A COMPARARTIVE STUDY OF OXYGENATION TECHNIQUES IN THE HYDROPONIC CULTIVATION OF *PELARGONIUM TOMENTOSUM.*

By

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Thesis submitted in fulfilment of the requirements for the degree

Master of Horticultural Science

In the Faculty of Applied Sciences

At the Cape Peninsula University of Technology

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Cape Town

December 2016

DECLARATION

I, Joshua David Butcher, declare that the contents of this thesis represent my own unaided work, and that the thesis has not been 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.

11/12/2016

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ABSTRACT

This aim of this study was to investigate the viability of growing *P. tomentosum* in deep water culture (DWC) hydroponics and to assess the effects of various methods of oxygenating the nutrient solution with regards to growth, development and chlorophyll responses. The experiment was conducted over a period of 74 days. In chapter 3, 16 different methods of oxygenation were applied to 9 replicates. The control had passive aeration. The treatments were made up of air-pumps, vortex oxygenators and the application of hydrogen peroxide (H_2O_2) at various frequency intervals; these were combined with each other and run as separate oxygenation methods.

In chapter 3 it was found that the highest chlorophyll content mean value (62.16 mg/$^{m-2}$) was achieved through a combination of high frequency application of H_2O_2, an air-pump and vortex oxygenation (APVHa). This treatment differed significantly ($P \leq 0.001$) from the control and the other treatments. Although high frequency dosage of H_2O_2 alone (Ha) had the highest level of DO (17.96 mg/l), the mean chlorophyll content value was slightly lower than when H_2O_2 was combined with vortex oxygenation and air-pump injection (in treatment 11/APVHa) and had a larger variance. These results indicate that to produce more photosynthetically active *Pelargonium tomentosum* plants in deep water culture hydroponics it is advisable that application of high frequency dosing of H_2O_2 be combined with other methods of oxygenation.

In chapters 4 and 5, 4 treatments were replicated 9 times. The experiment discussed in chapter 4 compared the application of H_2O_2 alone (used as the control) with treatments which combined H_2O_2 with; an air-pump, a vortex oxygenator, and a combination of both these devices. In this chapter it was found that the control (Ha) treatment yielded the highest readings of fresh shoot and root weights (71.1 g and 28.23 g respectively), as well as the greatest dry shoot weight (10.29 g) although not statistically different form treatment 1 (APVHa) in any of these comparisons. Treatment 1 (APVHa) yielded the highest readings for

dry root weights (2.14 g) suggesting that root development was benefited by the combination of H_2O_2 with both vortex oxygenation and an air-pump. For growers to increase the dry weight percentage of shoots and roots of *Pelargonium tomentosum* plants in DWC hydroponics it is advisable that application of H_2O_2 is combined with vortex oxygenation and air pumps.

In chapter 5 no treatments received H_2O_2; the control had passive aeration, the treatments consisted of air-pump injection, vortex oxygenation and a combination of these two methods of oxygen inclusion. In this chapter it was found that although the roots of the control (treatment 1) plants appeared to be less healthy than those in the actively aerated treatments (2/V, 3/AP & 4/APV) the shoots were not negatively affected by sub-optimal levels of DO within the experimental period. Plant height and leaf count were increased by aerating the solution with an air-pump connected to 'air-stone' dispersers in treatment 3 (AP). A slight increase of dry root weight percentage was achieved through the application of vortex oxygenation, treatment 2 (V). Although the above mentioned growth parameters were positively influenced by the respective oxygenation practices, these did not differ significantly than those within the control. These results indicate that production of *P. tomentosum* plants in deep water culture hydroponics is possible over the length of time of this experiment without additional aeration if the surface of the nutrient solution comes into sufficient contact with the atmosphere. Increased plant height (3.2%) and number of leaves (12%) can be achieved with the application of air pumps.

This study has found that *P. tomentosum* is a suitable plant for DWC hydroponic production, and that oxygenation/aeration of the nutrient solution affected the growth responses of *P. tomentosum*. The beneficial effects of oxygenation on root development were observed, shoot growth was also increased in treatments with higher levels of DO. When using H_2O_2 to oxygenate the nutrient solution, the development of roots, and the chlorophyll content is increased when combined with other methods of oxygenation.

ACKNOWLEDGEMENTS

I wish to thank:

- My mother, Susan Butcher, who provided me with emotional and financial support.
- My girlfriend, Amber Konstant, who's support, understanding and assistance was vitally important in the completion of this thesis.
- My supervisor, Prof. Charles Laubscher, for his willingness to assist and the guidance he provided toward the successful completion of this thesis.
- My co-supervisor, Prof. Johan Coetzee for his input and expertise.
- The Cape Peninsula University of Technology for financial support through the Mauerberger Foundation and the University Research Fund – without which these studies would not have been possible.
- The technicians of the Horticultural Sciences department, Deborah Erasmus and Timothy Jasson, for their assistance in the procurement of materials and logistical support.
- Members of the Cape Horticultural society for providing plant material for the experiment.

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CHAPTER 1

RESEARCH PROBLEM, AIMS, HYPOTHESIS AND OBJECTIVES

RESEARCH PROBLEM, AIMS, HYPOTHESIS AND OBJECTIVES

1.1 Research Problem

South Africa has a vast amount of indigenous flora, many of these species have high medicinal potential and useful metabolites. *Pelargonium* species are grown for ornamental, cosmetic, therapeutic and medicinal uses. *Pelargonium tomentosum* Jacq. is an herbaceous groundcover which produces a peppermint-like aroma and is known as the peppermint-scented pelargonium. Essential oils produced by *P. tomentosum* are used in the fragrance industry.

No studies investigating the viability of producing *P. tomentosum* in deep water culture (DWC) hydroponics have been published. Oxygen plays a vital role in plant metabolic processes. Plants grown in DWC have been shown to benefit from aeration/ oxygenation and recirculation of the nutrient solution. As DWC is one of the simplest and affordable kinds of hydroponic systems to install and maintain, this method could be utilized by farmers of limited financial and technical means. In order to make recommendations regarding the cultural practices involved in the production of *P. tomentosum* in DWC, various techniques of oxygenation have been compared.

1.2 Aims

This study aims to observe the effects on growth responses of *P. tomentosum* to different methods of oxygenation in DWC hydroponics. By comparing passive aeration, air-pump injection, vortex oxygenation, application of hydrogen peroxide at various frequency intervals; and combining these methods - the most effective way of oxygenating the nutrient solution to produce *P. tomentosum* in DWC may be established.

1.3 Hypothesis

The growth and development of *P. tomentosum* will be influenced by the method of aeration/ oxygenation in DWC.

1.4 Objectives of the research

1.4.1 Main objective

To investigate the effects of different methods of including oxygen into the hydroponic solution on the growth responses of *P. tomentosum* in deep water culture (DWC).

1.4.2 Specific objectives

1) To assess the effects of different oxygen inclusion techniques and the related level of dissolved oxygen on both the wet and dry weight of shoots and roots of *P. tomentosum*.

2) To measure the effects of different oxygen inclusion techniques and the related level of dissolved oxygen on the number of leaves of *P. tomentosum.*

3) To measure the effects of different oxygen inclusion techniques and the related level of dissolved oxygen on the length of shoots and roots of *P. tomentosum.*

4) To measure the effects of different oxygen inclusion techniques and the related level of dissolved oxygen on the level of chlorophyll production in the shoots of *P. tomentosum.*

CHAPTER 2

LITERATURE REVIEW

CHAPTER 2: LITERATURE REVIEW

2.1 Abstract

South Africa has a vast amount of indigenous flora, many of these species produce usable metabolites (Van Wyk, 2008). Although the country makes up less than 1% of the land surface of the earth, as much as 10% of the global population of flowering plants originate from here (Manning, 2009). *Pelargonium* species occur here naturally and are grown for ornamental, cosmetic, therapeutic and medicinal uses (Demarne & Van der Walt, 1989; Lawrence, 2002; Manning, 2009). *Pelargonium tomentosum* Jacq. is an herbaceous groundcover which produces a peppermint-like aroma and is known as the peppermint-scented pelargonium (Lawrence, 2002). The main constituents of the essential oil forming metabolites are menthone and isomenthone, these metabolites have both industrial and cosmetic potential. Considering the wealth of flora found in South Africa it is imperative that further research is conducted on indigenous plants to establish improvements in their cultivation.

Water scarcity and the lack of access to arable land has prompted the advent of horticultural technologies which are both conservative of the environment and have more efficient productivity than conventional field production. Cultivating plants in the protected environment provided by structures such as greenhouses and polytunnels has enabled growers to produce crops that would not grow in certain localities or in seasons which would make open field production unsuccessful. Although elements of hydroponic production have been utilized for potentially thousands of years in certain parts of the world, scientific research into this field began in earnest in the mid-1800s (Venter, 2010). For a long time, development of hydroponics was inhibited due to the large capital requirements of construction and installation of infrastructure, however access to affordable materials has made this type of plant production more feasible over the few decades preceding this study (Jensen, 1999).

Only a few studies on *P. tomentosum* have been published, none of which investigate the viability of hydroponic culture (Demarne & Van der Walt, 1989). Hydroponics is exponentially more productive than conventional field production; although the requirement for capital investment is significant when constructing such systems, the productivity in terms of time, space and resource efficiency makes hydroponics an attractive option (Jensen, 1999; Schröder & Lieth, 2002). Deep water culture (also referred to as a floating-raft system) is one of the simplest and cheapest methods of hydroponic production (Zeroni, 1983). Deep water culture (DWC) hydroponics is one of the simplest to construct and maintain. DWC is one of the more affordable hydroponic growing systems to install. Roots of plants are suspended directly in the nutrient solution, the large bulk body of water acts as an environmental buffer. As a result fluctuations in temperature, EC and pH are less dramatic than many other applications of hydroponic culture (Harris, 1992). Ease of installation, affordability and low energy input requirements make DWC a suitable method of plant production for semi-skilled rural farmers.

Plants in grown in DWC have shown positive growth responses when the nutrient solution has been recirculated and aeration practices employed (Zeroni *et al.*, 1983; Hu *et al.*, 2008). Oxygen is an essential requirement for plant life (Zheng, 2007), this element plays a critical role in the respiration process. Plants use oxygen and carbon dioxide to form carbohydrates; an absence of O_2 leads to impaired growth and the inability of roots to take in water and nutrients (Urrestarazu & Manzuela, 2005). Anaerobic respiration occurs when there is an insufficient level of available oxygen. When plants receive sufficient levels of oxygen, growth increases and so does general productivity. By comparing passive aeration with air-pump injection, vortex oxygenation, application of hydrogen peroxide at various frequency intervals; and combinations thereof - it is the aim of this study to establish the most effective method of oxygenation for the production of *P. tomentosum* in DWC. Findings of this work can be used to make recommendations for commercial producers and efficiency of production can be improved.

The use of hydroponic cultivation is highly relevant in areas where arable land and clean water is scarce, hydroponic culture offers growers an environmentally conservative choice to field production.

2.2 *Pelargonium tomentosum* Jacquin*;* peppermint-scented pelargonium.

The family Geraniaceae is comprised of five genera; *Pelargonium, Monsonia, Geranium, Erodium* and *Sarcocaulon.* There are approximately 220 species of *Pelargonium,* [although estimates have been as high as 300 (Meyers, 2006)]. Most of these species are indigenous to southern Africa. *P. tomentosum* occurs naturally in sandstone derived soils, in semi-shade. The distribution of this species is concentrated within the region of the Hottentots Holland and Langeberg in the Western Cape. *P. tomentosum* can be found occurring prolifically near Swellendam (Lawrence, 2002).

P. tomentosum is a sub-shrub or groundcover growing to a height of 0.5 m with lateral growth reaching 1.5 m. The shoots are herbaceous and become semi-woody as they mature. The leaves are palmatilobate to palmatipartite, with undulating margins, the petioles are clearly present (Demarne & Van der Walt, 1990; Lawrence, 2002). Fine hairs or trichomes cover the shoots, particularly the leaves. The inflorescence is a small pseudo-umbel containing white-petalled flowers with dark mauve nectar guides, this appears from October to January (southern hemisphere spring to mid-summer). The inflorescence protrudes above the foliage. Like other *Pelargonium* species; the posterior pair of petals is auricled and notably wider than the three anterior petals. Seven fertile stamens are usually found on each flower however, sterile male specimens occur often in nature (Demarne & Van der Walt, 1990). *P. tomentosum* produces a characteristic peppermint-like scent; the oil secreted by glandular hairs has therapeutic properties and cosmetic potential. It is important that techniques utilized in the production of this plant are studied in order to make recommendations to growers within the industry.

2.3 Water

Water covers a large part of the surface of the earth and has been detected in much of the investigated cosmos. Its exceptional molecular properties resulting from the combination of two out of the three of the most common elements in the universe has made it ubiquitous in the occurrence of organic processes and therefore; an essential component of life itself (Mottl *et al.*, 2007). The exemplary characteristics and peculiar behaviour observed in water have long been of interest and studied (Gikas & Angelakis, 2009), however there is still much yet to be established through further investigation into this field (Pollack, 2013).

The molecular structure of the water molecule dictates behaviour and interactions with other compounds. Water is a hydride of oxygen - each molecule is comprised of one oxygen atom and two atoms of hydrogen. When compared with other hydrides such as ammonia, hydrogen sulphide and methane, water is remarkable in that it remains in a fluid phase at room temperature and normal atmospheric pressure while others mentioned would volatize in these conditions. Water also exhibits distinct characteristics when in a solid phase (ice) in that it becomes less dense than the liquid form (Helmenstine, 2014).

Hydrogen bonds are by no means exclusively found in water; however, H_2O's characteristic molecular structure can facilitate the formation of four such bonds in any instance per molecule, it has 2 hydrogen-bond (HB) donor sites (the pair of protons) and 2 HB acceptor sites (the two electronic lone pairs of its oxygen atom). The possibility of the number of H-bonds being so similar to the number of covalent bonds forms the basis of the unique characteristics presented by this substance (Marechal, 2004). Water's density ranges from a maximum of 1.1-1.2 g/cm^3 to a minimum of 0.9-0.95 g/cm^3, this is influenced by atmospheric pressure and temperature, the number of resultant H-bonds correlates with these variables accordingly. At normal atmospheric pressure (water density = 1 g/cm^3) and temperature of 25 ^{o}C the 4 HB state is the most prevalent, the number of molecules with 4 hydrogen bonds outnumbers those with 2 or 3 and the less frequent 1 and 5 bonded molecules (Caffarena & Grigera, 2004). Simply put, in these conditions the structure of water is tetrahedral i.e. each molecule is joined to four adjacent molecules on average. “Quadruple bonding” is the mean observed over an experimental period, the hydrogen bond network in liquid water is dynamic – constantly creating and dissipating bonds within 100 – 200 femtoseconds (Anonymous, 2013). By investigating the behaviour of water as a substance, it may be possible to determine how best to manage irrigation schemes and hydroponic systems to ameliorate issues faced by growers worldwide.

2.3.1 Water, irrigation, and the evolution of agriculture

Water is synonymous with the evolution of life on this planet, a fulcrum for human development and thus of civilization. As ancient cultures began developing the means to grow their own food, steering away from the hunter-gatherer lifestyle and towards agriculture; a wide range of techniques were developed for managing and moving water to suit specific needs. From the early to middle Bronze Age or the Minoan era 1100 - 3000 BC (and quite possibly before) there is evidence of crop production in the Mediterranean, the Middle-East, India, China, as well as Central and South America (Tankersly *et al.*, 2011; Hadas, 2012; Riehl, 2012). The earliest known irrigation systems have been found between the Tigris and the Euphrates River in ancient Mesopotamia, these are said to have been developed by the Sumerians from the 5th millennium BC (de Châtel, 2014). At the time of this study irrigation technology is being applied worldwide in forms ranging from passive sub-feed, to drip, to high pressure mist and fogging systems - the development of sprayer nozzles and emitters has been significant within the last century. However, in the developing world it is often the simplest, ‘low-tech’ solutions which are of the greatest benefit - to rural farmers particularly. To determine the efficacy of alternative methods of plant production and water management principles it is essential that comparative studies between alternative and conventional methods be conducted.

