weight, dry shoot weight, wet root weight, chlorophyll A, chlorophyll B, and total chlorophyll values in week 11 are all expected to yield desirable results (See Chapter 3 and Chapter 4). At the end of the 11th week the plants are expected to be ready for harvesting. However, if a flowering plant is desired, an extended growth period and a change in nutrient ratios will be need so as to prompt the formation of floral buds (Follett et al., 1981; Stern, 2006).

5.10 DISCUSSION

In this study the effects of the pH and phosphorus treatments had a considerable influence on the growth and development of *S. chamelaeagnea* grown in hydroponics (See Table 5.1). During the rooting process of *S. chamelaeagnea* mortality rates were low. The successful formation of calluses and root growth in *S. chamelaeagnea* may be attributed to the pH of the rooting media at pH 6 not being very acidic or alkaline and thereby promoting root development opposed to hindering it (Hartmann & Kester, 2002; Loach, 1985).

Well rooted tip cuttings were placed into the hydroponic system and treatment applications began at a frequency of 8 times daily for a period of 15 minutes at a time to limit any chances of root rot due to over watering (Fare et al., 1992). Towards the end of the juvenile stage the applications of pH 4 treatments began to take effect, producing higher branch numbers when compared to the control (See Chapter 3), this development at a pH level of 4 was likely due to the high concentration of hydrogen ions at a low pH level having a significant effect on the ionic form of phosphates and the absorption there-of. As the pH level of a media becomes more alkaline the ionic form of phosphates changes from the absorbable univalent (H_2PO_4) to the less absorbable bivalent (HPO_4) and trivalent (PO_4) (Sutcliffe, 1962). This is in agreement with Honert, (1933) in which he studied the effect of pH and phosphate uptake on sugar cane and found that only univalent phosphate ions are absorbed in plants. This study supports the findings by Arnon et al., (1942) that at alkaline pH levels there was an inability to absorb sufficient phosphates.

Developing plants have higher photosynthetic and transpiration rates than that of cuttings, and therefore have an increased demand for water and nutrients (Marschner, 1995). For this reason, the irrigation frequency from the developing plant stage onward was increased to 15 times daily for 15 minutes at a time (Garcia-Navarro et al., 2011). As a result of increased irrigation in the developing and maturing stages, in improved heights, branch numbers and leaf colour were noticeable (See Chapters 3 and 4). However, overcrowding was becoming apparent. Should the experiment have carried on for longer than it did, plant spacing should be revised in order to limit the competition for light, spread of pests and chance of fungal attacks (Al-Ramamneh, 2009; Khazaie et al., 2008).

Plant development of height, branch numbers, root length, chlorophyll content, leaf colour and shoot weights in the pH 4 and 90 ppm treatment all yielded considerably higher results than that of the control in the mature plant stage (See Chapters 3 and 4). This is likely due to the increased absorption of P at a low pH level having a encouraging effect on P's fundamental roles in the composition of nucleic acids, coenzymes and phospholipids. In addition, the nutrient availability of N, Mn, Cu, Zn and B responsible for enzyme activity, starch formation, respiration and electron transport are readily available at this low pH level (Sutcliffe, 1962; Zhao et al., 2013). Similar results were recorded by Hoagland and Davis, (1923) whereby it was recorded that the absorption of nitrate and chloride was more rapidly absorbed into *Nitella* cells at an acidic pH level. Similarly, Hornet and Hooymans, (1955) found that nitrate uptake decreased as pH value increased. While the results recorded in this experiment will be of benefit to the cultivation of *S. chamelaeagnea,* little is known of the influences of the pH level 4 at 90 ppm P on the flower formation and the development there-of. Future studies should focus on a longer growth period, day length and temperature regulation in order to observe the effects of pH and supplementary P on the further development and flowering stages of *S. chamelaeagnea* (Booij & Meurs, 1995; Porat et al., 1995; Welander, 1984).

