Improved growth of hydroponically grown rough lemon (*Citrus jambhiri lush*) seedlings treated with kelp and vermicast extracts

by

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Submitted in partial fulfilment of the requirements for the degree
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Declaration

I, Lindsay Muchena do hereby declare that this dissertation, which I do hereby submit for the degree of Master of Science in Horticulture at University of Pretoria, is my own work and has never been submitted by myself at any other academic institution. Except where duly acknowledged, the research work reported herein is as a result of my own investigations.

L Muchena

Signature:

June 2017

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Improved growth of hydroponically grown rough lemon (*Citrus jambhiri lush*) seedlings treated with kelp and vermicast extracts

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Abstract

A substantial number of South African citrus trees are rooted on rough lemon rootstocks. In order to ensure the successful establishment of a high percentage of young citrus trees, it is necessary to ensure that farmers are provided with young vigorous nursery trees that have a large and healthy root system. Vermicast extracts (VE) and liquid extracts from the giant seaweed, *Ecklonia maxima* (EM), have been used on various crops over the past decades to promote plant growth and development. It is widely reported that plant growth, seedling vigour, water-use efficiency and nutrient-uptake of these crops improves with EM and VE treatments, thereby reducing excessive fertiliser applications. With the improved uptake of nutrients such as nitrogen (N), which is the main component of chloroplasts and proteins involved in the Calvin cycle, it is likely that the application of EM and VE to plants could lead to an improvement in the photosynthetic capacity of plants. The benefits from applications of EM and VE have been attributed to the presence of plant growth regulators/hormones (PGRs) such as auxins and cytokinins. Since seaweed extracts are manufactured using different methods, some of which may include the use of elevated temperatures, acid and/or alkaline hydrolysis of plant material,

it is not surprising that different seaweed extracts may contain various levels of PGRs which can lead to dissimilarities in their growth promoting effects. A hydroponic experiment was conducted to test if locally produced EM from two different suppliers and VE would increase root length, root volume, root dry mass, stem diameter, plant height, chlorophyll content, nutrient uptake and photosynthetic capacity of rough lemon seedlings. The other objective of this study was to determine if there was any difference in growth promoting effects of EM obtained from two different supplies due to the difference in the levels of plant growth regulators they contain. Since the action of EM and VE is dose dependant, it was also necessary to determine the optimum concentration of EM and VE for the growth of rough lemon seedlings.

This experiment was conducted in a glasshouse at the University of Pretoria experimental farm. Liquefied *Ecklonia maxima* from different manufactures; *Ecklonia maxima* - Kelpak® and Ecklonia maxima – Afrikelp® (EM1 & EM2 respectively); and vermicast extracts (VE) were applied separately to rough lemon seedlings as a drench (at 0.5%, 1% and 2% dilutions), at 14 day intervals. Deionized water and full strength Hoagland solution were used as controls. Root volume, root length, stem diameter, plant height, chlorophyll content and photosynthesis were measured weekly for 12 weeks. At the end of the trial, the average root dry mass and shoot dry mass for each treatment was determined. Upon termination of the trial, oven dried leaf samples were analysed for nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca). N analysis was done at an accredited commercial lab whilst P, K and Ca analyses were done at the University of Pretoria using ICP-OES.

Amongst the observed effects of EM and VE applications were the development of a vigorous root system. Rough lemon seedlings treated with EM and VE had significantly greater root volume and root dry mass than the Hoagland solution and deionised water controls. When all the EM and VE treatments were compared, the 2% VE resulted in the highest increase in root volume, root dry mass and shoot dry mass. EM1 and EM2 applied at lower concentrations

(0.5% and 1%) stimulated more root growth (root length, root volume and root dry mass), whilst the higher concentration (2%) negatively affected root development. Although the control had the highest significant root length, this was at the expense of dry mass accumulation as it had the least significant root dry mass. For EM1s, 1% EM1 induced the highest increase in stem diameter. Whilst 0.5% EM1 induced the highest increase in chlorophyll content, the differences between the Hoagland solution and 2% EM1 were not significant. The 1% EM2 significantly had the highest plant height, followed by EM1 and VE applied at the same concentration.

Results indicate that the different kelp and VE treatments resulted in an increase in root length, root dry mass, root volume, above ground dry matter and nutrient uptake. VE resulted in the largest increase in the root parameters measured while differences between the EM treatments were found. Root growth was also influenced by application concentration. A possible explanation for these differences in root growth between VE and EM's is that VE contains a higher NAA concentration than the other treatments as shown by some laboratory analyses results that were obtained. For the VE, the optimum concentration for root growth and above-ground plant growth was the 2% dilution, whilst for EM1 and EM2; the 1% dilutions generally exhibited better plant growth. The 2% dilutions for both EM1 and EM2 were mostly inhibitory to plant growth.