2.4 Hydroponics

The evolution of pumps has allowed for what could arguably be called the ultimate expression of irrigation, and that is, hydroponics. The term “Hydroponics” [the combination of the Greek words *hydros* (water) and *ponos* (labour/work)] was initially conferred to this system of plant cultivation by Prof. W.F. Gericke in the 1930's (Harris, 1992). The hydroponic method involves delivering nutrients to plants via an aqueous solution, using a sterile substrate for structural support as well as aeration in some cases (Harris, 1992; Venter, 2010). Some of the earliest expressions of hydroponics include the hanging gardens of Babylon (approximately 600 BC). Other early examples are traditional Chinese rice cultivation and riverside farming along the Nile in Egypt (Venter, 2010). In the 21st century hydroponics makes up a large portion of the global production of certain crops, a variety of applications have been defined - each with unique characteristics which cater to a diversity of crops and conditions. Although there are distinct methods of growing hydroponically; it is most common for systems to be hybridized combinations of various techniques, means of support, and the delivery and removal of nutrient solution. In the west, development of this field began about 500 years ago; early experimentation on the effects that different types of water and impurities had on plant growth was carried out by researchers such as Robert Boyle and John Woodward (Venter, 2010). By 1851 experiments were being carried out growing plants in silica, charcoal and other sterile substrates, researchers began identifying individual elements and compounds needed by plants and in which quantities. Many kinds of growing media have been tested and can now be used to grow plants in hydroponics. These range from saw dust to charcoal, gravel, rock-wool and coconut fibre. The development of processed minerals such as LECA® (lightweight expanded clay aggregate), perlite and vermiculite has broadened the application of hydroponic culture (Carmen, 2007; Boudaghpour & Hashemi, 2008; Gibeaut *et al.*, 1997; Harris, 1992). Techniques of supplying water differ significantly; from passive flow in deep water culture (DWC) to high pressure mist delivery in aeroponic systems. The evolution of hydroponics has been greatly influenced by the introduction of plastic – making cheap structural and glazing material for greenhouses, as well as the components of the growing systems themselves (Jensen, 1999). Considering the history and development of hydroponics informs and clarifies the intent of this study, the fundamental knowledge of plant requirements which has been formulated through research in hydroponics sets up a platform to investigate additional aspects of plant production.

2.4.1 Deep water culture

Hydroponics can be categorized generally into two types; water culture and soilless or aggregate culture. DWC was one of the first methods tested and regarded by some as being the only 'true' form hydroponics, the roots of plants grow directly into the nutrient solution (Harris, 1992). Large volumes of water are held in tanks or furrows, the bulk water acts as a buffer to fluctuations in EC and pH making DWC systems somewhat simpler to manage than other systems. Polystyrene sheets are sometimes used as floating 'rafts' which support plants as they extend their roots into the nutrient solution, the meeting point between the shoot and root systems is often held in a net-pot inserted into the polystyrene sheet (Tyson *et al.*, 1999). DWC can either be run as a passive non-circulating system (Kratky, 2010) or actively recirculated; aeration and distribution of nutrients is encouraged by recirculating the solution (Hu *et al.*, 2008; Zeroni *et al.*, 1983). The addition or inclusion of oxygen into the root zone of plants has shown to increase metabolic processes and growth (Chen *et al*, 2011; Morimoto *et al.*, 1989), this is especially notable with consideration to DWC as the bulk water has a proportionately small surface area and therefore less potential area for gaseous exchange within the atmosphere to take place (Ben-Yaakov & Ben-Asher, 1982). DWC is a pragmatic, affordable hydroponic choice for growers; although a large volume of water is used to fill the reservoir; the water can be circulated for considerable lengths of time if the ability of the technician/ grower allows for the accurate identification of nutrient deficiency exhibited in the crop and steps are taken to ameliorate these symptoms. If managed correctly DWC can be a water conserving method of hydroponic production.

2.5 Oxygen

Oxygen is the third most abundant element in the known universe, it has a high reactivity and can combine with most other elements; it makes up 21% of the earth's atmosphere. Oxygen's atomic number is 8, the atomic weight 15.9994 (Anonymous, 2015c). There are eight electrons, two inner on an inner "orbit" – six on the outer; this results in the outer shell of the atoms being 'incomplete' and hence highly reactive (Pappas, 2014). The name is formed from the greek *oxys* and *genes* which means "acid-forming" and was initially conferred by Antoine Lavoisier who believed that oxygen was necessary to form all acids (Anonymous, 2015a). Cyanobacteria were most likely responsible for the introduction of oxygen into the atmosphere of the earth, as the first photosynthetic organisms – they "exhaled" oxygen (Pappas, 2014). There are 3 stable isotopes of Oxygen; ^{16}O, ^{17}O and ^{18}O; which are found in the following percentages respectively 99.8%, 0.04% and 0.2% (Stewart, 2012). Understanding of the reactive nature of oxygen is required to optimize the application of oxygen into hydroponic systems.

2.5.1 Plant requirements and oxygen

Plants utilize oxygen for respiration; it can be taken up through the stomata within the leaves and through the roots (Douglas, 1970). Although the initial stage of plant respiration does not require O_2, oxygen is needed to complete the process. Stern *et al.* (2008) continues this statement with a simplified equation below to explain the process of respiration which starts in the cytoplasm of a cell and completed in the mitochondria;

$$C_6H_{12}O_6 \text{ (glucose)} + 6O_2 \text{ (oxygen)} \xrightarrow{\text{enzymes}} 6CO_2 \text{ (carbon dioxide)} + 6H_2O \text{ (water)} + \text{energy}$$

Anaerobic respiration occurs when there is a lack of available O_2, the by-products of which include ethyl alcohol and lactic acid which become cytotoxic at certain levels. Anaerobic respiration is about 18 times less efficient than aerobic respiration (Stern *et al.*, 2008).

2.5.2 Methods of aeration and dissolving oxygen into solution

Oxygenation in hydroponics is centred on the rate of diffusion of O_2 and the surface area of the solution. The factors which affect the rate of oxygen dissolution in water are; air and water temperatures, atmospheric pressure, turbulence, purity of water and salt content (Ben-Yaakov & Ben-Asher, 1982; Morimoto, 1989; Kepenyes & Váradi, 2015). Increasing the surface area of solution is possible by spraying the solution through pressurized pipe/nozzle manifolds or by letting solution flow from a height into a reservoir creating turbulence on entry, disrupting the surface layers and increasing gaseous exchange with the atmosphere (Schröder & Lieth, 2002). Venturi oxygenators and counter-flow column gas recyclers are other examples of aeration devices (Kepenyes & Váradi, 2015). Wojtowics (2013) reported that vortex flow regulators can effectively increase the dissolved oxygen content in waste and storm water management schemes. Application of hydrogen peroxide (H_2O_2) is another method of oxygenation into aqueous solutions.

2.5.3 Air-pumps

Using air-compressors or air-pumps to generate bubbles within the solution is commonly practiced, the size of these bubbles effects how much oxygen is dissolved and therefore the amount available for plant root uptake. Park and Kurata (2009) found that microbubbles (≤50 µm) were more effective in stimulating root growth than macrobubbles (>50 µm), this result was most likely due to the exponential increase in surface area of the gas-liquid interface. Positive growth trends have been recorded in plants produced in DWC using air-pump aeration (Tesi *et al.*, 2003).

2.5.4 Hydrogen peroxide

The application of hydrogen peroxide (H_2O_2) is a commonly practiced oxygenation technique in hydroponics (Kessler, 2015). H_2O_2 breaks down readily when introduced into a nutrient solution, releasing a molecule of water and a reactive O-molecule; which can bind with another O-molecule (forming a stable O_2 molecule dissolved in the solution), this O-molecule can also react with organic compounds (which often results in degradation of the compound) (Fredrickson, 2015). Increased water/ solution temperature results in less oxygen dissolution as well as an increase of pathogenic microbes (Anonymous, 2011a). In such instances the reactive behaviour of hydrogen peroxide is beneficial, on application dissolved oxygen levels are increased and the growth of harmful bacteria, fungi and algae is inhibited by the free O- molecules.

2.5.5 Vortex oxygenation

Vortices occur in nature where energy is being expended in a fluid phase, as can be observed as a wake in water behind a boat; or trailing from the fin of a turbine (Revstedt, 1998). The spiralling motion observed in such formations is replicated by water mixing technologies to discourage stagnation and stratification in standing reservoirs (Oppenheimer, 2008). Patented devices such as the 'water activator and fluids revitalizer' made by ACQUAPHI™, the 'vortex water revitalizer'- a product of INNOVATIVE SEALING SOLUTIONS, INC, the 'Water vitalizer plus™' (which is actually described as an advanced oxygenation system) and the 'energizers' and 'harmonizers' sold by the Centre for Implosion Research™ are all examples of the facilitation of the toroidal flow of water through various flow apparatus. These industrial manufacturers have borrowed the somewhat esoteric notions put forth by natural philosophers such as Viktor Schauberger and Rudolph Steiner. The observations of these individuals have founded a range of alternative water management and agricultural practices such as biodynamic agriculture (Reganold, 1995); the esoteric nature of certain aspects of these systems makes them somewhat unscientific so in this regard no claim is being made as to the credibility of the overall movements they inspired. Although some scientific publications have investigated biodynamic principles compared with conventional methods, research into this field is still largely undeveloped (Reganold, 1995). There is some evidence of the effectiveness of water vortices dissolving substantial amounts of oxygen into the liquid (Wojtowics *et al.*, 2013); this correlates with the aspects of the anecdotal data presented by the above-mentioned Austrians and the suggestion that the quality of water is improved when encouraged to flow in a toroidal manner. Fertilization by means of 'preparations' in biodynamic farming involves stirring solutions and facilitating the formation of vortices are regarded by practitioners as being integral to the process of manufacturing these solutions, no studies have been published which specifically measure the effect these vortices have on the solutions. The various properties of flow dynamics and temperature related oxygen content were harnessed by Schauberger in the development of highly efficient log flumes (Alexanderson, 2002).

Potential benefits of facilitating the occurrence of vortices in water with regards to application in plant production have yet to be investigated, technology for facilitating this kind of flow within hydroponics needs to be tested to assess efficacy.

2.6 Development of test apparatus

Preliminary experimentation showed that vortex oxygenation increased the dissolved oxygen content of the nutrient solution to a greater extent than the other methods of aeration tested (air-pumps and spraying). Figure 2.1 below shows simplified sketch diagrams for the original vortex oxygenator (on the left) and the streamlined version used for this study (on the right).

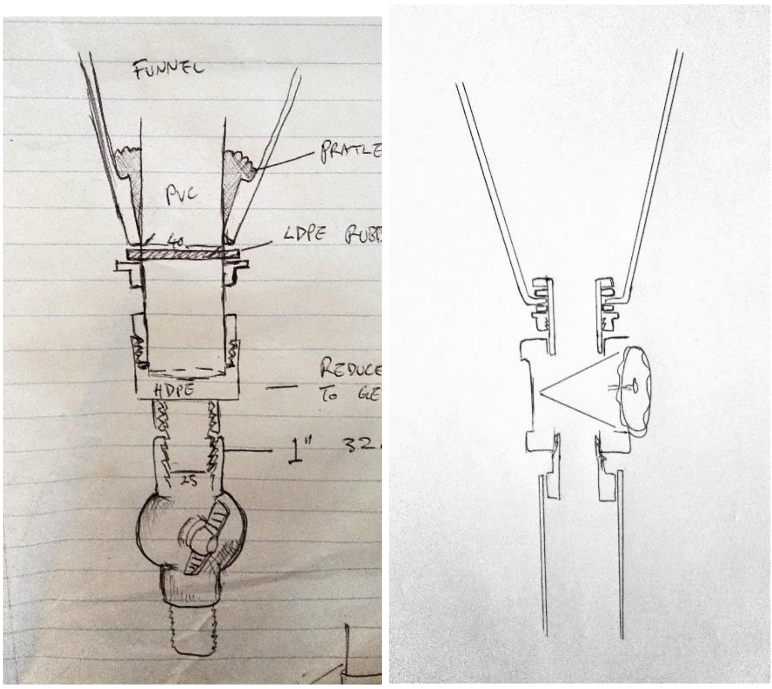

Figure 2.1

Essentially the apparatus is similar to the original although alterations to the types of fittings used to connect the various components were reviewed and simplified. Rather than a PVC pipe fitted directly into the funnel and sealed with putty, a tank connector was inserted through the funnel aperture which could be sealed with rubber washers. A brass gate valve was used instead of a PVC ball valve which greatly increased the flow control capability at the funnel outlet. The final design was constructed for the experiment and is shown in a photograph below (Fig. 2.2).

Figure 2.2

2.7 Measuring dissolved oxygen

There are several methods of testing for dissolved oxygen in liquid water (Anonymous, 2009); the Winkler Method which involves “fixing” the dissolved oxygen (DO) from a water sample with the addition of a series of acid compound-forming reagents, thereafter titrating the mixture with a neutralising compound and achieving a reaction which results in a change of colour called the “endpoint” (Bruckner, 2015). The other methods of measuring DO are electrochemical analysis and membrane electrode techniques. Measuring DO content within the separate treatments of this test could assist in providing data pertaining to the efficacy of the various oxygenation techniques.

2.7.1 ORBECO HELLIGE® series 150 for measuring DO

The ORBECO HELLIGE® series 150 (6456 Parkland Dr, Sarasota, FL 34243) is a meter capable of measuring multiple parameters; EC, pH, water temperature and dissolved oxygen. An electrolyte solution is held inside the probe and capped by a gas permeable membrane. This membrane allows oxygen molecules to flow into the chamber with the electro-chemical gradient when the probe is exposed to the atmosphere or a solution. Electrodes measure the different strength of electrical current passing through a charged wire before and after exposure to the measured environment; this difference correlates with the amount of oxygen present (Anonymous, 2015b).

2.8 Chlorophyll and plant health

Photosynthesis in higher plants depends mainly on chlorophyll pigments; these are located in the thylakoid membrane of chloroplasts. Photosynthetic units are made up of several different pigments. Pigment *a* is more abundant and is blue-green in colour, pigment *b* is yellow-green and can absorb light of different frequencies within the spectrum; increasing the efficiency of the photosynthetic process, *b* pigments pass absorbed energy on to *a* pigments in order for the Hill reaction (the so called ‘light-dependant’ phase of photosynthesis) to take place (Stern *et al.*, 2008; Anonymous, 2015a). Energy is the transferred to photosystem 1 (PSI) and 2 (PSII) where the ‘light-independent’ phase of photosynthesis takes place. The amount of chlorophyll within a plant’s leaves correlates with the accumulation of nitrogen and is indicative of the overall health of the plant. By measuring chlorophyll researchers and technicians can assess the rate at which nutrients in the form of fertilizer are being taken up by crops, as well as detecting the quality of water used for irrigation thereby making treatment and application schedules more efficient (Anonymous, 2015d). Chlorophyll content indicates stress levels and productivity of crops, using chlorophyll content as a parameter for measurement is an effective tool for the investigation of plant health (Marsh, 2016).

2.8.1 Measuring chlorophyll

Fluorescence is light which is not absorbed by a leaf, this light is re-emitted at a different wavelength to that which the leaf receives from the environment, the Kautsky effect describes this difference in wavelengths (Maxwell & Johnson, 2000; Anonymous 2016). The chlorophyll fluorescence ratio (CFR) is a comparative measurement created by exposing a leaf to a pre-determined level of light energy and measuring the amount that is re-emitted as fluorescence (Anonymous, 2011b). Fluorometers are devices which can give accurate field readings of the level of fluorescence and use the CFR to calculate the amount of chlorophyll within leaves (Maxwell & Johnson, 2000). The OPTI-SCIENCES® CCM-300 (8 Winn Ave, Hudson, NH 03051, USA) is a fluorometer which uses light frequencies of 735nm the range of 700–710nm to measure fluorescence (Marsh, 2016), Gittelson *et al.* (1999) found that these frequencies can be used to precisely measure the amount of chlorophyll present in a leaf sample.

If measuring the chlorophyll production within plants can help determine the efficacy of cultivation practices it would be a useful method of investigation to establish the ideal cultivation practices of *P. tomentosum* in DWC hydroponics.