The use of a closed hydroponic system in this study proved to be very effective in that the treatments did not take long to produce results. Although the absorption of P has been successful in hydroponic culture, it may have some negative implications in the landscape and nursery industries (Sutcliffe, 1962). The use of Leca clay, while ideal for providing sufficient oxygen to the roots in hydroponics, will not be suitable for use in domestic and landscape plantings due to its initial costs and low water holding capacity due to its porosity. Future studies on other media, such as; coco, perlite and coarse sand among others, should be carried out so as to identify a medium that is suitable for the commercial production of *S. chamelaeagnea* (Bradley & Marulanda, 2000; Parks et al., 2009; Jones, 2005).

5.11 CONCLUSION

In conclusion, this paper provides insight into the cultivation requirements of *S. chamelaeagnea* and indicates that a pH level of 4 and a phosphorus concentration of 90 ppm significantly effect the development, yields and marketability of *S. chamelaeagnea,* cultivated for its applications in the medicinal and domestic plant trades in South Africa. In addition this study may aid in reducing costs of labour and fertilizers while saving water and reducing the risk of nutrient wastes leeching into neighbouring soils.

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5.14 TABLE

Table 5.1: A proposed hydroponic production schedule for the commercial cultivation of *Salvia chamelaeagnea* grown at 16 - 28 °C in a controlled green house.

Week	Development stages	Actions	Media	pH	Irrigation	Treatments	Growth Measurements
1-3	Cuttings	-Propagate cuttings in plug trays. - Monitor and feed.	50 % coarse sand and 50 % perlite.	6.0	-Misting every 30 minutes for 2 minutes. - Light watering 4 times daily for 10minutes in week 3.	Seradix 2 rooting hormone, Light foliar nutrient application of CHEMICULT® once a week	Low mortality rate.
4-5	Juvenile plant	-Transplant rooted cuttings at a spacing of 30cm. - Pinch apical buds.	Leca clay	4.0	8 times daily for 15 minutes	Hoagland solution supplemented with 90 ppm P	Noticeable increase in branch numbers
6-8	Developing Plant	Organic pesticide.	Leca clay	4.0	15 times daily for 15 minutes	Hoagland solution supplemented with 90 ppm P	Leaf colour and branch numbers yielded higher results when compared to the control.
9-10	Maturing plant	Organic pesticide	Leca clay	4.0	15 times daily for 15 minutes	Hoagland solution supplemented with 90 ppm P	Height, leaf colour and branch numbers produced significantly higher results than the control.
11	Mature plant	Plants ready for harvesting	Leca clay	4.0	15 times daily for 15 minutes	Hoagland solution supplemented with 90 ppm P	Height, branch numbers, root length, wet shoot weight, dry shoot weight , wet root weight, chlorophyll A, chlorophyll B, and total chlorophyll produced significantly higher results that the control.

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSION

6.1 GENERAL DISCUSSION

The abundance of research pertaining to cultivation practices, pH, mineral nutrition, phosphorus, salt stress on increasing crop yields express that nutrient composition and mineral nutrient availability of a medium are crucial factors in the crop production process, and stresses that if these fundamental factors were not to be addressed may lead to a decrease in growth rates, yields, quality and marketability of a crop.

The pH level of a media or nutrient solution is an indication of its acidity or alkalinity, on a scale of 0 to 14, 0 being very acidic and 14 very alkaline. At these differing pH levels nutrient availability is affected. For example, at a pH level of 5 and below mineral nutrients such as Mn, Fe and Zn are abundantly available while Mg, K and Ca are in short supply. Adversely, at pH levels of 8 and above Ca, K and Mg are more readily available while Mn and Fe become less available.

Phosphorous (P) and its use in cultivating plants is an essential part of the growth and development of crop plants due to its many roles in, respiration, formation of seeds, deoxyribonucleic acid (DNA), ribonucleic acid (RNA) and adenosine tri-phosphate (ATP) synthesis and in energy transfer from light energy to useable chemical energy and the storage thereof. P has also been documented to play a large role in ameliorating crop productivity under conditions of low soil fertility. P deficiency symptoms in plants have a negative impact on growth and development and is characterised by stunted growth, a build up of sucrose and starch, purpling of the lower leaves, reduced seed development and a decrease in photosynthesis.