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List of abbreviations

AgriLASA	Agricultural Laboratory Association of Southern Africa
ANOVA	Analysis of variance
AtIPS1	Arabidopsis thaliana gene induced by phosphate starvation1
BL	Brassinolide
CKs	Cytokinins
CS	Castasterone
CBM	Cell burst method
CMP	Cold micronization process
dhZ	Dihydrozeatin
dhZR	Dihydrozeatin riboside
DW	Deionized water
EM	*Ecklonia maxima*
EM1	*Ecklonia maxima* – Kelpak®
EM2	*Ecklonia maxima* – Afrikelp®
GA1-GA7	Gibberellin1-gibberellin7
GS-MS	Gas chromatography-mass spectrometry
HNO_3	Nitric acid
HS	Hoagland's nutrient solution
HPLC-ESI-MS/MS	High performance liquid chromatography electrospray ionization tandem mass spectrometry
IAA	Indole-3-acetic acid
IAAGlu	N-indole-3-yl-acetyl glutamic acid
IBA	Indole-3-butyric acid
ICP-OES	Inductively coupled plasma optical emission spectrometry

iP	N^6-isopentenyladenine
2-iP	Isopentyladenine
iPA	Isopentyladenosine
KIN	Kinetin
LCMS	Liquid chromatography mass-spectrometry
LC-MS/MS	Liquid chromatography tandem mass spectrometry
NAA	Naphthalene acetic acid
NPA	N-(1-napthyl) phtalamic acid
PAs	Polyamines
PAR	Photosynthetically active radiation
PPFD	Photosynthetic photon flux density
PGRs	Plant growth regulators
SCA	Seaweed concession areas
SWEs	Seaweed extracts
TIBA	2, 3, 5-triiodobenzoic acid
tZ	Trans-Zeatin
VE	Vermicast extracts
Z	Zeatin
Z-O-Glu	Zeatin-O-glucoside
ZR	Zeatin riboside

CHAPTER 1 General introduction

Cultivated citrus trees primarily consist of two combined parts, the rootstock and the scion (Lee et al. 2009). The scion is the fruit-bearing part of the tree whilst the rootstock provides the tree with a root system. The first rootstock to be commercially used in South Africa was rough lemon (Lee et al. 2009). Use of rough lemon as the dominating commercial rootstock dates back to as early as 1906 until the 1970's when commercial use of another type of rootstock, the trifoliate, started (Miller et al. 2003). A significant number of South African citrus farmers (10.88%) are, however, still using rough lemon rootstocks (Citrus Improvement Scheme 2013). In order to guarantee the viability of the South African citrus industry, it is necessary to ensure that farmers are provided with nursery trees of the highest possible quality (Lee et al. 2009). The rise in energy prices and the improved awareness of ecological issues like excessive fertiliser and pesticide use increases the need to find other cheaper, environmentally friendly ways to improve plant growth (Metting et al. 1990). One approach for achieving this is by using agricultural biostimulants. Agricultural biostimulants are non-fertiliser products that have beneficial effects on plant growth (Russo & Berlyn 1992). They increase plant growth and seedling vigour through increased efficiency of water and nutrient-uptake thereby reducing the need for excessive fertiliser applications (Russo & Berlyn 1991). Additionally, they are biodegradable, non-toxic, non-polluting and non-hazardous to humans, animals and birds (Dhargalkar & Pereira 2005). Seaweed extracts (SWEs) derived from kelps and vermicast extracts (VE) produced through vermicomposting are currently being used extensively as agricultural biostimulants (Stirk & Van Staden 1997).

Vermicomposting is the practice of transforming and stabilising organic wastes into humus through the joint action of earthworms and microorganisms (Yuan 2016). The composition of the resulting product, commonly known as vermicast, worm castings or vermicompost is more homogeneous than the source material, has reduced levels of contamination, contains organic acids such as humic and fulvic acids; and has high levels of plant growth regulators (PGRs) and symbiotic microorganisms (Arancon et al. 2006; Edwards et al. 2006; Arancon et al. 2008; Gupta and Garg, 2016). Vermicast extracts (aqueous extracts of vermicompost) have been shown to increase plant growth, nutritive quality and yield by enhancing the mineral nutrient status of plants, enhancing the action of beneficial microorganisms; and by inducing the production of defence compounds that have favourable bioactivities (El-Haddad et al. 2014). In addition, water-extractable PGRs extracted from the vermicompost may also positively affect initial root and plant development (Pant et al. 2009; Lazcano et al. 2010; Wang et al. 2014;). Vermicompost extracts may be produced under aerated or non-aerated conditions and there are various factors which affect the quality of VE namely; quality of water used during the extraction process, aeration, additives, compost stability and brewing time (El-haddad et al. 2014). Hence for the purposes of this study, the VE were obtained from the same manufacturer to control the variation in quality. Many studies have shown that VE enhance plant growth in a wide range of agricultural crops such as tomatoes, strawberries, apples, grapes and green beans (Pant et al. 2009; Lazcano et al. 2010). However, little work has been done to investigate the effects of vermicast extracts on growth and nutrient uptake of citrus seedlings grown under hydroponic conditions. Hence one of the objectives of this study was to test whether additional application of VE on rough lemon seedlings stimulates plant growth and to identify the effects of different concentrations of these VE on the growth of rough lemon seedlings grown hydroponically under controlled greenhouse conditions.