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CHAPTER 3

EFFECTS OF VARIOUS OXYGENATION TECHNIQUES ON THE CHLOROPHYLL RESPONSES OF *PELARGONIUM TOMENTOSUM* GROWN IN DEEP WATER CULTURE HYDROPONICS

EFFECTS OF VARIOUS OXYGENATION TECHNIQUES ON THE CHLOROPHYLL RESPONSES OF *PELARGONIUM TOMENTOSUM* GROWN IN DEEP WATER CULTURE HYDROPONICS

3.1 ABSTRACT

Pelargonium tomentosum Jacq.; the peppermint-scented pelargonium, is an herbaceous groundcover indigenous to the Western Cape of South Africa. Volatile oils are produced by this plant which are used in the fragrance industry. Studies on other *Pelargonium* species have shown chlorophyll content may affect the yield of essential oils. This study was carried out to investigate the viability of growing *P. tomentosum* in deep water culture (DWC) hydroponics and how best to aerate/ oxygenate the nutrient solution to increase the chlorophyll content within leaves. The experiment was conducted over a period of 74 days, 16 different methods of oxygenation were applied to 9 replicates. The control had passive aeration; the treatments were made up of air-pumps, vortex oxygenators and the application of hydrogen peroxide at various frequency intervals; these were combined with each other and run as separate oxygenation methods. The measurement of the chlorophyll content with plants' leaves has been established to be an accurate way of establishing vigour, health and levels of stress. It was found that the combination of high frequency application (every third day) of hydrogen peroxide, vortex oxygenation and air-pump injection (both operational for 24hr/day); treatment 11/ APVHa, yielded the highest (62.16 mg/$^{m-2}$) production of chlorophyll within all the replicates differing significantly ($P \leq 0.001$) from the control and the other treatments in the APVH combination group. The increased production of chlorophyll is likely due to healthy root growth and respiration within a nutrient solution where high levels of dissolved oxygen and turbulence were facilitated.

Keywords: Hydroponic nutrient solution, deep water culture (DWC), vortex, chlorophyll, chlorophyll fluorescence ratio (CFR).

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3.2 INTRODUCTION

Chlorophyll is an accumulation point for increased levels of nitrogen in plants. Observation of the chlorophyll content within a leaf can be indicative of a plant's ability to take up nitrogen, develop chloroplasts and successfully photosynthesize. Measurement of chlorophyll content can provide information regarding the overall health of a plant (Manetas *et al.*, 1998; Ling *et. al.,* 2011; Marsh, 2016). Chlorophyll pigments *a* and *b* are found within chloroplasts and are vital for the process of photosynthesis to occur in both higher and lower plants (Stern *et al.*, 2008; Anonymous, 2015a). Photosynthesis involves a light dependant phase where light is absorbed by a chlorophyll-protein complex dubbed the "antenna", the energy is then transferred into Photosystem 1 & 2. By freeing an electron the excitation energy is converted into a chemical form which continues to the NADP in Photosystem 1. By further processes which make up the 'dark phase' of photosynthesis; the formation of ATP and $NADPH_2$ is enabled (Pavlović *et al.*, 2014).

3.2.1 Chlorophyll fluorescence

Light energy which is not absorbed by leaves to drive photosynthesis dissipates as heat or is re-emitted as fluorescence (Anonymous, 2016). Due to the different wavelengths at which light is re-emitted as fluorescence to those which are absorbed by a leaf described by the Kautsky effect, a direct correlation to the level of photosynthesis (photochemistry) can be observed and the content of chlorophyll within a leaf ascertained (Maxwell & Johnson, 2000). The proliferation of easy-to-use chlorophyll fluorometers over the few decades preceding this study has made chlorophyll fluorescence a widely-investigated parameter in scientific botanical research in both laboratory and field studies (Van Kooten & Snel, 1990; Manetas *et al.,* 1998; Maxwell & Johnson, 2000; Ling *et al.,* 2011). Not only has observation of chlorophyll within plants given researchers insight into the stress levels experienced in certain environments but also into quality of water used for irrigation and the effects of fertilizer application (Schlemmer *et al.*, 2005; Anonymous, 2015c).

3.2.2 The role of oxygen in plant growth

Oxygen plays a vital role in plant metabolic processes (Zheng *et al.*, 2007), respiration in particular, requires oxygen to be carried out effectively; it can be taken up through the stomata within the leaves or via the root system (Douglas, 1970). Respiration involves both carbon dioxide and oxygen in the formation of carbohydrates. In plants oxygen deficiency has rapid deleterious effects on the ability of roots to absorb water and other essential compounds (Urrestarazu & Manzuela, 2005). Although glycolysis does not depend solely on the presence of O_2 (Salisbury & Ross, 1985); the resultant pyruvate and NADH will accumulate and result in anaerobic respiration if there is an absence.

3.2.3 Oxygenation in hydroponics

Plant growth has been shown to increase when irrigated with additional oxygen in the solution (Soffer *et al.*, 1990) therefore in hydroponics it is important that oxygen is dissolved in the solution around the roots of plants. The level of oxygen able to be held within the nutrient solution is affected by factors such as water temperature, air pressure, salt content and purity (Ben-Yaakov & Ben-Asher, 1982; Morimoto, 1989; Kepenyes & Váradi, 2015). Oxygen injection at low pressures using air compressors or pumps into the growing medium has been shown to have some positive trends even in field grown crops (Chen *et al.*, 2011).

There are several methods employed to dissolve oxygen into hydroponic nutrient solution; these include increasing the surface area for gaseous exchange by forming droplets through spraying the solution at pressure; creating turbulence or agitation and by gaseous injection with air-pumps (Schröder & Leith, 2002; Bonachela *et al.*, 2005). Venturi oxygenators, surface agitators, paddles and counter-flow column gas recyclers are methods mentioned by Kepenyes and Váradi (2015). The size of the bubbles of atmospheric gas which enter the nutrient solution have an effect on plant development, root growth has been shown to be enhanced by the application of microbubbles (50 µm and smaller) compared with macrobubbles which are larger than 50 µm (Park & Kurata, 2009).

Gaseous bubbles in the nutrient solution contain oxygen however this must be dissolved for submerged plant roots to utilize it (Bonachela *et al.,* 2005). Air injection by using air pumps with dispersers has become the conventional method for aerating the solution in hydroponics. These simple devices are easy to install, and have shown beneficial effects on plant growth in hydroponics (Tesi *et al.*, 2013).

Although there are few published findings of oxygenating water by means of vortices, Wojtowics (2013) found that atomization of water by vortex flow regulators used in waste and storm water management schemes had a marked increase on dissolved O_2 (DO) levels. The effect of a venturi on fluid flow is a decrease in pressure and an increase in velocity, this can facilitate the formation of a visible spinning depression in the fluid (described as a vortex) where air or atmosphere is drawn toward the constriction in the flow apparatus due to the pressure differential. The uniform and predictable mixing of fluids (gas and liquid) created by venturi and the associated vortex formation is utilized by carburettors within internal combustion engines (Earls, 1997) to ensure efficient fuel/air mixing.

Oxygenation of the solution can also be achieved with the addition of hydrogen peroxide (H_2O_2) - this is a common practice in hydroponic plant cultivation (Kessler, 2015). H_2O_2 is an unstable compound in the molecular sense, when the substance breaks down one molecule of water is released as well as one highly reactive molecule of oxygen (O-) which either binds to another O- and results in a stable molecule of oxygen (Fredrickson, 2014) or reacts with an organic compound (usually degrading the said compound). The warmer water gets (above 2^0C) the less oxygen is able to be held in the solution, the resulting combination means an increase of the growth of anaerobes, fungi and bacteria (Anonymous, 2011a). The dual function of hydrogen peroxide as an oxygenator and sterilizing agent make it an attractive option in some cases despite the risk of oxidative stress (Cheeseman, 2007).

3.2.4 Measuring dissolved oxygen

There are several methods of testing for dissolved oxygen in liquid water (Anonymous, 2009); the Winkler Method which involves “fixing” the DO from a water sample with the addition of a series of acid compound-forming reagents, thereafter titrating the mixture with a neutralising compound and achieving a reaction which results in a change of colour called the “endpoint” (Bruckner, 2015). The other methods of measuring DO are electrochemical analysis and membrane electrode techniques.

3.2.5 *Pelargonium tomentosum* Jacquin*;* peppermint-scented pelargonium

The peppermint scented pelargonium, or *Pelargonium tomentosum,* is indigenous to South Africa (Lawrence, 2002). *P. tomentosum* is an herbaceous groundcover (Height of 0.5 m) with a sprawling habit (1.5 m). Possibly the most remarkable feature of *P. tomentosum* is the prominent peppermint scent; the volatile oils and resultant aroma make this a potentially useful therapeutic plant, it is used in the perfume industry. The essential oil secreted by glandular hairs is made up of (-)-isomenthone (59-62%) and (+)-methone (25-33%), terpenic hydrocarbons make up the remainder (Demarne & Van der Walt, 1990). Production of geranial oil in *Pelargonium graveolens* was increased and chlorophyll content was higher after application of 24-epibrassinolide (Swamy & Rao, 2009). Optimal biomass production, photosynthetic rate and essential oil synthesis was observed in *P. graveolens* when zinc was applied at a rate of 0.025 g/m^{-3} (Misra *et al*., 2005). These findings indicate a link between the chlorophyll content (and resulting rate of photosynthesis) and the production of essential oils in this species of *Pelargonium*, as it is possible that increasing the chlorophyll content within *P. tomentosum* could translate to higher yields of cosmetically valuable essential oil. To date no studies on the effects of oxygenation techniques on *P. tomentosum* have been carried out, this study aims to investigate the growth responses to various methods of oxygenation and aeration in deep water culture hydroponics by measuring the chlorophyll production within leaves.

3.3 MATERIALS AND METHODS

3.3.1 Greenhouse experiment

The experiment was conducted in the research greenhouse facility at the Cape Peninsula University of Technology, Bellville, Cape Town, South Africa; GPS co-ordinates - 33° 55ˈ 45.53S, 18° 38ˈ 31.16E. The nature of the structure in this location ensured control of the environment in which the experiment was conducted. A 40% Alunet shade cloth was installed 4 m above the ground level, an additional 40% polyethylene black shade was suspended at a height of 2 m to provide acceptable lighting conditions for the shade-loving plants.

3.3.2 Plant preparation

All the propagation material was procured from a single stock plant of *P. tomentosum* situated in a private garden on the outskirts of Scarborough near to Cape Point. Only cuttings taken using homogenous methods; i.e. stem cuttings, ±15 cm long with a stem thickness of approx. 9 mm were used for the experiment. Two hundred cuttings were made in order to ensure that the minimum number of 144 rooted plants were available for the test. Once rinsed with municipal water the cuttings were placed into propagation trays containing sterile rooting media (Lawrence, 2002) made up of equal parts river sand and perlite to allow for ample drainage. Polystyrene plug trays which allow for isolated rooting to take place were used; these trays were placed onto a hot bed in a climate controlled glasshouse. The cuttings were sprayed twice weekly with fungicide (BIOGROW COPPER-SOAP, P/Bag X 15, Suite 115, Hermanus, Western Cape, RSA, 7200) mixed with municipal water at 20 ml/l to discourage microbial pathology. After 6 weeks, the cuttings had developed roots and were transplanted into 10 cm plastic pots, the growth media consisted of 1 part river sand, 1 part coconut peat, and ¼ part sifted compost. These plants were placed in a grid layout 50–100 cm away from an evaporative cooling pad which ensured cool conditions and ample ventilation. The plants were hand watered sparingly, fungicide application continued at the same frequency mentioned above with the addition of NITROSOL® (EFEKTO, PO Box 652147, Benmore, Johannesburg, Gauteng, 2010) at 5 ml/l of municipal water.

3.3.3 Hydroponic experiment

Once the plants reached the desired degree of homogeneity they were removed from the 10 cm pots, thoroughly rinsed with municipal water, and transplanted into 12.5 cm pots filled with lightweight expended clay aggregate or LECA® (PO Box 3749, Office 519, Galadari Building, Baniyas Street, Dubai, UAE). LECA® was the medium of choice as it is chemically inert, lightweight and maintains structural integrity (Boudaghpour & Hasemi, 2008) which is ideal for a DWC system. Eleven additional holes of 8 mm diameter cut out from the lower third of each pot to allow for root expansion, the pots were placed with their lower portions were submerged in the nutrient solution, while the upper part of the pots were held in place by the lids of the plastic reservoir containers.

Each treatment had an individual reservoir; ADDIS® (Sacks Circle, Bellville, CT, 7535) 'Roughtote' 150 l storage boxes fitted with extra drainage valves were used for this purpose. The hydroponic solution was formed by adding NUTRIFEED™ (Manufactured by STARKE AYRES Pty.Ltd. Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, 1619) to municipal water at 90 g per 150 l to achieve an electrical conductivity (EC) ranging from 700–1000 µS/cm (Anonymous, 2015d). EC was measured every third day with an ORBECO HELLIGE® (6456 Parkland Dr, Sarasota, FL 34243) series 150 multiparameter meter.

A randomized block design, made up of 16 replicates each with 9 individual plants was used to study the effects of various methods of including oxygen into the hydroponic solution on *P. tomentosum*. After plants were introduced to the DWC system a half strength nutrient solution was used to fill the reservoirs to allow for acclimatization within the new environment. Treatments began on the 15th of April 2016 and the experiment was conducted over a period of 74 days. Every 9 days the system was flushed; i.e. the nutrient solution was renewed (Harris, 1992). The pH of the solution was measured with an ORBECO HELLIGE® series 150 multiparameter meter every third day, adjusted and kept within a range of pH 5.5–6.5. Due to the high buffering capacity offered by comparatively large bodies of water there was little deviation from the range mentioned above.

3.3.4 Treatment application

The separate treatments were comprised of different methods of aerating and dissolving oxygen into the nutrient solution, as described below; the treatments' codes are followed by their definitions. Each treatment has been allocated a number for the purposes of discussion.

- (1) C = Passive aeration in the form of gaseous exchange at the surface of the nutrient solution (Kratky, 2010); this is the treatment that was used as the control.

The 'Pure' group:

- (2) V = Vortex oxygenator.
- (3) AP = A single air-pump attached to two aquarium aeration stones in the reservoir/ sump.
- (4) APV = Combination of an air-pump with two aquarium aeration stones and a vortex oxygenator.

The 'VH' group:

- (5) VHa = Vortex oxygenation plus a high frequency dose of H_2O_2.
- (6) VHb = Vortex oxygenation plus a mid-frequency dose of H_2O_2.
- (7) VHc = Vortex oxygenation plus a low frequency dose of H_2O_2.

The 'AP' group:

- (8) APHa = A single air-pump attached to two aquarium aeration stones in the reservoir/ sump plus a high frequency dose of H_2O_2.
- (9) APHb = A single air-pump attached to two aquarium aeration stones in the reservoir/ sump plus a mid-frequency dose of H_2O_2.
- (10) APHc = A single air-pump attached to two aquarium aeration stones in the reservoir/ sump plus a low frequency dose of H_2O_2.

The 'APVH' group:

- (11) APVHa = Combination of an air-pump with two aquarium aeration stones and vortex oxygenator, plus a high frequency dose dosage of H_2O_2.
- (12) APVHb = Combination of an air-pump with two aquarium aeration stones and vortex oxygenator, plus a mid-frequency dose of H_2O_2.
- (13) APVHc = Combination of an air-pump with two aquarium aeration stones and a vortex oxygenator, plus a low frequency dose of H_2O_2.

The 'H' group:

- (14) Ha = High frequency dose of H_2O_2.
- (15) Hb = Mid-frequency dose of H_2O_2.
- (16) Hc = Low-frequency dose of H_2O_2.

Each individual reservoir contained a recirculating pump which operated 24 hr/day (LIFETECH AP1000 aquarium pump AC220-240V, H:0.65 m, 400 l/hr), in the vortex treatments this pump was fitted to the vortex device - in the others the pumps were not connected to any piping. The pump curve specifications were used to determine the head and flow-rate relationship, the pumps were adjusted to account for the head of pressure required to lift water into the funnels whilst still maintaining a homogenous flow rate with the other treatments.