This series of investigations evaluated the effects of three combinations of pH levels and four phosphorus concentrations on the number of branches, heights, root lengths, wet and dry root and shoot weights, leaf colour, chlorophyll a, chlorophyll b, total chlorophyll and the nutrient up take of hydroponically grown *S. chamelaeagnea*, in attempts to identify an efficient cultivation method for its commercial production, and as a result reduce wastes and damages to the environment.

In this study the effects of pH regulation and phosphorus concentrations on the growth parameters has shown that *S. chamelaeagnea* performs well at a low pH level with a moderate concentration of available P. Greater plant heights, branch numbers, root lengths and shoot weights were all recorded in pH 4 treatments receiving either 90 ppm or 180 ppm of supplementary phosphorus. This shows that the micro nutrient availability, of Mn, Cu, Zn and B, which are responsible for enzyme activity, starch formation, respiration and electron transport, at a low pH in conjunction with a moderate amount of supplementary phosphorus, has a positive effect on the plants utilization of available mineral nutrients and thus its productivity. This is in agreement with Khan et al., (2000), Naeem and Khan, (2009) and in which P has been documented to have positive effects on the growth and development of plants in poor soils.

The chlorophyll responses studied in this experiment showed that P applications had a minimal effect while pH treatments had a significant effect. Higher chlorophyll and leaf colour values were recorded in the treatments receiving a pH of 4 with no supplementary P followed by 90 ppm P and 210 ppm P. However, nutrient uptake values were higher in plants receiving a nutrient solution with a pH 8. Despite these high nutrient uptake values in plants receiving a nutrient solution with a pH of 8, chlorosis of their leaves was apparent throughout the growth period. This suggests that the uptake of some essential nutrients responsible for chlorophyll development, photosynthesis, respiration, cell division and protein formation namely; Cu, B, N and Fe were insufficient at this high pH level. High leaf colour, chlorophyll a, chlorophyll b and total chlorophyll values recorded in treatments offering a pH level of 4 with little to no supplementary P, are in agreement with Lafarga-De la Cruz., et al (2006), Minotta and Pinzauti, (1996) and Eriksen and Iversen, (1995), in which improved chlorophyll responses were documented in soils with a lower availability of nutrients. This suggests that supplied nutrient solution that is acidic and low in available N, K and Ca will largely increase chlorophyll production of *S. chamelaeagnea* grown in hydroponics. Interestingly these conditions mimic that of the fynbos biome soils, in which *S. chamelaeagnea* naturally occurs.

The use of a closed hydroponic system in this study proved to be very effective for research purposes, in that the treatments did not take long to produce noticeable results. Development of plant heights, branch numbers, root lengths, chlorophyll contents, leaf colours and shoot weights in the pH 4 at 90 ppm P treatment all yielded much higher results than that of the control in the mature plant stage. However, overcrowding of the plants in the later weeks showed that a plant spacing of 30 cm was insufficient, and so should the experiment have carried on for longer plant spacing would need to be increased to limit the competition for light, spread of pests and chance of fungal attacks. In addition, future studies should focus on a longer growth period, day length and temperature regulation in order to promote the flowering stages of *S. chamelaeagnea* grown in hydroponics. Though this particular hydroponic system proved to be effective for research purposes it may have some negative real world implications. For example, Leca clay is ideal for providing sufficient oxygen to the roots in hydroponics, however it will not be suitable for use in domestic and landscape plantings due to its prohibitive cost implications. Therefore future studies should explore the possibilities of using another hydroponic media which will be more favourable for transplanting into soils after being cultivated in hydroponics.

6.2 CONCLUSION

In conclusion these results give us insight into the unknown growth requirements of *S. chamelaeagnea* produced for the medicinal and landscape trades, and illustrates that a nutrient solution with a pH level of 4 and a moderate concentration of supplementary phosphorus can significantly improve the growth, development and marketability of *S. chamelaeagnea.* Furthering studies into the extended day length, companion planting of nitrogen fixing plants and inoculation of arbuscular mycorrhiza in *S. chamelaeagnea* cultivation programs would be beneficial so as to further reduce the effects of large scale cultivation systems on the environment.

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CHAPTER SEVEN

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7. REFERENCES

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