Apart from the VE, SWEs derived from kelp can also be used in agriculture as biostimulants and they will also be tested in this study. The seaweeds are commonly found in the western coast of southern Africa. The western coast of southern Africa is affected by the cold Benguela current as well as the accompanying coastal upswelling that is created by offshore winds thereby supplying nutrient-rich water (Anderson & Lucas 2009). In the southerly Cape of Good Hope seawater temperatures range between 12-13°C whilst as one moves further north towards Walvis Bay, Namibia, temperatures gradually increase up to around 15-16°C (Anderson & Lucas 2009). These nutrient-rich waters are inhabited by highly prolific beds of kelp which mainly comprise of *Ecklonia maxima* (Osbeck) Papenfuss, *Laminaria pallida* Greville ex. J. Agardh, *L. pallida* var. *schintzii and Macrocystis angustifolia* Bory (Stirk et al. 2004). Since 1979, *E. maxima* has been gathered for commercial use at Kommetjie, South Africa and processed at a factory in Simon's Town by Kelp Products (Pty) Ltd. Roughly 100 t dry *E. maxima* is harvested annually to make the SWEs, Kelpak® (Stirk et al. 2014). By using a cell burst method (CBM), the washed *E. maxima* are distributed over a sequence of cutters during which they are cut into fine particles. These fine particles are then exposed to high pressure and then passed at high velocity through a low pressure chamber. Energy is released during this process which causes the cell walls to expand until they surpass their elastic limit, thereby bursting and discharging their cellular contents (Stirk & Van Staden 1997). The resulting seaweed extract is then marketed under the trade name Kelpak®. As the CBM does not include any use of heat, chemicals and dehydration procedures which may denature the active components in the kelp, it results in the production of a high value product (Papenfus et al. 2012). Contrary to this, other seaweed extracts are produced using detrimental acidic or alkaline solutions at elevated temperatures. Afrikelp® is produced using a cold micronization process (CMP) by Afrikelp (Pty) Ltd, Milnerton, South Africa. By using CMP, kelp is treated without using detrimental chemicals and elevated temperatures followed by being mechanically processed to release the natural PGRs into a solution. The solution is then filtered to remove any particles greater than 30µm in size to produce the final product which is sold as Afrikelp® (http://www.afrikelp.com/?m=3).

Seaweed extracts such as Kelpak® and Afrikelp® have been applied on several types of crops in South Africa such as flowering plants, vegetables, monocotyledons and tree crops. They induce beneficial effects which include enhanced seed germination, seedling establishment, flowering, crop and fruit yield, shelf-life and resistance to pests and diseases (Stirk & Van Staden 1997; Papenfus et al. 2012; Sharma et al. 2014). Physiologically, SWEs improve nutrient partitioning and mobilisation, chlorophyll content and leaf area, development of a vigorous root system and retard leaf senescence (Stirk & Van Staden 2004). The relatively low application rates required mean that the beneficial effects cannot be accounted for by an increase in the supply of macro and micronutrients available in the extracts (Crouch & Van Staden 1993; Craigie 2011). Due to the wide scope of physiological effects induced by SWEs and the low concentrations required, it is thought that several PGRs (e.g. auxins and cytokinins) and other elicitor molecules (e.g. polysaccharides and oligomers) are the active components (Crouch et al. 1990; Crouch & Van Staden 1992; Crouch & Van Staden 1993; Khan et al. 2009). Recently, some cytokinins (free bases, O-glucoside derivatives and aromatic cytokinins) and auxins (indole-3- acetic acid, four amino acid conjugates and three other conjugates) (Stirk & Van Staden 2004, Lötze & Hoffman 2016) and polyamines have been detected in Kelpak® (Papenfus et al. 2012). Overally, the cytokinin content was 5 pmol ml^{-1} of Kelpak® whilst the auxin content was much higher at 34 pmol ml^{-1} Kelpak® (Stirk et al. 2014).

A number of studies have shown that horticultural industries ought to completely exploit and adopt SWEs in their production systems (Khan et al. 2009; Mattner et al. 2013). However, applied knowledge to increase the consistency of crop responses to SWEs and better understanding of the mechanisms involved is still needed to achieve this. Information on optimum application rates, dilutions, timing and application methods for specific crops in different environments still needs to be developed (Craigie 2011). For example, few researchers have examined the effects of SWEs rough lemon seedlings grown hydroponically. Often, the SWEs are sold with a certain guaranteed hormonal content such as 100 ppm cytokinin content, but this can differ with kelp species, season and commercial extraction method utilised (Mattner et al. 2013; Bulgari et al. 2015). The purpose of this study was also to test whether additional application of SWEs from *E. maxima* obtained from two different manufacturers stimulates plant growth in rough lemon seedlings grown hydroponically and if there is a difference in growth promoting effects of the two products owing to the different manufacturing methods. This study was also designed to identify the effects of different concentrations of these seaweed extracts and to identify their optimum concentration for growth of rough lemon seedlings grown hydroponically under controlled greenhouse conditions.