3.3.5 Oxygenation technology

3.3.5.1 Vortex oxygenation

Each vortex oxygenation device was constructed by inserting a plastic funnel (300 mm long, with 200 mm opening at the base tapering to 32 mm hole at the opposite end), with the larger opening facing upwards, into a PVC pipe (110 mm diam.) which provided structural support. A fish tank pump placed at the bottom of the PVC support was attached to a 700 mm length of class C low density polyethylene (LDPE) with a diameter of 15 mm that was secured to the outside of the funnel. Two 15 mm 90^0 elbows were attached to the end of this length of pipe and fed through the upper part of the funnel at an angle to deliver nutrient solution tangentially; this pipe is referred to as the inlet. At the lowest part of the funnel a 1 inch gate valve restricted the flow of solution as it drained from the funnel (this is referred to as the outlet); by manually adjusting this valve the formation of vortices in the solution above was facilitated. Another length of LDPE (12 mm diameter) was fitted 27 mm below the inlet and functioned as an over-flow, when setting the rate of flow through the outlet and during periods of low atmospheric pressure a rise in the level of the solution within the funnel occurred – the over-flow pipe directed surplus solution back into the reservoir directly.

3.3.5.2 Air-pump specification

DARO™ twin aquarium air-pumps (DARO™, 7 Dwyka Street, Stikland Industria, Bellville, 7530) were used to supply oxygen to the solution of treatments specified above. The air-pumps were connected to two 'air-stone' dispersers within each of the selected treatment reservoirs with flexible LDPE tube.

3.3.5.3 Hydrogen peroxide; H_2O_2

H_2O_2 (33%) was sourced from SCIENCEWORLD™ (26A Stellenberg rd., Parow Industrial, Cape Town, 7499). Low-frequency dosage of H_2O_2 was applied at a rate of 1.7 ml per litre of solution only during the replacement of nutrient solution, i.e. once every 9 days. Mid-frequency dosing was carried out every 4.5 days. The high-frequency application of H_2O_2

occurred once every three days; a dose was administered with the renewal of the solution, and twice again at three day intervals throughout the 9 day flush cycle.

3.3.6 Determination of plant growth

3.3.6.1 Chlorophyll content

At the end of the test period the chlorophyll content of the foliage of individual plants was measured by utilizing non-destructive fluorometer analysis (Manetas *et al.*, 1998). The CCM-300 chlorophyll meter produced by OPTI-SCIENCES®, Inc. (8 Winn Ave, Hudson, NH 03051, USA) uses a chlorophyll fluorescence ratio (CFR) to measure the chlorophyll content within plants' leaves. Two readings from separate, fully developed leaves of each plant were averaged and used to create data sets. A fibre-optic probe connected to an LED diode and two high sensitivity detectors enables this device to direct light at the leaf surface at one wavelength and measure the re-emitted light (fluorescence) at another wavelength. Readings were obtained by holding the probe against the adaxial side of the leaves. The ratio between the fluorescence at 735 nm and within the range of 700-710 nm has been established as an accurate measure for determining the chlorophyll content within leaves (Gitelson *et al.,* 1999). These are the wavelengths utilized by the CCM-300 to measure chlorophyll content (Marsh, 2016). A study conducted with another non-destructive chlorophyll measuring device, the SPAD-502 (Konica Minolta, Spectrum Technologies, Plainfield, Illinois) which used a similar mechanism of dual wavelength transmittance to measure chlorophyll content showed that such apparatus was accurate even when used to on pubescent leaves (Manetas *et al.,* 1998).

3.3.6.2 Dissolved oxygen content

Once a week throughout the experimental period the DO content within each treatment's reservoir was measured using an ORBECO HELLIGE® series 150 multiparameter meter. The probe was inserted into the upper portion of the nutrient solution through an inspection hole in each respective reservoir lid. The DO probe is capped with a gas-permeable membrane and contains and electrolyte solution which facilitates the formation of an electro-chemical gradient enabling the flow of oxygen from the aqueous solution once the probe is inserted. Within the probe were two electrodes which sensed the amount of electrical current passing through a charged wire before and after being exposed to the electrolyte mixture; the charge disturbance is indicative of the amount of oxygen. The meter is capable of measuring oxygen concentrations within a range between 0–20 mg/l of water (Anonymous, 2015b).

3.3.7 Statistical Analysis

All data was analysed using one-way analysis of variance (ANOVA), using the computing software program STATISTICA 13. Occurrence of statistical difference was determined by using the Fisher Least Significance Difference (L.S.D.) at values of $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$ levels of significance (Steel & Torie, 1980).

3.4 RESULTS AND DISCUSSION

3.4.1. The APVH group

The chlorophyll content readings for the APVH group (treatments 11/APVHa, 12/APVHb and 13/APVHc) and the control (treatment 1) are shown in figure 3.1. The plants in the APVHa (treatment 11) had the highest mean value, the readings indicate significant difference ($P \leq 0.001$) between this treatment, the control and the other treatments. Treatments 12 (APVHb) and 13 (APVHc) were higher than the control (10% and 35% respectively), although not significantly different. Considering chlorophyll production has been shown to correlate with N accumulation and overall plant health (Marsh, 2016) the increased level of chlorophyll detected in these plants indicates they were more vigorous and metabolically active. This could be attributed to the significantly higher ($P \leq 0.01$) level of dissolved oxygen content (Ehret *et al.*, 2010; Kessler, 2015) within the nutrient solution (see Fig. 3.2) facilitated by the combination of pressurized air injection (air-pump), a vortex oxygenator and high frequency dosing with H_2O_2. There was no significant difference detected between the water temperatures or EC levels of the different treatments (data not shown) which indicates that differences in DO were a direct result of the various methods used to oxygenate the solution. A study conducted by Shete *et al.* (2013) on spinach plants grown in an aquaponics system showed that chlorophyll production increased with longer periods of mechanical circulation of the nutrient solution. The turbulence within the solution of the treatments may have had a similar effect in the present study. Turbulence created by the combination of air pumps and vortex devices would also have affected the amount of gaseous exchange between the nutrient solution and the atmosphere by exposing a higher proportion of the solution to the surface gas-liquid interface (Peng *et al.*, 1995; Morse & Kytömaa, 2011). The increased level of mixing within these reservoirs discouraged stratification in the solution thereby avoiding the occurrence of oxygen deficient zones and a loss in water quality (Chapman, 1996; Oppenheimer, 2008).

3.4.2 The APH group

Statistical difference was found in the APH group (treatments 8/APHa, 9/APHb & 10/APHc) when compared with the control as shown in figure 3.3. APHa (treatment 8) and APHc (treatment 10) had significantly higher chlorophyll content than the control ($P \leq 0.05$). Treatment 9 (APHb) yielded slightly higher (21%) chlorophyll content readings than the control plants however did not differ significantly. Treatment 10 (APHc) yielded the highest mean within the group; these readings correspond with the elevated levels of DO displayed in figure 3.4, which indicates this treatment had the highest level of DO and differed significantly to the control ($P \leq 0.05$). Both other treatments (APHa and APHb) had significantly higher DO levels than the control ($P \leq 0.05$) however yielded lower DO levels than treatment 10 (APHc). Soybean and rice plants grown in aerated solution (by using air-pumps) in a test carried out by Boru *et al.* (2003) showed higher levels of chlorophyll content than those grown in non-aerated solution which supports the findings of the current study. Three cultivars of spinach were grown in an experiment conducted by Tesi *et al.* (2003)

where two methods of oxygenating the nutrient solution (i. air injection by an air pump, ii. mechanical circulation of solution through pump and length of pipe returning the solution at the surface of the reservoir) were compared with a non-oxygen enriched control. Both methods yielded plants which had significantly higher levels of chlorophyll than those from the control in the summer crop test (Tesi *et al.*, 2003), results of the current experiment agree with these findings.

3.4.3. The H group

Figure 3.5 indicates significantly ($P \leq 0.05$) increased chlorophyll content in the plants from treatment 14 (Ha) compared with those from the control. Treatment 15 (Hb) and 16 (Hc) both showed higher chlorophyll content means (31% and 31.5% respectively) than the control however, they did not differ significantly. Treatments Ha (treatment 14), Hb (treatment 15) and Hc (treatment 16) all differed significantly from the control ($P \leq 0.01$) but were not significantly different from one another when DO was measured (see Fig. 3.6). Although treatment 14 (Ha) had a DO mean value of only 2.84 mg/l higher than treatment 16 (Hc), the variance indicated by the error bar for treatment 14 (Ha) was the largest among the treatments (Fig. 3.6). At certain points throughout the experiment treatment 14 (Ha) had DO levels of 23.05 mg/l which may explain why the chlorophyll readings from this treatment were significantly higher than the control (Fig. 3.6) whilst those from treatment 15 (Hb) and 16 (Hc) were higher than the control but not significantly so. Khandaker *et al.* (2012) detected increased chlorophyll production in *Persea* plants when leaves had been treated with foliar applications of H_2O_2, although the method of application is different, the present study supports these findings.

3.4.4. The VH and Pure group

A comparison of chlorophyll content for the VH group (treatments 5/VHa, 6/VHb & 7/VHc) and the control showed that although treatment 7 (VHc) had a higher mean value than the other treatments, this was not significantly different (data not shown). Comparison of the chlorophyll content readings measured in the pure group (treatments 2/V, 3/AP & 4/APV) and the control indicated that none of these treatments yielded results of significant difference (data not shown). None of these treatments showed any significantly different levels in DO (data not shown) to the control.

3.5 CONCLUSION

The highest chlorophyll content mean value was achieved through a combination of high frequency application of H_2O_2, an air-pump and vortex oxygenation (treatment 11/ APVHa). Although treatment 14 (Ha) had the highest level of DO, the mean chlorophyll content value was slightly lower than treatment 11 (APVHa) and had a larger variance. These results indicate that to produce more photosynthetically active *P. tomentosum* plants in deep water culture hydroponics it is advisable that application of high frequency dosing of H_2O_2 be combined with other methods of oxygenation; when used with air-pumps (treatment 8/APHa) significantly higher amounts of chlorophyll were produced although not as high as when combined with an air pump and vortex oxygenation. The increase in chlorophyll content could affect the essential oil yield, additional testing is required to establish the link between chlorophyll content and the production of essential oils in *P. tomentosum.* Further research is necessary to investigate the effectiveness of this oxygenation practice on chlorophyll production on other plant species.

3.6 ACKNOWLEDGEMENTS

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

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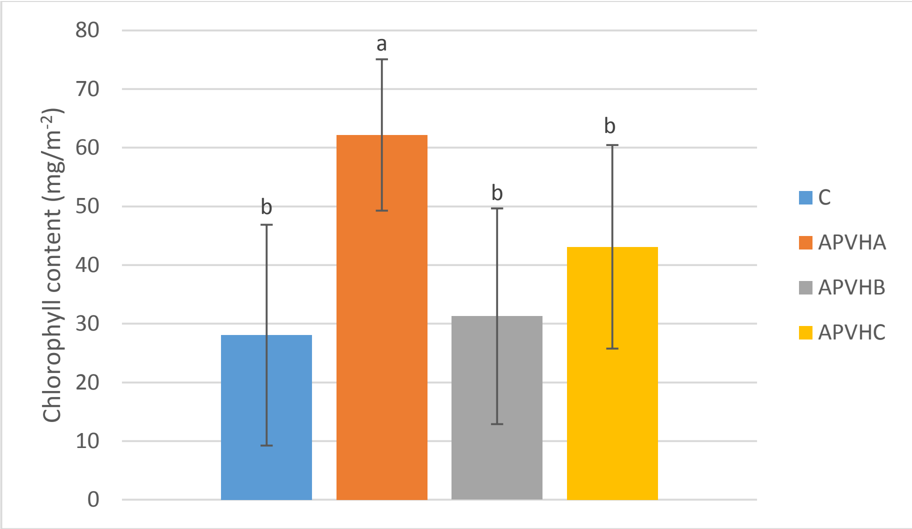

Figure 3. 1

Effects of treatments 11 (APVHa; air-pump, vortex oxygenator plus high frequency dosage of H_2O_2), 12 (APVHb; air-pump, vortex oxygenator plus mid frequency dosage of H_2O_2) and 13 (APVHc; air-pump, vortex oxygenator plus low frequency dosage of H_2O_2) on the chlorophyll content of leaves compared with the control (treatment 1). Bars indicate mean values ±SD. The mean values represented by the bars annotated with different letters differ significantly at $P \leq 0.001$ as calculated by Fisher's least significant difference. Bars annotated with the same letter are not significantly different. The one-way ANOVA F-statistic is 7.398.

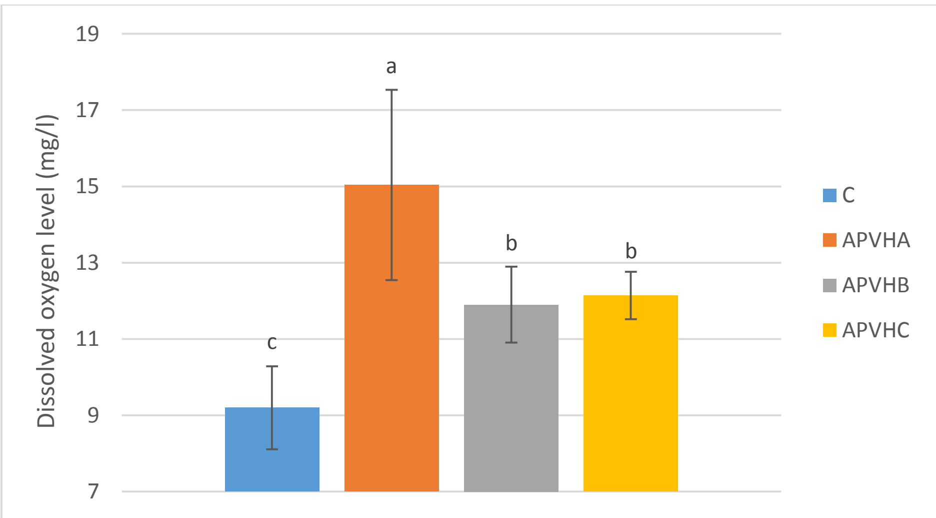

Figure 3.2

Effects of treatments 11 (APVHa; air-pump, vortex oxygenator plus high frequency dosage of H_2O_2), 12 (APVHb; air-pump, vortex oxygenator plus mid frequency dosage of H_2O_2) and 13 (APVHc; air-pump, vortex oxygenator plus low frequency dosage of H_2O_2on the level of dissolved oxygen compared with that of the control (treatment 1). Bars indicate mean values ±SD. The mean values represented by the bars annotated with different letters differ significantly at $P \leq 0.01$ as calculated by Fisher's least significant difference. The one-way ANOVA F-statistic is 5.513.

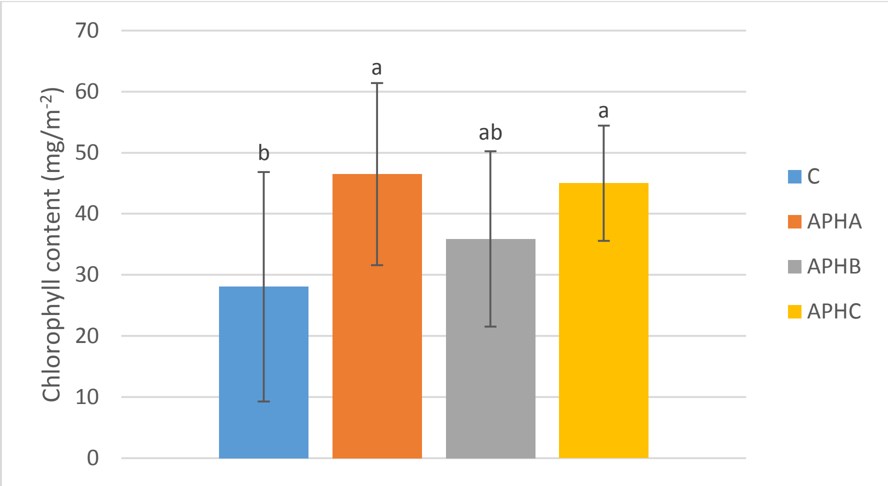

Figure 3.3

Effects of air-pumps combined with various frequency dosing of hydrogen peroxide treatments 8 (APHa; high frequency dosage), 9 (APHb; mid frequency dosage) and 10 (APHc; low frequency dosage) on the chlorophyll content of leaves compared with the control (treatment 1). Bars indicate mean values ±SD. The mean values represented by the bars annotated with different letters differ significantly at P ≤0.05 as calculated by Fisher's least significant difference. The one-way ANOVA F-statistic is 3.049.