1.1 Hypotheses

The following hypotheses were tested:

i. Commercial kelp and vermicast extracts contain auxins and cytokinins which are thought to improve growth of rough lemon seedlings.
ii. An increase in concentration of commercial kelp will lead to an increase in root length, root volume, root dry mass, stem diameter, plant height, chlorophyll content, nutrient uptake and photosynthesis of rough lemon seedlings.
iii. A difference exists in the growth-promoting effect of the two commercial kelp products due to the difference in manufacturing procedures used, with *Ecklonia maxima* – Kelpak® (EM1) being expected to perform better than *Ecklonia maxima* – Afrikelp® (EM2). Where EM1 and EM2 are liquid seaweed products made from the giant seaweed, *Ecklonia maxima* (EM) obtained from two commercial companies situated in the Western Cape, Kelpak® (Pty) Ltd and Afrikelp® (Pty) Ltd respectively.
iv. An increase in the concentration of vermicast extracts will lead to an increase in root length, root volume, root dry mass, stem diameter, plant height, chlorophyll content, nutrient uptake and photosynthesis of rough lemon seedlings.

v. There will be an optimum concentration of commercial kelp and vermicast extracts for root length, root volume, root dry mass, stem diameter, plant height, chlorophyll content, nutrient uptake and photosynthesis of rough lemon seedlings.

1.2 Objectives

The objectives of this study were:

1.1. To determine the auxin and cytokinin content of different commercial kelps and vermicast extracts.

1.2. To determine if an increase in the concentration of commercial kelp from 0.5%, 1% up to 2% would increase root length, root volume, root dry mass, stem diameter, plant height, chlorophyll content, nutrient uptake and photosynthesis of rough lemon seedlings.

1.3. To compare the effect of the two commercial kelps on root length, root volume, root dry mass, stem diameter, plant height, chlorophyll content, nutrient uptake and photosynthesis of rough lemon seedlings.

1.4. To determine if an increase in the concentration of vermicast extracts from 0.5%, 1% up to 2% would increase root length, root volume, root dry mass, stem diameter, plant height, chlorophyll content, nutrient uptake and photosynthesis of rough lemon seedlings.

1.5. Identify the optimum concentration of commercial kelp and vermicast extracts for root length, root volume, root dry mass, stem diameter, plant height, chlorophyll content, nutrient uptake and photosynthesis of rough lemon seedlings.

CHAPTER 2 Literature review

2.1 Introduction

Seaweeds are multicellular, macroscopic marine algae which thrive in coastal ecosystems of the world (Sangha et al. 2014). Although a few selected species are cultivated on land-based systems or in open water facilities for commercial use, most of them exist naturally on their own attached to hard substrata. About 10 000 seaweed species exist which are classified into three phyla: Chlorophyta, Phaeophyta / Ochrophyta and Rhodophyta; that is the green, brown and red seaweeds respectively (Khan et al. 2009; Papenfus et al. 2012). The brown seaweed species are the second most available group with an estimated 2000 species belonging to this group and they are the type mostly used in agriculture (Sangha et al. 2014). Among them are species such as *Ecklonia maxima*, *Ascophyllum nodosum*, *Laminaria spp*, *Fucus spp*, *Turbinaria spp* and *Sargassum spp* (Khan et al. 2009; Sangha et al. 2014; Sharma et al. 2014). About 15 million metric tonnes of seaweed extracts (SWEs) (liquid and powder forms) are manufactured every year and most of these are used as biostimulants to enhance plant growth, yields and fertiliser use efficiency (Russo & Berlyn 1992). In addition, they are also used to improve tolerance to various biotic and abiotic stresses such as water, nutrient and salinity stress (Russo & Berlyn 1992). In addition to SWEs, various vermicast extracts (VE) have been widely used to promote plant growth and resistance to adverse growing conditions. Vermicast extracts can be described as aqueous extracts of vermicompost in which particulate organic matter, microorganisms, nutrients and PGRs are passed from the vermicompost to the extract either through active aeration of non-aerated extraction for a defined period of time at room temperature (Scheuerell & Mahaffee 2002; Scheuerell & Mahaffee 2004). Liquid extracts of compost have been used in agriculture since 1920 and experimental evidence demonstrated that application of VE in particular, enhanced nutritional quality, yields, suppressed plant diseases; and increased overall plant health (Scheuerell & Mahaffee 2004; Edwards et al. 2006). Application of VE enhances nutritional quality, plant health and yields through supplying plant growth regulators (PGRs), organic acids, microbial biomass, fine particulate organic matter and soluble mineral nutrients to plants and soils (Edwards et al. 2006; Sinha et al. 2010). The mechanisms through which VE suppress plant diseases comprise of antibiosis, competition and induced resistance (Scheuerell & Mahaffee 2002; Scheuerell & Mahaffee 2004). Thus there is clear evidence of protection imparted on different crops exposed to different environmental stresses as well as the bio-stimulatory effect of SWEs and VE, which necessitates further research on their use in citrus nursery tree production.