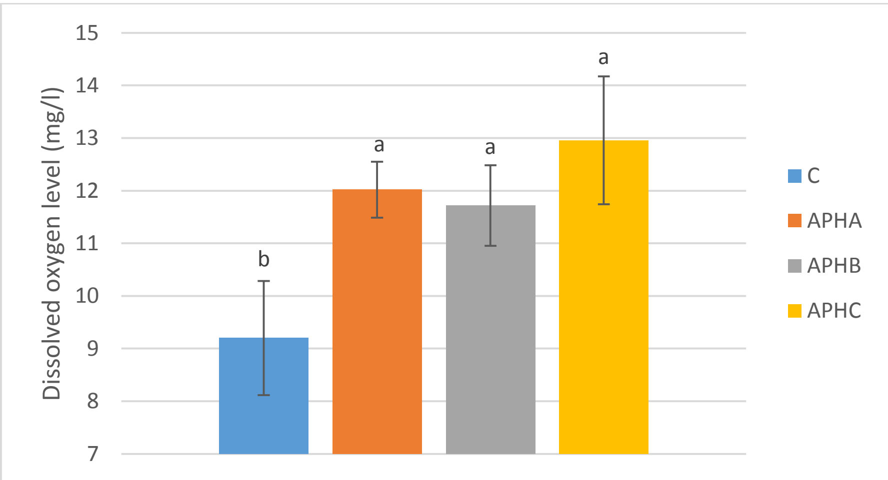

Figure 3.4

Effects of air-pumps combined with various frequency dosing of hydrogen peroxide treatments 8 (APHa; high frequency dosage), 9 (APHb; mid frequency dosage) and 10 (APHc; low frequency dosage)) on the level of dissolved oxygen compared with that of the control (treatment 1). Bars indicate mean values ±SD. The mean values represented by the bars annotated with different letters differ significantly at P ≤0.05 as calculated by Fisher's least significant difference. The one-way ANOVA F-statistic is 14.647.

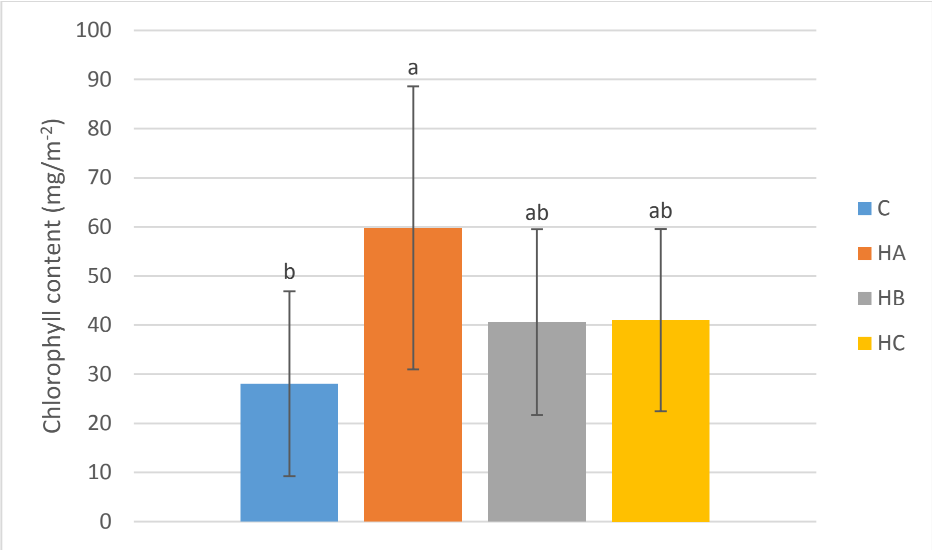

Figure 3.5

Effects of various frequency dosing of hydrogen peroxide treatments 14 (Ha; high frequency), 15 (Hb; mid frequency) and 16 (Hc; low frequency) on the chlorophyll content of leaves compared with the control (treatment 1). Bars indicate mean values ±SD. The mean values represented by the bars annotated with different letters differ significantly at P ≤0.05 calculated by Fisher's least significant difference. The one-way ANOVA F-statistic is 3.266.

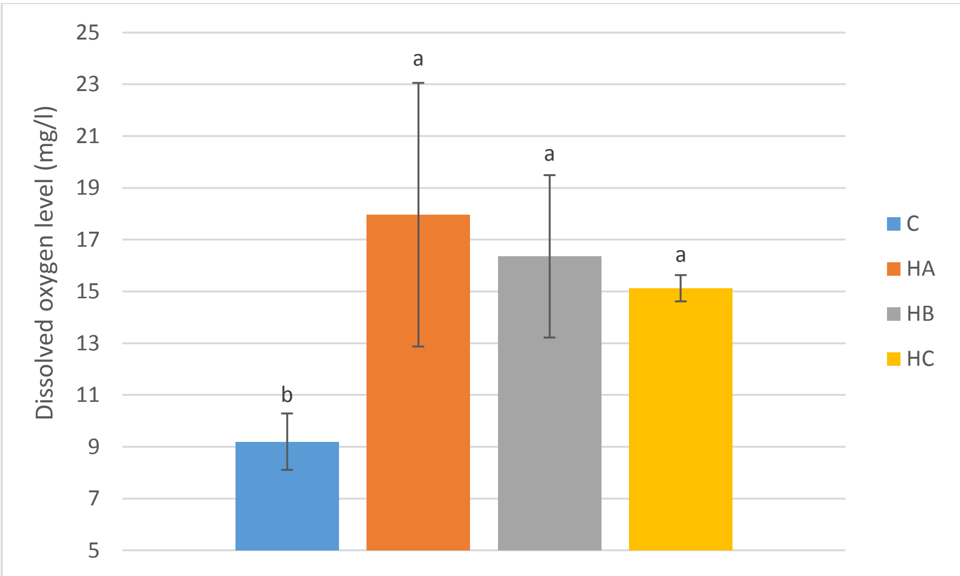

Figure 3.6

Effects of various frequency dosing of hydrogen peroxide treatments 14 (Ha; high frequency), 15 (Hb; mid frequency) and 16 (Hc; low frequency) on the level of dissolved oxygen of the solution compared with the control (treatment 1). Bars indicate mean values ±SD. The mean values represented by the bars annotated with different letters differ significantly at P ≤0.01 calculated by Fisher's least significant difference. The one-way ANOVA F-statistic is 7.843.

CHAPTER 4

EFFECTS OF HYDROGEN PEROXIDE COMBINED WITH VORTEX OXYGENATORS AND AIR-PUMPS ON THE FRESH AND DRY WEIGHT OF *PELARGONIUM TOMENTOSUM* GROWN IN DEEP WATER CULTURE HYDROPONICS.

EFFECTS OF HYDROGEN PEROXIDE COMBINED WITH VORTEX OXYGENATORS AND AIR PUMPS ON THE FRESH AND DRY WEIGHT OF *PELARGONIUM TOMENTOSUM* GROWN IN DEEP WATER CULTURE HYDROPONICS.

4.1 ABSTRACT

Pelargonium tomentosum Jacq.; the peppermint-scented pelargonium, is an herbaceous groundcover indigenous to the Western Cape of South Africa. Volatile oils are produced by this plant which are used in the fragrance industry. This study was carried out to investigate the effectiveness of applying hydrogen peroxide (H_2O_2) as a method of oxygenation and combining this with other oxygenation/ aeration techniques to increase the biomass of *P. tomentosum* grown in deep water culture (DWC) hydroponics. The experiment was conducted over a period of 74 days, 4 different methods of oxygenation were applied to 9 replicates. The control (treatment 4/Ha) received H_2O_2, the treatments were comprised of H_2O_2 application and; air-pump injection (treatment 2/APHa), vortex oxygenation (treatment 3/VHa) and the combination of these two oxygenation/ aeration techniques (treatment 1/APVHa). Dissolved oxygen levels were highest in the control (treatment 4/Ha). The control (Ha) yielded the highest fresh and dry shoot weights (71.1 g and 10.29 g respectively) followed closely by treatment 1 (APVHa), these weights were statistically similar. Treatment 1 (APVHa) had the highest dry root weight mean (2.14 g) although not significantly higher than that measured in the control. Essential oils are obtained from the shoot system of *P. tomentosum*, it was found that application of H_2O_2 was the most efficient method of oxygenation the solution and produced plants with the greatest shoot biomass.

Keywords: Hydrogen peroxide, dissolved oxygen, vortex oxygenator, air-pump, volatilization, turbulence, deep water culture hydroponics (DWC)

Clarification of terms: When referring to weight the terms ‘fresh’ and ‘wet’ are treated as synonyms.

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4.2 INTRODUCTION

Oxygenation of the nutrient solution in a hydroponic system can be achieved with the addition of inorganic forms of H_2O_2 and is a common practice in hydroponic plant cultivation (Kessler, 2015). In some cases this compound forms part of the pre-treatment of both sexual and vegetative propagation (Khandaker *et al.*, 2012). H_2O_2 as can be seen by its abbreviated name is, simply put, a water molecule with an extra oxygen atom attached to it (Kessler, 2015). When hydrogen peroxide breaks down in the presence of transitional metals, one molecule of water is released as well as one highly reactive molecule of oxygen (O-). This negatively charged atom either binds to another O- and results in a stable molecule of oxygen (Fredrickson, 2014), or reacts with an organic compound usually degrading said compound.

H_2O_2 is found naturally occurring within plants as a catalytic metabolite, involved in a host of processes such as the differentiation and lignification of xylem cells, root hair development, reactive oxygen species (ROS) signalling and stress responses (Cheeseman, 2007). Application of H_2O_2 is used to disinfect water as it sterilizes fungal spores and other microbial pathogens (Khandaker *et al.*, 2012). The warmer the solution; the less oxygen can be held, this combination can result in an increase of the growth of anaerobes, fungi and bacteria (Anonymous, 2011) which is undesirable in hydroponic culture. The dual function of hydrogen peroxide as an oxygenator and sterilizing agent make it an attractive option in some cases despite the risk of oxidative stress (Cheeseman, 2007).

Foliar application of diluted H_2O_2 (at 20 mM) has been shown to increase the dry weight of leaves, increase fruit set, biomass and quality of fruit as well as decreasing bud drop in wax apple crops (Khandaker *et al.*, 2012). Increase of salt stress tolerance in maize plants has been shown to be linked with spraying the leaves with aqueous solution containing H_2O_2 (Gondim *et al.*, 2012). This study investigates the effects of H_2O_2 when applied alone, when combined with vortex oxygenators, with air-pumps and when combined with both these methods of oxygenation together on the wet weight and dry weight of shoots and roots of *P. tomentosum* plants grown in DWC hydroponics.

4.3 MATERIALS AND METHODS

4.3.1 Greenhouse experiment

The experiment was conducted in the research greenhouse facility at the Cape Peninsula University of Technology, Bellville, Cape Town, South Africa; GPS co-ordinates - 33° 55ˈ 45.53S, 18° 38ˈ 31.16E. The nature of the structure in this location ensured control of the environment in which the experiment was conducted. A 40% Alunet shade cloth was installed 4 m above the ground level, an additional 40% polyethylene black shade was suspended at a height of 2 m to provide acceptable lighting conditions for the shade-loving plants.

4.3.2 Plant preparation

All the propagation material was procured from a single stock plant of *P. tomentosum* situated in a private garden on the outskirts of Scarborough near to Cape Point. Only cuttings taken using homogenous methods; i.e. stem cuttings, ±15 cm long with a stem thickness of approx. 9 mm were used for the experiment. Fifty cuttings were made in order to ensure that the minimum number of 35 rooted plants were available for the test. Once rinsed with municipal water the cuttings were placed into propagation trays containing sterile rooting media (Lawrence, 2002) made up of equal parts river sand and perlite to allow for ample drainage. Polystyrene plug trays which allow for isolated rooting to take place were used; these trays were placed onto a hot bed in a climate controlled glasshouse. The cuttings were sprayed twice weekly with fungicide (BIOGROW COPPER-SOAP, P/Bag X 15, Suite 115, Hermanus, Western Cape, RSA, 7200) mixed with municipal water at 20 ml/l to discourage microbial pathology. After 6 weeks, the cuttings had developed roots and were transplanted into 10 cm plastic pots, the growth media consisted of 1 part river sand, 1 part coconut peat, and ¼ part sifted compost. These plants were placed in a grid layout 50–100 cm away from an evaporative cooling pad which ensured cool conditions and ample ventilation. The plants were hand watered sparingly, fungicide application continued at the same frequency mentioned above with the addition of NITROSOL® (EFEKTO, PO Box 652147, Benmore, Johannesburg, Gauteng, 2010) at 5 ml/l of municipal water.

4.3.3 Hydroponic experiment

Once the plants reached the desired degree of homogeneity they were removed from the 10 cm pots, thoroughly rinsed with municipal water, and transplanted into 12.5 cm pots filled with lightweight expended clay aggregate or LECA® (PO Box 3749, Office 519, Galadari Building, Baniyas Street, Dubai, UAE). LECA® was the medium of choice as it is chemically inert, lightweight and maintains structural integrity (Boudaghpour & Hasemi, 2008) which is ideal for a DWC system. Eleven additional holes of 8 mm diameter cut out from the lower third of each pot to allow for root expansion, the pots were placed with their lower portions were submerged in the nutrient solution, while the upper part of the pots were held in place by the lids of the plastic reservoir containers.

Each treatment had an individual reservoir; ADDIS® (Sacks Circle, Bellville, CT, 7535) 'Roughtote' 150 l storage boxes fitted with extra drainage valves were used for this purpose. The hydroponic solution was formed by adding NUTRIFEED™ (Manufactured by STARKE AYRES Pty.Ltd. Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, 1619) to municipal water at 90 g per 150 l to achieve an electrical conductivity (EC) ranging from 700–1000 µS/cm (Anonymous, 2015d). EC was measured every third day with an ORBECO HELLIGE® (6456 Parkland Dr, Sarasota, FL 34243) series 150 multiparameter meter.

A randomized block design, made up of 16 replicates each with 9 individual plants was used to study the effects of various methods of including oxygen into the hydroponic solution on *P. tomentosum*. After plants were introduced to the DWC system a half strength nutrient solution was used to fill the reservoirs to allow for acclimatization within the new environment. Treatments began on the 15th of April 2016 and the experiment was conducted over a period of 74 days. Every 9 days the system was flushed; i.e. the nutrient solution was renewed (Harris, 1992). The pH of the solution was measured with an ORBECO HELLIGE® series 150 multiparameter meter every third day, adjusted and kept within a range of pH 5.5–6.5. Due to the high buffering capacity offered by comparatively large bodies of water there was little deviation from the range mentioned above.

4.3.4 Treatment application

The separate treatments were comprised of different methods of aerating and dissolving oxygen into the nutrient solution, as described below; the treatments' codes are followed by their definitions. Each treatment has been allocated a number for the purposes of discussion.

- (1) APVHa = Combination of an air-pump with two aquarium aeration stones, a vortex oxygenator and H_2O_2.
- (2) APHa = A single air-pump attached to two aquarium aeration stones in the reservoir/ sump plus H_2O_2.
- (3) VHa = Vortex oxygenation plus H_2O_2.
- (4) Ha = H_2O_2 only; this treatment was used as the control.

Each individual reservoir contained a recirculating pump which operated 24 hr/day (LIFETECH AP1000 aquarium pump AC220-240V, H:0.65 m, 400 l/hr), in the vortex treatments this pump was fitted to the vortex device - in the others the pumps were not connected to any piping. The pump curve specifications were used to determine the head and flow-rate relationship, the pumps were adjusted to account for the head of pressure required to lift water into the funnels whilst still maintaining a homogenous flow rate with the other treatments.

4.3.5 Oxygenation technology

4.3.5.1 H_2O_2

H_2O_2 (33%) was sourced from SCIENCEWORLD™ (26A Stellenberg rd., Parow Industrial, Cape Town, 7499). H_2O_2 was applied at a rate of 1.7 ml per litre of solution once every three days; a dose was administered with the renewal of the solution, and twice again at three day intervals throughout the 9-day flush cycle.

4.3.5.2 Air-pumps

DARO™ twin aquarium air-pumps (DARO™, 7 Dwyka Street, Stikland Industria, Bellville, 7530) were used to supply additional oxygen to the solution of treatments 1 (APVHa) and 2 (APHa). The air-pumps were connected to two 'air-stone' dispersers within each of the selected treatment reservoirs with flexible LDPE tube.