2.2 Manufacturing of vermicompost extracts

Recently, there has been a growing interest in waste degradation and composting using earthworms as this is proving to be a rapid process which results in the production of a nearly odourless product that is highly nutritive, detoxified and disinfected unlike compost from conventional composting technologies (Sinha et al. 2010). At their optimum performance, most earthworms consume half their body weight of organic waste per day (Visvanathan et al. 2005). The main species of earthworms used for vermicomposting, *Eisenia fetida,* can consume organic waste material at a rate equal to their body weight on a daily basis. By using earthworms, the natural biodegradation and decomposition of organic wastes is increased by approximately 60 to 80% (Sinha 2009). Two main methods are used to produce VE that is, aerated and non-aerated methods. These methods can also be described as active and passive or aerobic and anaerobic methods respectively (Scheuerell & Mahaffee 2002). Both methods include immersing the vermicompost in water for a given period at room temperature. During aerated VE production, the mixture is aerated by pumping air through the water containing vermicompost in order to keep oxygen levels above 5 ppm (Weltzein & Ketterer 1986; Ingham 2005). Humic acid, kelp extract, grain, fish emulsion and sugars are frequently incorporated as additives whilst extracting the aerated extracts to increase microbial activity of the end product (Pant et al. 2009; Sinha 2009). The non-aerated methods are methods which involve no disturbance or minimal disturbance of the mixture during the extraction process. The non-aerated method may require 1-2 weeks steeping time unlike the aerated method which requires only 1-2 days steeping time and results in reduced odour problems (Ingham 2005). However, the non-aerated production method does not involve any specialised equipment besides a steeping vessel and is associated with low energy input or low costs whilst the aerated method entails constant aerating and stirring of great volumes of liquid. A number of reports have shown that aerated extracts can be inconsistent in their effect on plant growth and disease control compared to non-aerated extracts whilst other studies have shown that aerated extracts can be more consistent. There are also various factors which affect the quality of VE such as the brewing time, aeration, additives and the quality of water used to produce VE (Ingham 2005). Hence for the purposes of this study, the VE were obtained from one manufacturer to avoid any discrepancies due to the above-mentioned factors.

2.3 Global distribution of kelps

Seaweed growth is determined by interactions amongst temperature, nutrient availability, and light. Global seaweed forests grow on shallow rocky coastlines in a mid-latitude group where light and ocean conditions permit their growth and development (Figure 2-1). They comprise chiefly of brown algae in the order Laminariales (Dayton 1985). There are three groups of seaweeds as demarcated by the canopy height of their fronds. The dominant among these is the gigantic seaweed, *Macrocystis* spp. which can grow up to 45m in length and are dominant in the west coasts of South and North America. They are also found in scattered places in the South Pacific Ocean comprising South Africa, New Zealand, southern Australia and a number of sub-antarctic islands (Figure 2-1). Smaller canopy seaweeds comprise of *Alaria fistulosa* in the Pacific coast of Asia and Alaska, *Nereocystis leutkeana* commonly found between Alaska and

Central California; and the Southern Hemisphere kelp, *Ecklonia maxima* which is prevalent in South Africa. Their usual length is around 10m (Dayton 1985). Another group of seaweeds are the “stipitate kelps” which are apprehended above the benthos by firm stipes. They include *Laminaria* spp. from the Pacific Northwest and Europe, *Ecklonia* from New Zealand and southern Australia and *Lessonia* from Chile. Although a few *Laminaria* spp grow up to 10m in length, most of these species are less than 5m in length (Abbott & Hollenberg 1976).

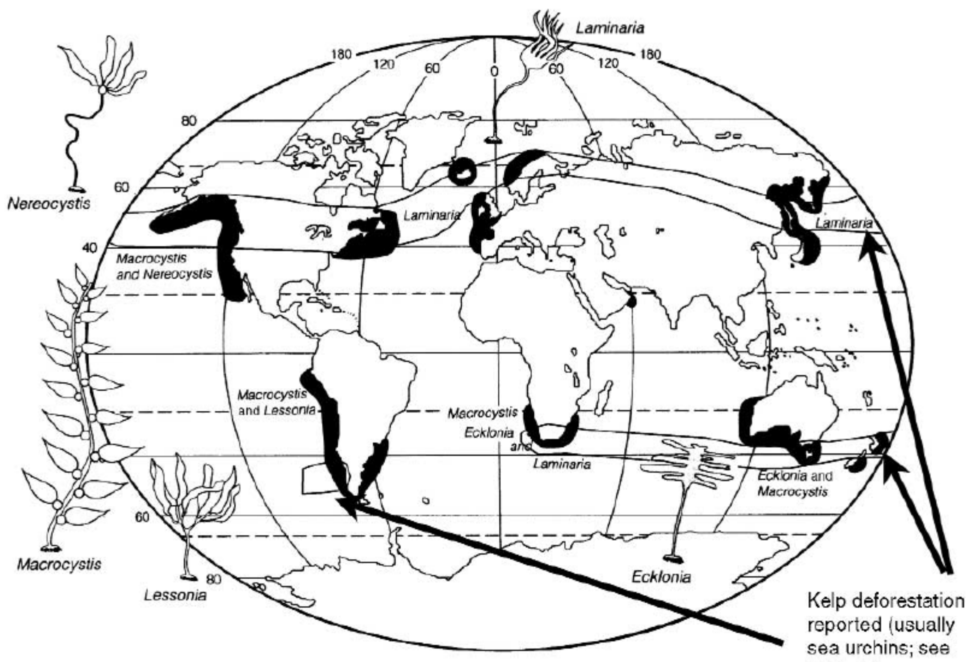


Figure 2-1 Global distribution of kelp forests and the dominant genera (Raffaelli & Hawkins 1996)