4.3.5.3 Vortex oxygenation

Each vortex oxygenation device was constructed by inserting a plastic funnel (300 mm long, with 200 mm opening at the base tapering to 32 mm hole at the opposite end), with the larger opening facing upwards, into a PVC pipe (110 mm diam.) which provided structural support. A fish tank pump placed at the bottom of the PVC support was attached to a 700 mm length of class C low density polyethylene (LDPE) with a diameter of 15 mm that was secured to the outside of the funnel. Two 15 mm 90^0 elbows were attached to the end of this length of pipe and fed through the upper part of the funnel at an angle to deliver nutrient solution tangentially; this pipe is referred to as the inlet. At the lowest part of the funnel a 1 inch gate valve restricted the flow of solution as it drained from the funnel (this is referred to as the outlet); by manually adjusting this valve the formation of vortices in the solution above was facilitated. Another length of LDPE (12 mm diameter) was fitted 27 mm below the inlet and functioned as an over-flow, when setting the rate of flow through the outlet and during periods of low atmospheric pressure a rise in the level of the solution within the funnel occurred – the over-flow pipe directed surplus solution back into the reservoir directly.

4.3.6 Determination of plant growth

4.3.6.1 Plant weight

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

4.3.6.2 Dissolved oxygen content:

Once a week throughout the experimental period the DO content within each treatment's reservoir was measured using an ORBECO HELLIGE® series 150 multiparameter meter. The probe was inserted into the upper portion of the nutrient solution through an inspection hole in each respective reservoir lid. The DO probe is capped which a gas permeable membrane and contains and electrolyte solution which facilitates the formation of an electro-chemical gradient enabling the flow of oxygen from the aqueous solution once the probe is inserted. Within the probe were two electrodes which sensed the amount of electrical current passing through a charged wire before and after being exposed to the electrolyte mixture; the charge disturbance is indicative of the amount of oxygen. The meter is capable of measuring oxygen concentrations within a range between 0–20 mg/l of water (Anonymous, 2015b).

4.3.7 Statistical Analysis

All data was analysed using one-way analysis of variance (ANOVA), using the computing software program STATISTICA 13. Occurrence of statistical difference was determined by using the Fisher Least Significance Difference (L.S.D.) at values of $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$ levels of significance (Steel & Torie, 1980).

4.4 RESULTS AND DISCUSSION

4.4.1 Dissolved oxygen (DO)

Figure 4.1 shows the comparison between levels of DO in the nutrient solution of the separate treatments. The control (Ha) had the highest mean level of DO (17.96 mg/l) and differed significantly ($P \leq 0.05$) from treatments 2 (APHa) and 3 (VHa). All the treatments had DO levels which were above the minimum level of plant requirements [3 mg/l (Bonachela *et al.*, 2010)]. There was no significant difference detected between the water temperatures or EC levels of the different treatments (data not shown) which indicates that differences in DO were a direct result of the various oxygenation techniques. The evaporation rate of water is strongly influenced by the degree of turbulence within a specific water body causing exposure of solution to the liquid-gas surface interface (Morse & Kytömaa, 2011). Turbulent conditions cause the surface layer to be continuously replaced by the solution from lower portions of the bulk liquid (Peng *et al.*, 1995). The higher the degree of turbulence within the reservoirs - the more likely the volatilization of excess oxygen molecules is to occur. The bubbling action of the air pump (treatment 2/APHa) and the increased exposure of the solution's surface to the atmosphere facilitated by vortex oxygenation (treatment 4/VHa) may have caused the additional oxygen supplied by H_2O_2 to volatize, decreasing the overall level of DO. The low degree of turbulence within the reservoir of the control (treatment 4/Ha) resulted in the diffusion rate of H_2O_2 being slower than that of the other treatments, decreasing volatilization and allowing a build-up of DO. This suggests that the benefits of using H_2O_2 to increase levels of DO may be negated at a certain degree of turbulence, however the sterilizing action of H_2O_2 was not noticeably inhibited i.e. neither root rot nor algae was detected. Treatment 2 (APVHa) had the second highest level of DO (15.04 mg/l), although not significantly different from the other treatments; the reservoir for this treatment had the highest degree of turbulence and therefore it is possible that increased diffusion of O_2 from the atmosphere into the solution (Schröder & Lieth, 2002) may have compensated for the volatilization loss of O_2 supplied by hydrogen peroxide.

4.4.2 Plant root weights

Figure 4.2 shows the effect the different combinations of oxygenation had on the wet root weight of the sample plants. Treatment 1 (APVHa) and 4 (Control/Ha) yielded the heaviest root systems, these fresh weights were similar to each other but significantly different ($P \leq 0.05$) to those measured from treatment 3 (VHa). Treatment 2 (APHa) yielded wet root weight readings that were higher than treatment 3 (VHa), lower than treatments 1 (APVHa) and the control (treatment 4/Ha), but did not differ significantly from any of these treatments. A comparison of the dry root weights of plants from treatments 1–4 is shown in figure 4.3. Treatment 1 (APVHa) yielded the highest readings and were significantly different ($P \leq 0.001$) to those from the other treatments. A test carried out on cucumber plants showed a proliferation of roots when plants were grown in oxygen enriched hydroponic media (Ehret *et al.*, 2010). Marfà *et al.* (2005) conducted a study and found that the dry weight of fine roots (<2 mm diam.) produced by pepper plants grown in hydroponic culture were significantly greater in oxygen enriched treatments. Roots produced by the *P. tomentosum* plants in this experiment were similar in diameter to those mentioned above, and likewise yielded significantly greater ($P \leq 0.001$) dry weights in the treatments which had higher levels of dissolved oxygen. Although the dissolved oxygen levels of treatment 1 (APVHa) and the control were similar (Fig. 4.1) the higher degree of turbulence facilitated by the combination of a vortex oxygenator and an air-pump had an observable positive effect on the root growth. This turbulence may also have increased the availability of nutrients within the root zone which would have improved root development. Although treatment 2 (APHa) yielded slightly lower (9.9%) dry root weights than the control, they were statistically similar.

4.4.3 Plant shoot weights

The treatments' comparative wet and dry shoot weights are shown in figure 4.4 and 4.5 respectively. In both sets of data the control (treatment 4/Ha) yielded the highest readings, (71.1 g fresh, 10.29 g dry) differing significantly ($P \leq 0.001$) from treatments 2 (APHa) and 3 (VHa). Figure 4.4 indicates the wet shoot weights measured in treatment 1 (APVHa) differed significantly ($P \leq 0.001$) from treatment 3 (VHa) only and were similar to both the control (Ha) and treatment 2 (APHa); however, figure 4.5 shows the dry shoot weight mean of treatment 1 (APVHa) was similar to the control (treatment 4/Ha) but significantly greater ($P \leq 0.001$) than treatments 2 (APHa) and 3 (VHa). *Ficus* plants grown in DWC hydroponics with elevated levels of DO were found to grow more vigorously and have greater biomass in an experiment conducted by Soffer *et al*. (1991), this study supports these findings. Treatment 3 (VHa) yielded the lowest wet shoot weights of all the treatments (Fig. 4.4), however figure 2.5 shows the dry weights of shoots were higher (6.2%) although not significantly different to those measured for treatment 2 (APHa). The DO content of treatment 3 (VHa) was marginally greater than that of treatment 2 (APHa), as shown in figure 4.1, suggesting that the slightly higher level (1.1%) of DO had more of an effect on dry weight than fresh weight of shoots.

4.5 CONCLUSSION

The efficiency of supplying dissolved oxygen in deep water culture hydroponics through the application of hydrogen peroxide (H_2O_2) is affected when combined with other methods of oxygenation. DO levels measured in the combination treatments were all lower than that of the control (Ha). This is attributed to the volatilization of excess oxygen molecules due to the degree of turbulence caused by the bubbling action of air-pumps and the increased exposure of the solution surface to the atmosphere and subsequent fluid-mixing action of vortex oxygenation. However, at the highest degree of turbulence the loss of O_2 (supplied by H_2O_2) through volatilization was somewhat compensated by increased diffusion of oxygen from the atmosphere into the solution. The control treatment (Ha) yielded the highest readings of fresh shoot and root weights, as well as the greatest dry shoot weights although not statistically different form treatment 1 (APVHa) in any of these comparisons. Treatment 1 (APVHa) yielded the highest readings for dry root weights suggesting that root development was benefited by the combination of H_2O_2 with both vortex oxygenation and an air pump. As essential oils are produced in the shoot system of *P.tomentosum,* these results indicate that application of H_2O_2 is an effective way of increasing shoot biomass and can be recommended as a method for oxygenating the nutrient solution in DWC. Further research is necessary to establish the efficacy of these oxygenation techniques on the growth responses of other plant species.

4.6 ACKNOWLEDGEMENTS

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

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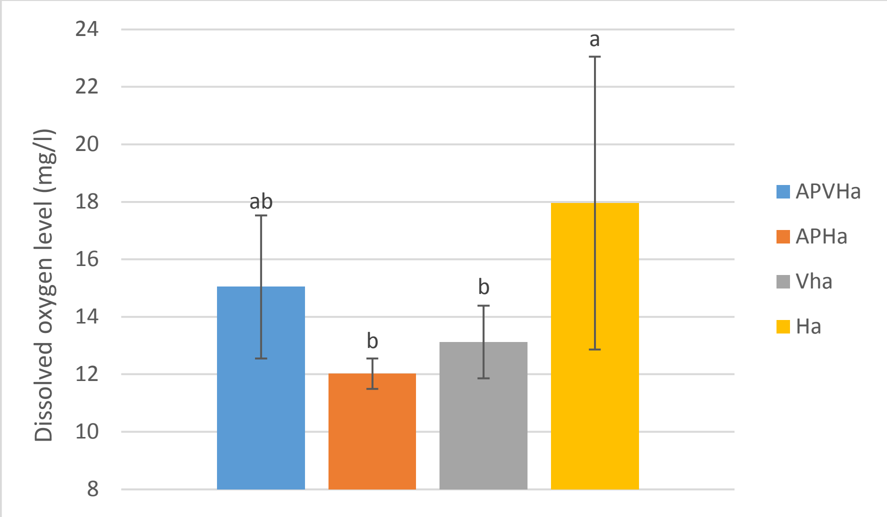

Figure 4.1

Effects of H_2O_2combined with vortex oxygenation plus an air-pump (treatment 1/APVHa); H_2O_2 with air-pump only (treatment 2/APHa), and H_2O_2 with vortex oxygenation only (treatment 3/VHa) on the level of dissolved oxygen compared with the control (treatment 4/Ha). Bars indicate mean values ±SD. The mean values represented by the bars annotated with different letters differ significantly at $P \leq 0.05$ as calculated by Fisher's least significant difference. The one-way ANOVA F-statistic is 3.9812.

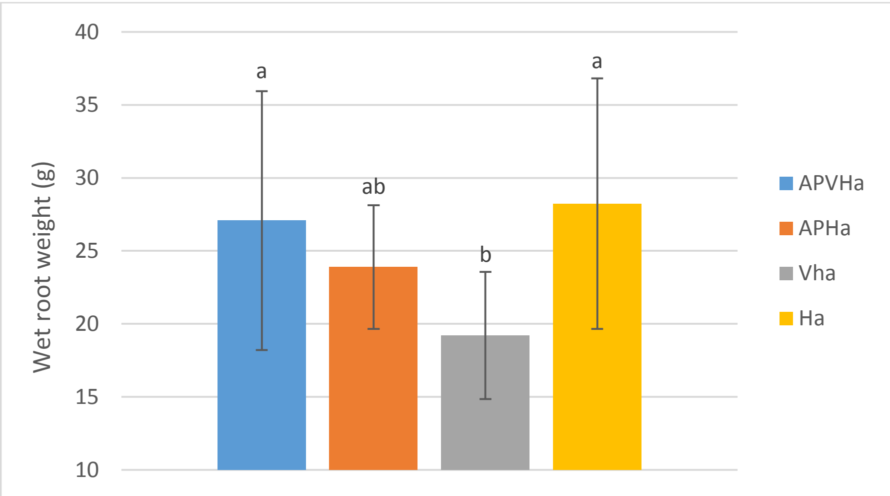

Figure 4.2

Effects of H_2O_2combined with vortex oxygenation plus an air-pump (treatment 1/APVHa); H_2O_2 with an air-pump only (treatment 2/APHa), and H_2O_2 with vortex oxygenation only (treatment 3/VHa) on the wet root weight compared with the control (treatment 4). Bars indicate mean values ±SD. The mean values represented by the bars annotated with different letters differ significantly at $P \leq 0.05$ as calculated by Fisher's least significant difference. The one-way ANOVA F-statistic is 3.1067.

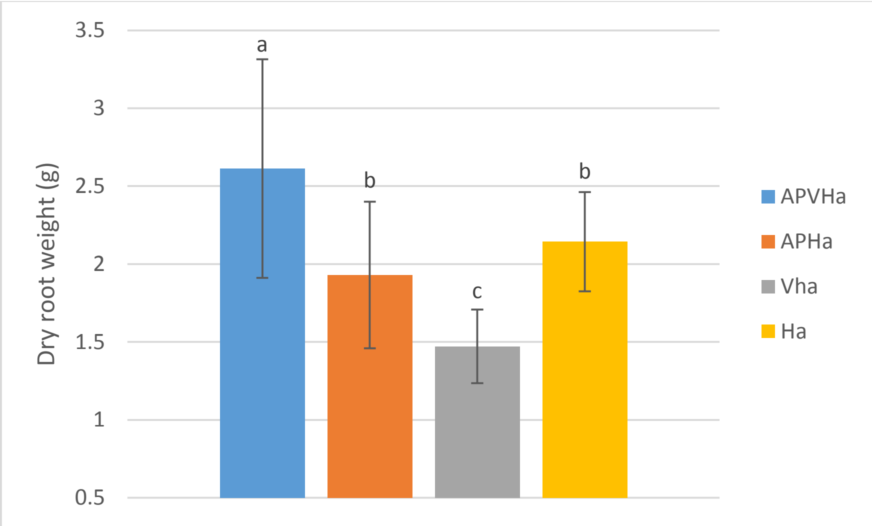

Figure 4.3

Effects of H_2O_2combined with vortex oxygenation plus an air-pump (treatment 1/APVHa); H_2O_2 with an air-pump only (treatment 2/APHa), and H_2O_2 with vortex oxygenation only (treatment 3/VHa) on the dry root weight compared with the control (treatment 4). Bars indicate mean values ±SD. The mean values represented by the bars annotated with different letters differ significantly at P ≤0.001 as calculated by Fisher's least significant difference. The one-way ANOVA F-statistic is 9.3084.

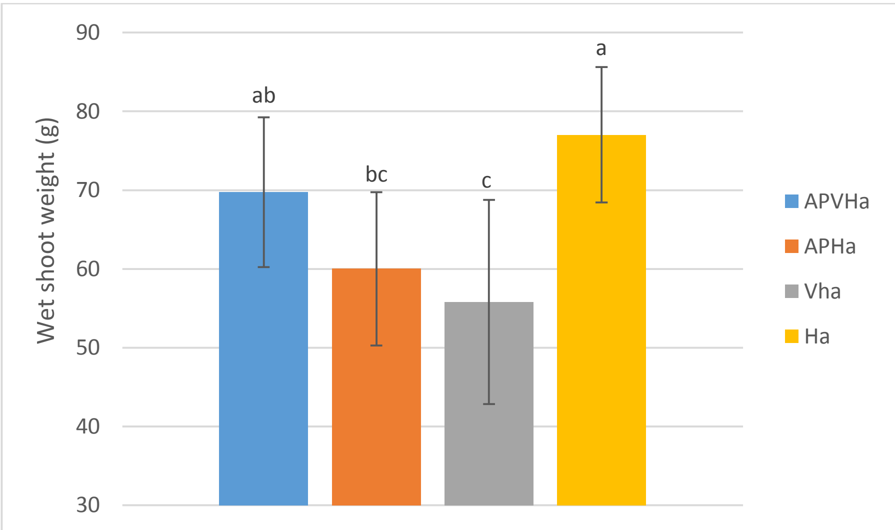

Figure 4.4

Effects of H_2O_2 combined with vortex oxygenation plus an air-pump (treatment 1/APVHa); H_2O_2 with an air-pump only (treatment 2/APHa), and H_2O_2 with vortex oxygenation only (treatment 3/VHa) on the wet shoot weight compared with the control (treatment 4). Bars indicate mean values ±SD. The mean values represented by the bars annotated with different letters differ significantly at P ≤0.001 as calculated by Fisher's least significant difference. The one-way ANOVA F-statistic is 7.71.

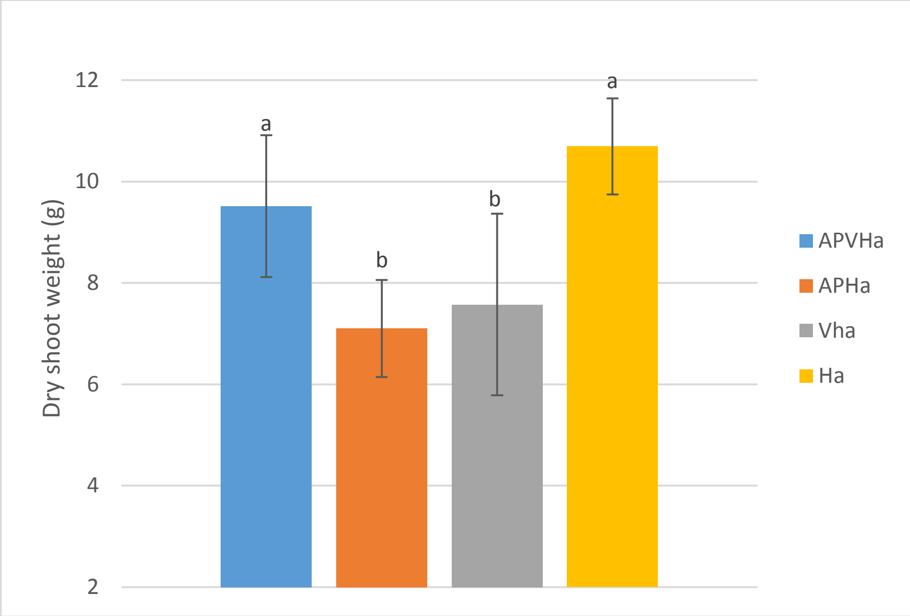

Figure 4.5

Effects of H_2O_2 combined with vortex oxygenation plus an air-pump (treatment 1/APVHa); H_2O_2 with an air-pump only (treatment 2/APHa), and H_2O_2 with vortex oxygenation only (treatment 3/VHa) on the dry shoot weight compared with the control (treatment 4). Bars indicate mean values ±SD. The mean values represented by the bars annotated with different letters differ significantly at $P \leq 0.001$ as calculated by Fisher's least significant difference. The one-way ANOVA F-statistic is 14.537.

CHAPTER 5

ADAPTABILITY OF *PELARGONIUM TOMENTOSUM* TO SUB-OPTIMAL LEVELS OF DISSOLVED OXYGEN AND GROWTH RESPONSES TO VARIOUS TYPES OF AERATION IN THE FORM OF AIR-PUMP INJECTION AND VORTEX OXYGENATORS IN DEEP WATER CULTURE HYDROPONICS.

ADAPTABILITY OF *PELARGONIUM TOMENTOSUM* TO SUB-OPTIMAL LEVELS OF DISSOLVED OXYGEN AND GROWTH RESPONSES TO VARIOUS TYPES OF AERATION IN THE FORM OF AIR-PUMP INJECTION AND VORTEX OXYGENATORS IN DEEP WATER CULTURE HYDROPONICS.

5.1 ABSTRACT

Pelargonium tomentosum Jacq.; the peppermint-scented pelargonium, is an herbaceous groundcover indigenous to the Western Cape of South Africa. Volatile oils are produced by this plant which are used in the fragrance industry. This study was carried out to investigate the adaptability of *P. tomentosum* to sub-optimal dissolved oxygen (DO) and the effects of applying different methods of oxygenation on the growth responses of *P. tomentosum* grown in deep water culture (DWC) hydroponics. The experiment was conducted over a period of 74 days, 4 different methods of oxygenation were applied to 9 replicates. The control (treatment 1) received passive aeration (the Kratky method), treatment 2 (V) received vortex oxygenation, treatment 3 (AP) was aerated through air-pump injection, treatment 4 (APV) received a combination of air-pump injection and vortex oxygenation. Plant height and total leaf count were greatest in treatment 3 (AP) and significantly different ($P \leq 0.05$) to treatments 2 (V) and 4 (APV). The roots of the control plants were the least healthy compared to the other treatments, however the plant heights and leaf count were only slightly lower than, and statistically similar to the air-pump treatment (3/AP). These findings suggest that although root growth of *P. tomentosum* is inhibited by sub-optimal levels of DO, shoot growth is not adversely affected.

Keywords: Dissolved oxygen, air-pump, vortex oxygenation.

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

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5.2 INTRODUCTION

5.2.1 Oxygen deficiency and adaptability of plants

Environmental stress can adversely affect plants; flooding which results in a lack of oxygen in the root-zone accounts for a significant loss in agricultural crops (Ismond *et al.*, 2003). Waterlogging is especially prevalent in clay predominated soils (Dennis *et al.*, 2000). Hypoxia or anoxia (limited access to oxygen or lack thereof) usually results in higher plants manifesting symptoms of stress, impaired growth and even senescence. The deleterious effects of oxygen deficiency and the resulting build-up of phytotoxins created by anaerobic microbes can have a severe impact on the root health of terrestrial plants (Armstrong *et al.,* 1991). Some species have developed mechanisms to tolerate and even thrive in low oxygen containing environments (Perata & Alpi, 1993), however the ability of terrestrial crops to adapt to low oxygen environments (waterlogging) varies greatly (Ismond *et al.*, 2003).

Although adaptations of these tolerant species differ, some general traits have been identified. For example, within rice crops the production of energy in form of adenine tri-phosphate (ATP) via mitochondrial oxidative phosphorylation can be minimized or even ceased switching to anaerobic respiration (fermentation). Certain proteins are required to metabolise the carbohydrates created through fermentation, tolerant species have been identified for their capacity to produce these proteins for extended periods. Tolerance of hypoxia/anoxia is a combined result of adaptations from physiological, molecular and phytochemical aspects (Perata & Alpi, 1993). Anoxia avoidance mechanisms include superficial rooting (formation of adventitious roots) and increased gas space within the root cortex (Drew, 1983). In conditions where roots are produced in sub-optimal levels of oxygen they have been found to be long and thin; maximizing their surface area in relation to their volume enables them to utilize the marginal O_2 content (Armstrong *et al.,* 1991).

Plants grown in hydroponics, especially in large bodies of water such found in deep water culture (DWC) systems develop most of their roots outside of the naturally aerated surface layer of nutrient solution, therefore making artificial aeration necessary to maintain healthy root development (Ben-Yaakov & Ben-Asher, 1982). Root mass of tomato plants has been shown to increase when grown in oxygen enriched nutrient solution (Ehret *et al.*, 2010). Kessler (2015) states that most hydroponic crops grow vigorously when supplied with nutrient solution containing dissolved oxygen at a level of 9 ppm (9mg/l). To date no research into the adaptability of *Pelargonium tomentosum* to sub-optimal levels of oxygen availability has been published. In this experiment; *P. tomentosum* was grown in DWC hydroponics supplied with various levels of dissolved oxygen (DO). Different methods of oxygenation (i.e. passive aeration, air injection by air-pumps fitted with 'air-stone' dispersers, vortex oxygenators and combinations thereof) were used to determine the effect of oxygenation on growth parameters and thus give insights into the adaptability of this plant in passively-aerated DWC hydroponic culture.

5.3 MATERIALS AND METHODS

5.3.1 Greenhouse experiment

The experiment was conducted in the research greenhouse facility at the Cape Peninsula University of Technology, Bellville, Cape Town, South Africa; GPS co-ordinates - 33° 55ˈ 45.53S, 18° 38ˈ 31.16E. The nature of the structure in this location ensured control of the environment in which the experiment was conducted. A 40% Alunet shade cloth was installed 4 m above the ground level, an additional 40% polyethylene black shade was suspended at a height of 2 m to provide acceptable lighting conditions for the shade-loving plants.

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All the propagation material was procured from a single stock plant of *P. tomentosum* situated in a private garden on the outskirts of Scarborough near to Cape Point. Only cuttings taken using homogenous methods; i.e. stem cuttings, ±15 cm long with a stem thickness of approx. 9 mm were used for the experiment. Fifty cuttings were made in order to ensure that the minimum number of 35 rooted plants were available for the test. Once rinsed with municipal water the cuttings were placed into propagation trays containing sterile rooting media (Lawrence, 2002) made up of equal parts river sand and perlite to allow for ample drainage. Polystyrene plug trays which allow for isolated rooting to take place were used; these trays were placed onto a hot bed in a climate controlled glasshouse. The cuttings were sprayed twice weekly with fungicide (BIOGROW COPPER-SOAP, P/Bag X 15, Suite 115, Hermanus, Western Cape, RSA, 7200) mixed with municipal water at 20 ml/l to discourage microbial pathology. After 6 weeks, the cuttings had developed roots and were transplanted into 10 cm plastic pots, the growth media consisted of 1 part river sand, 1 part coconut peat, and ¼ part sifted compost. These plants were placed in a grid layout 50–100 cm away from an evaporative cooling pad which ensured cool conditions and ample ventilation. The plants were hand watered sparingly, fungicide application continued at the same frequency mentioned above with the addition of NITROSOL® (EFEKTO, PO Box 652147, Benmore, Johannesburg, Gauteng, 2010) at 5 ml/l of municipal water.

5.3.3 Hydroponic experiment

Once the plants reached the desired degree of homogeneity they were removed from the 10 cm pots, thoroughly rinsed with municipal water, and transplanted into 12.5 cm pots filled with lightweight expended clay aggregate or LECA® (PO Box 3749, Office 519, Galadari Building, Baniyas Street, Dubai, UAE). LECA® was the medium of choice as it is chemically inert, lightweight and maintains structural integrity (Boudaghpour & Hasemi, 2008) making LECA® ideal for a DWC system. Eleven additional holes of 8 mm diameter cut out from the lower third of each pot to allow for root expansion, the pots were placed with their lower portions were submerged in the nutrient solution, while the upper part of the pots were held in place by the lids of the plastic reservoir containers.

Each treatment had an individual reservoir; ADDIS® (Sacks Circle, Bellville, CT, 7535) ‘Roughtote’ 150 l storage boxes fitted with extra drainage valves were used for this purpose. The hydroponic solution was formed by adding NUTRIFEED™ (Manufactured by STARKE AYRES Pty.Ltd. Hartebeesfontein Farm, Bredell Rd, Kaalfontein, Kempton Park, Gauteng, 1619) to municipal water at 90 g per 150 l to achieve an electrical conductivity (EC) ranging from 700–1000 µS/cm (Anonymous, 2015d). EC was measured every third day with an ORBECO HELLIGE® (6456 Parkland Dr, Sarasota, FL 34243) series 150 multiparameter meter.

A randomized block design, made up of 16 replicates each with 9 individual plants was used to study the effects of various methods of including oxygen into the hydroponic solution on *P. tomentosum*. After plants were introduced to the DWC system a half strength nutrient solution was used to fill the reservoirs to allow for acclimatization within the new environment. Treatments began on the 15th of April 2016 and the experiment was conducted over a period of 74 days. Every 9 days the system was flushed; i.e. the nutrient solution was renewed (Harris, 1992). The pH of the solution was measured with an ORBECO HELLIGE® series 150 multiparameter meter every third day, adjusted and kept within a range of pH 5.5–6.5. Due to the high buffering capacity offered by comparatively large bodies of water there was little deviation from the range mentioned above.

5.3.4 Treatment application

The separate treatments were comprised of different methods of aerating and dissolving oxygen into the nutrient solution, as described below; the treatments' codes are followed by their definitions. Each treatment has been allocated a number for the purposes of discussion.

- (1) C = Passive aeration in the form of gaseous exchange at the surface of the nutrient solution (Kratky, 2010); this is the treatment that was used as the control.
- (2) V = Vortex oxygenator.
- (3) AP = One air-pump attached to two aquarium aeration stones in the reservoir/ sump.

- (4) APV = Combination of air-pump with two aquarium aeration stones and a vortex oxygenator.

Each individual reservoir contained a recirculating pump which operated 24 hr/day (LIFETECH AP1000 aquarium pump AC220-240V, H:0.65 m, 400 l/hr), in the vortex treatments this pump was fitted to the vortex device - in the others the pumps were not connected to any piping. The pump curve specifications were used to determine the head and flow-rate relationship, the pumps were adjusted to account for the head of pressure required to lift water into the funnels whilst still maintaining a homogenous flow rate with the other treatments.

5.3.5 Oxygenation technology

5.3.5.1 Air-pumps

DARO™ twin aquarium air pumps (DARO™, 7 Dwyka Street, Stikland Industria, Bellville, 7530) were used to supplement oxygen in the solution of treatments 1 (APVHa) and 2 (APHa). The air pumps were connected to two ‘air-stone’ dispersers within each of the selected treatment reservoirs with flexible LDPE tube.

5.3.5.2 Vortex oxygenation

Each vortex oxygenation device was constructed by inserting a plastic funnel (300 mm long, with 200 mm opening at the base tapering to 32 mm hole at the opposite end), with the larger opening facing upwards, into a PVC pipe (110 mm diam.) which provided structural support. A fish tank pump placed at the bottom of the PVC support was attached to a 700 mm length of class C low density polyethylene (LDPE) with a diameter of 15 mm that was secured to the outside of the funnel. Two 15 mm 90^0 elbows were attached to the end of this length of pipe and fed through the upper part of the funnel at an angle to deliver nutrient solution tangentially; this pipe is referred to as the inlet. At the lowest part of the funnel a 1 inch gate valve restricted the flow of solution as it drained from the funnel (this is referred to as the outlet); by manually adjusting this valve the formation of vortices in the solution above was facilitated. Another length of LDPE (12 mm diameter) was fitted 27 mm below the inlet and functioned as an over-flow, when setting the rate of flow through the outlet and during periods of low atmospheric pressure a rise in the level of the solution within the funnel occurred – the over-flow pipe directed surplus solution back into the reservoir directly.

5.3.6 Determination of plant growth

5.3.6.1 Plant weight

Weight of plants was measured before planting using a standard laboratory scale to ensure homogeneity within the sample. Post-harvest, shoot and root systems were separated and the fresh/ wet weight recorded. The plant material was then oven dried at 55^{o}C in a LABTECH™ model LDO 150F (Daihan Labtech India. Pvt. Ltd. 3269 Ranjit nagar, New Dehli, 110008) oven until weight stabilised; this dry weight was measured and recorded. The difference between the wet and dry weights correlated with the amount of water held within the plants' tissues.

5.3.6.2 Plant height and root length

Before transplanting, the plants' shoots and root length were measured and recorded to ensure homogeneity. Length of shoots was determined by using a standard metric tape measure held alongside the plants with the zero-mark placed at the level of the highest root. This same point was used as the zero point to determine the length of the root system. A similar procedure was carried out post-harvest.

5.3.6.3 Leaf count

Measurements were taken on the same days as plant heights were measured. Leaf numbers for individual plants were manually counted and recorded. Buds that had not opened were not included in this data set, buds which had begun to open were included.

5.3.6.4 Dissolved oxygen content

Once a week throughout the experimental period the DO content within each treatment's reservoir was measured using an ORBECO HELLIGE® series 150 multiparameter meter. The probe was inserted into the upper portion of the nutrient solution through an inspection hole in each respective reservoir lid. The DO probe is capped which a gas permeable membrane and contains and electrolyte solution which facilitates the formation of an electro-chemical gradient enabling the flow of oxygen from the aqueous solution once the probe is inserted. Within the probe were two electrodes which sensed the amount of electrical current passing through a charged wire before and after being exposed to the electrolyte mixture; the charge disturbance is indicative of the amount of oxygen. The meter is capable of measuring oxygen concentrations within a range between 0–20 mg/l of water (Anonymous, 2015b).

5.4 Statistical Analysis

All data was analysed using one-way analysis of variance (ANOVA), using the computing software program STATISTICA 13. Occurrence of statistical difference will be determined by using the Fisher Least Significance Difference (L.S.D.) at values of $P \leq 0.05$; $P \leq 0.01$ and $P \leq 0.001$ levels of significance (Steel & Torrie, 1980).