Seaweed forests are mostly in the tropics of Cancer and Capricorn alongside the western coasts of southern California to Mexico, Western South Africa, Western Australia and northern Chile to Peru (Figure 2-1). In these areas, cool ocean currents flowing toward the equator, or upwelling direct cool, nutrient-rich water to the seaweed forests. Although seaweeds can grow in sub-Antarctic and Arctic regions, they are less diverse and abundant because of light limitations (Henley & Dunton 1997). Hence they seldom grow beyond latitudes of around 60°.

2.4 Seaweed concession areas in southern Africa

The main region of interest includes Namibia, Ciskei, Transkei and the coasts of the Cape Province of South Africa, which jointly form an economic and geographic continuum. Oceanographically, the region is described as warm temperate with two main divisions, a western subdivision from Cape Peninsula to Walvis Bay, Namibia, characterised by an average annual temperature fluctuating between 12 and 16°C and a southern subdivision from the eastern border of Transkei to Cape Agulhas having average annual temperatures fluctuating between 17.2 and 18.2°C. Both subdivisions are linked by a transition area (Bolton 1986). The section from Cape Peninsula to Walvis Bay is frequently marked by irregular pulses of nutrient-rich cold water (Bolton 1986). Each subdivision contains distinctive flora, hence the seaweeds *Ecklonia maxima* and *Laminaria pallida* are constrained to the western and transition areas, whilst *Gelidium pristoides* is restricted in the southern and transition areas. Figure 2-2 shows the distribution of these and other economically important seaweeds. With the exclusion of Ciskei and Transkei, the 17 Seaweed Concession Areas (SCA) as elected by South Africa for the exploitation of its seaweed resources are also shown in Figure 2-2. All concessions are currently assigned to five commercial companies.

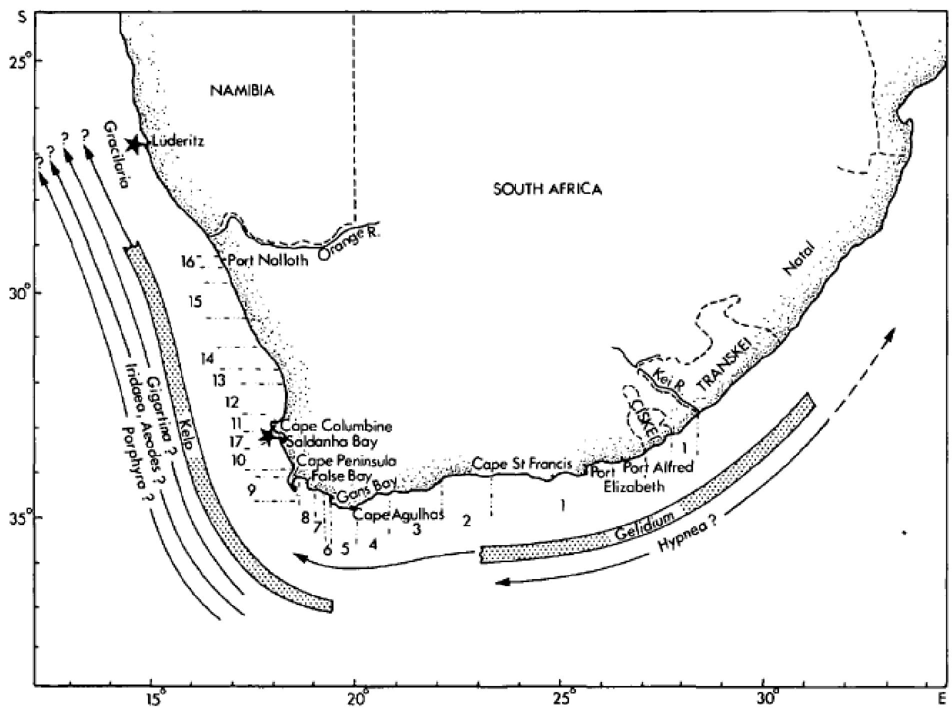


Figure 2-2 Southern Africa map showing seaweed concession areas which are numbered from 1 to 17. Shaded bars or stars show places where certain seaweeds are currently being exploited whilst finer lines indicate areas where there are other potential seaweed resources (Troell et al. 2006).

2.5 Manufacturing of seaweed extracts

The first method for developing liquid extracts from seaweeds was developed in 1949 (Milton 1952). Currently, commercial SWEs are mostly manufactured using the brown seaweeds *E. maxima*, *A. nodosum*, *Laminaria spp.*, *Durvillaea spp.* and *Sargassum spp.* However, species such as *Enteromorpha intestinalis, Fucus serratus, Kappaphycus alvarezii* and *Ulva lactuca* have also been used (Stirk & Van Staden 1997; Rathore et al. 2009). The selection of a particular species is usually governed by the availability of great quantities of biomass rather than the presence of growth promoting elements (Blunden et al. 1978). As an example, about 6-8 tonnes (fresh mass) of *E. maxima* is harvested per day at Kommetjie, South Africa (Figure 2-3) and used for making the liquid SWEs, Kelpak® (Stirk & Van Staden 2006). Generally, the aqueous SWEs differ in colour from dark brownish-black to colourless. Viscosities, solids, odours and particulate matter are also wide-ranging. SWEs are most commonly manufactured by stirring macerated seaweeds in hot water, through acid or alkaline hydrolysis together with steam or without steam, or through physically disrupting the seaweeds by low temperature milling which results in a “micronized” suspension of fine particles (Herve & Roullier 1977; Metting et al. 1990). The micronized seaweed suspensions are mildly acidic and greenish-brown to green in colour. In most cases the resulting extracts have a pH ranging between 7 and 10 (Milton 1952). Formaldehyde is often added to the resulting extracts as a preservative to lessen microbial contamination (Verkleij 1992). The SWEs, Afrikelp® is produced using a cold micronization process (described in Chapter 1). During the acid or alkaline hydrolysis methods an acidic or alkaline solution is used to weaken the cell walls of the seaweeds which results in the releasing of cellular contents into the liquid medium which is then filtered and dried into a water soluble powder (Stirk & Van Staden 1997; Stirk & Van Staden 2006). Another widely used method requires heating the seaweeds with alkaline potassium or sodium solutions. During this process, the reaction temperature is elevated by pressurizing the reaction vessels (Milton 1952). The seaweeds may also be liquefied at ambient temperature. An alternative method is the cold CBM used in the product-ion of Kelpak®. During this process fresh kelp is harvested, milled and homogenised to about 50 µm in diameter (Figure 2-3, Figure 2-4). The milled kelp is then exposed to high pressure (> 40 000 kPa) and distributed at high velocity through a low pressure area in which the quick change in pressure forces the cell walls to inflate and rupture (Figure 2-4). This bursting of the cell walls results in the releasing of the cytosolic constituents into the liquid medium which are obtained from the filtered liquid extracts (Stirk & Van Staden 2016). The main advantage of this CBM is that it does not involve the use of acids, alkalis, organic solvents and heat which may denature some of the active components of SWEs (Stirk & Van Staden 1997).

Figure 2-3 Harvesting of *E. maxima* at Kommetjie, South Africa, for the production of the seaweed extract, Kelpak® (Stirk & Van Staden 2006).

Figure 2-4 Production of Kelpak® from *E. maxima* using the cold Cell burst method (Stirk & Van Staden 2006).

2.6 Modes of action of seaweed extracts

The various components of SWEs such as vitamins, amino acids, cytokinins, auxins and other PGRs influence cellular metabolism in plants on which they have been applied resulting in improved growth and yields (Crouch et al.1990; Crouch & Staden 1992; Crouch & Staden 1993). Even though their mode of action is not completely known, some suggestions indicate that all these components act synergistically to induce the observed benefits due to application of SWEs (Papenfus et al. 2012). They are bioactive at very low concentrations which can be as low as 1:1000 dilutions (Khan et al. 2009). Figure 2-5 illustrates some growth responses stimulated by SWEs and possible mechanisms involved.