5.5 RESULTS AND DISCUSSION

5.5.1 DO

Treatment 4 (APV) had the highest mean DO readings (11.16 mg/l) of the experiment (data not shown); although not greater at a statistically significant level (17.5%) than the control which had the lowest mean DO content (9.2 mg/l), this did influence growth parameters. All treatments yielded DO levels higher than the minimum level for plant requirements (3 mg/l) as determined by Bonachela *et al.* (2010). There was no significant difference observed in level of EC or water temperature of the different treatments (data not shown); effects on DO levels and plant responses can therefore be correlated with the various methods of oxygenation. Kessler (2015) mentioned that vigorous growth of a wide range of plant species can be achieved by supplying DO at 9 mg/l. Although the mean value of DO content of the control was above 9 mg/l; the deviation from the mean indicates levels dropped to 8.15 mg/l at certain times, therefore plants were subjected to sub-optimal levels of DO at points within the duration of the experiment.

5.5.2 Plant height

Plant height (shown in Fig. 5.1) of treatment 3 (AP) was the greatest (532.11 mm) and significantly different (P ≤0.05) to treatment 4 (APV) which had the lowest mean (447.22 mm). The control (treatment 1) had the second highest mean value (512.11 mm); this was greater and significantly different (P ≤0.05) to treatment 4 (APV) however did not differ significantly from treatment 3 (AP) or treatment 2 (V). Although treatment 4 (APV) had the highest DO mean value (11.16 mg/l) this did not differ significantly to that measured in treatment 3 (AP). Although the DO values of the treatments aerated by vortex oxygenators were higher than the control (treatment 1) it is possible that limited circulation within the reservoir due to the pump-vortex oxygenator reticulation system resulted in plants situated further from the outlet (Figure 5.4) being exposed to solution which had depleted levels of DO. Zeroni *et al.* (1983) found that levels of DO within the solution of a deep water hydroponic system decreased proportionally with distance from the point of aeration, plants closer to the aeration point could metabolise the oxygen before the plants situated further away were exposed to the solution. The findings of this study agree with these results.

5.5.3 Leaf count

Treatment 3 (AP) had the highest (56.33) leaf count mean value (Fig. 5.2) and differed significantly (P ≤0.05) from treatments 2 (V) and 4 (APV). The control (treatment 1) yielded a leaf count mean value (49.55) that was lower than that for treatment 2 (AP) but not significantly different when compared to this or the other treatments (Fig. 5.2). This outcome suggests that shoot growth of *P. tomentosum* was not adversely affected by sub-optimal DO levels (recorded in the control treatment) within the experimental period.

5.5.4 Plant weights

Fresh and dry shoot weights are shown in table 5.1; both the control (treatment 1) and treatment 3 (AP) showed significantly higher fresh ($P \leq 0.05$) weights compared to treatment 2 (V). The control (treatment 1) fresh shoot weight mean was higher than treatment 4 (APV) and lower than treatment 3 (AP), but did not differ significantly from either of these two treatments. The dry shoot weight mean of the control (treatment 1) was the highest recorded (13.7 g) and differed significantly ($P \leq 0.01$) from both treatment 2 (V) and 4 (APV), although this mean was slightly higher than the mean of treatment 3 (AP) no significant difference was detected.

Treatment 3 (AP) had the heaviest fresh (25.24 g) and dry (1.96 g) root weights although not significantly different to the other treatments. The dry root weight percentage of treatment 2 (V) was higher than the other treatments although not significantly so. The control (treatment 1) had the lowest root weights compared with other treatments but were not significantly different from the other treatments; due to the limited duration of the test it is not possible to comment on the prolonged exposure of *P. tomentosum* roots to sub-optimal levels of DO.

5.5.5 Plant root length

Root length (data not shown) was affected in an inversely proportional manner when considering the level of DO (11.16 mg/l) in treatment 4 (APV) these roots were the shortest (398.2 mm). The control (treatment 1) produced the longest roots (583.5 mm), although difference was not significant when analysed statistically. This indicates *P. tomentosum* may be able adapt to low-oxygen conditions to some extent for a certain period by maximizing root surface area, Armstrong (*et al.,* 1991) describes this adaptive process in other plants.

5.5.6 Visual observation of roots

Figure 5.3 shows a visual comparison of the root systems from the different treatments. Treatment 3 (AP) were the densest and most developed, filling a greater portion of the growing pot region than the control (treatment 1) and other two treatments. The smell given off by roots of the control plants on harvesting was indicative of the occurrence of anaerobic respiration; the colour was visibly more brown than what was observed in the aerated treatments which indicates roots were not as healthy as in the other treatments (roots in treatments 2/V, 3/AP and 4/APV showed no visible signs of discolouration and no detectable scent of anaerobic respiration). Anaerobic respiration in roots results in the accumulation of harmful by-products such as ethanol and acetaldehyde (Drew, 1983) which lead to the deterioration of roots in plants which are intolerant to oxygen deficiency. If the experiment had been run for a longer time-period it is possible that the control plants' roots may have exhibited increased stress symptoms.

5.6 CONCLUSION

Although the roots of the control (treatment 1) plants appeared to be less healthy than those in the actively aerated treatments (2/V, 3/AP & 4/APV) the shoots were not negatively affected by sub-optimal levels of DO within the experimental period. Plant height and leaf count were increased by aerating the solution with an air-pump connected to 'air-stone' dispersers in treatment 3 (AP). Production of *P. tomentosum* plants in deep water culture hydroponics is possible without additional aeration if the surface of the nutrient solution comes into sufficient contact with the atmosphere, however increased plant height and number of leaves can be achieved with the application of air-pumps.

5.7 ACKNOWLEDGEMENTS

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

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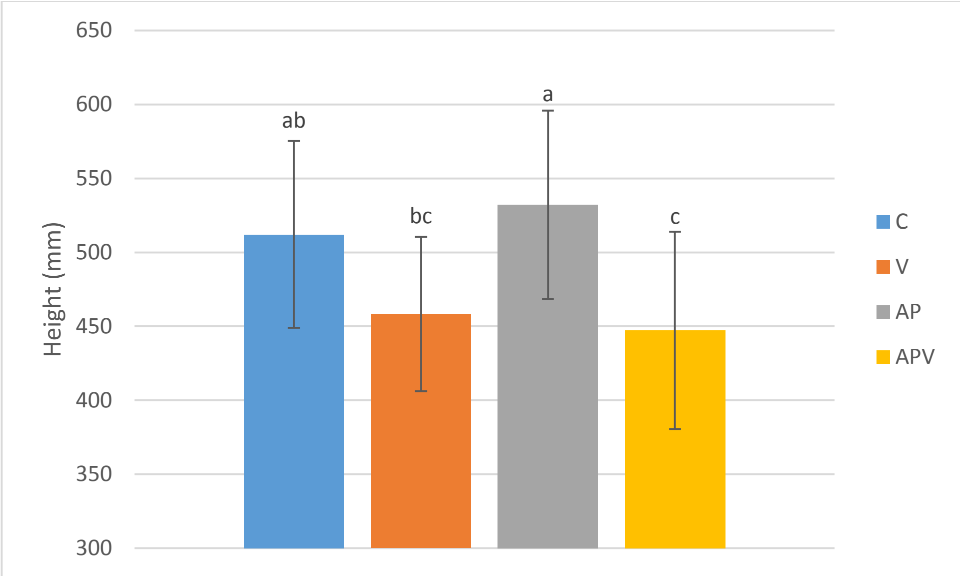

Figure 5.1

Effects of different methods of oxygenation; treatment 2 (V/vortex oxygenation), 3 (AP/air-pump) and 4 (APV/air-pump vortex oxygenator combination) on plant height compared with the control (treatment 1). Bars indicate mean values ±SD. The mean values represented by bars annotated with different letters differ significantly at P ≤0.05 as calculated by Fisher's least significant difference. The one-way ANOVA F-statistic is 4.004.

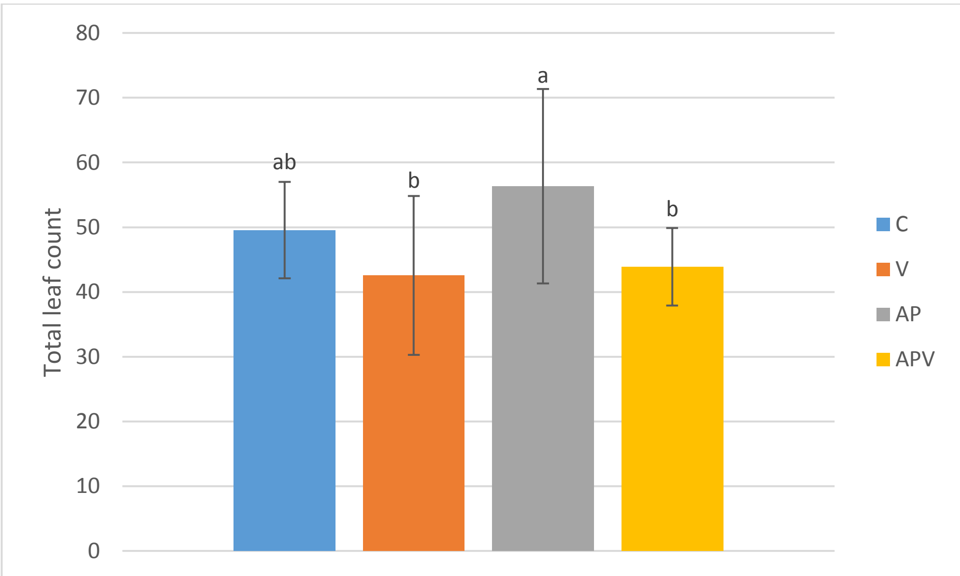

Figure 5.2

Effects of different methods of oxygenation; treatment 2 (V/vortex oxygenation), 3 (AP/air-pump) and 4 (APV/air-pump vortex oxygenator combination) on the total number of leaves compared with the control (treatment 1). Bars indicate mean values ±SD. The mean values represented by bars annotated with different letters differ significantly at P ≤0.05 as calculated by Fisher's least significant difference. The one-way ANOVA F-statistic is 0.0432.

Figure 5.3

Photographs showing the visual comparison of quality and development of roots within the different treatments at the time of harvest. The treatments are shown from left to right in numerical order; the control (treatment 1), treatment 2 (V/vortex oxygenation), treatment 3 (AP/air-pump injection) and treatment 4 (APV/combination of air-pump and vortex oxygeanation).

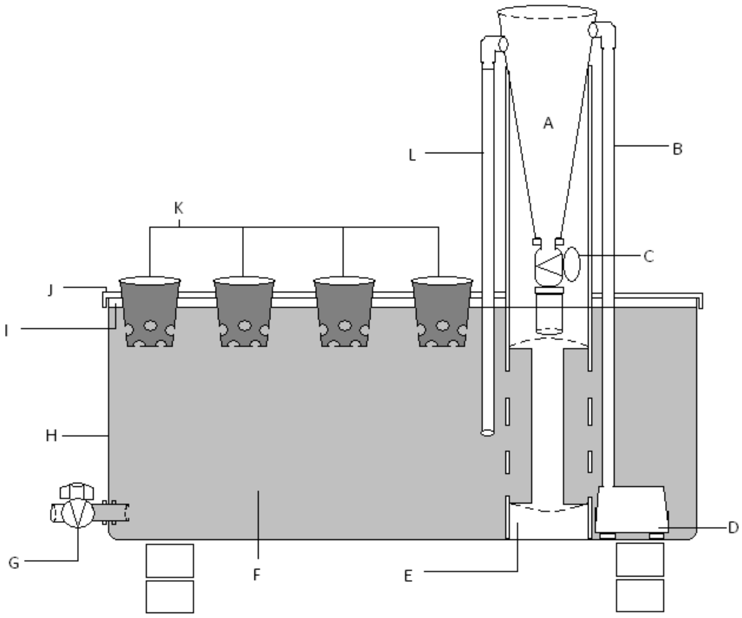

Figure 5.4

A schematic diagram of the DWC system with individual components annotated alphabetically, a vortex oxygenation unit is shown on the right-hand side of the diagram. The parts which have been labelled are; A: funnel/venturi, B: inlet, C: gate valve (outlet), D:

pump, E: PVC support, F: nutrient solution, G: flush-valve, H: reservoir, I: air-gap, J: lid, K: pots, L: over-flow.

Table 5.1

Effects of different methods of oxygenation; treatment 2 (V/vortex oxygenation), 3 (AP/ air-pump) and 4 (APV/ air-pump vortex oxygenator combination) on the post-harvest weight of plant material compared with the control (treatment 1).

Treatment	Wet shoot weight (g)	Dry shoot weight (g)
Control	107,91±20,39ab	13,7±13,12a
V	85,21±15,81c	9,85±1,89b
AP	110,22±27,07a	13,51±3,69a
APV	86,722±6,98bc	9,59±2,31b
ANOVA F-statistic	3,0322 *	5,6239 **

Mean values ±SD are shown in columns. The mean values followed by different letters are significantly different at P ≤0.05 (*) and P ≤0.01 (**) as calculated by Fisher's least significant difference.

CHAPTER 6

GENERAL DISCUSSION AND CONCLUSSION

6.1 GENERAL DISCUSSION AND CONCLUSSION

In chapter 3 it was found that the highest chlorophyll content mean value was achieved through a combination of high frequency application of hydrogen peroxide (H_2O_2), an air-pump and vortex oxygenation; treatment APVHa. The same frequency application of H_2O_2 alone produced the highest level of dissolved oxygen (DO) but the mean chlorophyll content value was slightly lower and had a larger variance than when combined with an air-pump, and vortex oxygenation. These results indicate that to produce more photosynthetically active *P. tomentosum* plants in deep water culture hydroponics it is advisable that application of high frequency dosing of H_2O_2 be combined with other methods of oxygenation. When H_2O_2 was combined with an air-pump a significantly higher content of chlorophyll was produced than the control, although not as high as when combined with an air-pump and vortex oxygenation. The increase in chlorophyll content could affect the essential oil yield however additional research is required to establish the link between chlorophyll content and the production of essential oils in *P. tomentosum.* Further research is also necessary to investigate the effectiveness of the oxygenation practice on chlorophyll production on other plant species.

In chapter 4 it was found that the efficiency of supplying oxygen in deep water culture hydroponics through the application of H_2O_2 is affected when combined with other methods of oxygenation. DO levels measured in the combination treatments were all lower than that of the low frequency dose of H_2O_2. This was attributed to the volatilization of excess oxygen molecules due to the degree of turbulence caused by the bubbling action of air pumps and increased exposure of the solution surface to the atmosphere as well as the fluid-mixing action of vortex oxygenation. At the highest degree of turbulence; the loss of O_2 (supplied by H_2O_2) through volatilization was somewhat compensated by increased diffusion of oxygen from the atmosphere into the solution. Application of H_2O_2 alone yielded the highest readings of fresh shoot and root weights, as well as the greatest dry shoot weights although none of these results were statistically different from the treatment where air-pump injection, vortex oxygenation and application of H_2O_2 were all combined. The latter treatment yielded the highest readings for dry root weights suggesting that root development was benefited by the combination of H_2O_2 with both vortex oxygenation and an air pump. Further research is necessary to establish the efficacy of these oxygenation techniques on other plant species.

In chapter 5 it was found that although the roots of the passive aeration treatment (C) appeared to be less healthy than the actively aerated treatments, the shoots were not negatively affected by sub-optimal levels of DO within the experimental period. Plant height and leaf count were increased by aerating the solution with an air pump connected to 'air-stone' dispersers treatment (AP). It is thus recommended that the production of *P. tomentosum* plants in deep water culture hydroponics is possible without additional aeration if the surface of the nutrient solution comes into sufficient contact with the atmosphere, however increased plant height and number of leaves can be achieved with the application of air-pumps which could be beneficial in the production of essential oils.

This study has found that *P. tomentosum* is a suitable plant for DWC hydroponic production, and that oxygenation/aeration of the nutrient solution affected the growth responses of *P. tomentosum*. The research has shown that beneficial effects of oxygenation on root development were observed and shoot growth was increased in treatments with higher levels of DO. When using H_2O_2 to oxygenate the nutrient solution, the development of roots and the chlorophyll content were increased when combined with air-pump injection and vortex oxygenation. Additional research is required to investigate the relationship between production of essential oils related to the chlorophyll content of *P. tomentosum*, further studies are necessary to investigate the effects of the various methods of oxygenation in DWC on other plant species.

CHAPTER 7

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