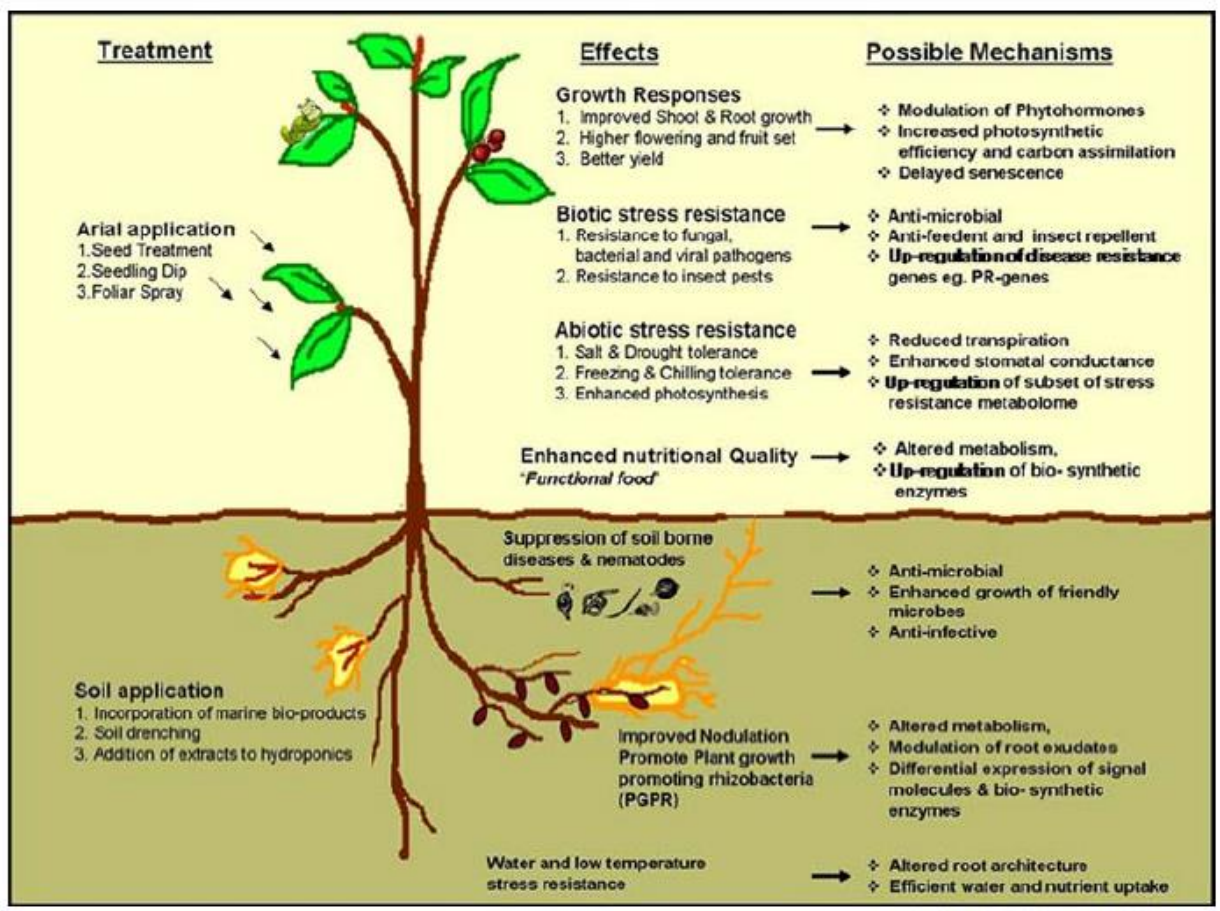


Figure 2-5 Physiological responses stimulated by seaweed extracts and potential mechanism(s) of bioactivity (Khan et al. 2009)

2.7 Plant growth hormones in seaweed extracts and vermicast extracts

As mentioned earlier, the levels of nutrients present in SWEs alone cannot account for the growth effects induced by application of these SWEs. Since the SWEs are effective at relatively low concentrations, this has led to the assumption that SWEs contain PGRs. Plant growth hormones are naturally occurring organic molecules which are capable of influencing physiological processes at low concentrations. These processes may include growth, development and differentiation. Moreover, the wide variety of growth reactions induced implicates the availability of more than one class of PGRs (Crouch & Van Staden 1993). Seaweeds and their extracts contain high levels of auxins and auxin-like compounds (Crouch & Van Staden 1993). By using gas chromatography/mass spectroscopy, Crouch and Van Staden (1993) indicated the occurrence of indole compounds, together with IAA in a seaweed concentrate made from *E. maxima.* In a similar study, Stirk and Staden (2004) discovered the existence of three indole conjugates of IAA and four amino acids in the extracts of *E. maxima* and *Macrocystis pyrifera*. Likewise, cytokinins were also identified in seaweeds and their extracts (Brain et al. 1973). These include trans-zeatin riboside, trans-zeatin and their dihydro-derivatives (Stirk & Van Staden 1997). Analysis of 31 seaweeds from different groups through liquid chromatography/mass spectroscopy (LC/MS) showed that isopentenyl conjugates (IP) and zeatin are the major cytokinins in seaweeds (Khan et al. 2009). Furthermore, Featonby-Smith and Van Staden (1984) observed that application of Kelpak ® led to a rise in cytokinin levels in all plant parts unlike the control plants in which cytokinins appeared in the fruits only. By using high performance liquid chromatography electrospray ionization tandem mass spectrometry (HPLC-ESI-MS/MS), Pant et al. (2011) also reported the presence of PGRs in VE. These included auxins [indole-3-acetic acid (IAA), N-indole-3-yl-acetyl-glutamic acid (IAAGlu), and N-indole-3-yl-acetyl-aspartic acid (IAAAsp)]; cytokinins [isopentyladenine (2iP), zeatin riboside (ZR), isopentyladenosine (iPA), zeatin (Z), dihydrozeatin (dhZ), zeatin-O-glucoside(Z-O-Glu) and dihydrozeatin riboside (dhZR)], abscisic acid and gibberellins. Vermicompost extracts also contain humic acid (Sinha et al. 2010). According to Kangmin (1998), these humic acids are essential as they enable plants to extract nutrients from the soil, stimulate root growth and help plants overcome stress. In this study, more focus will be on the auxins and cytokinins as these two groups of plant hormones are the main growth stimulants in commercial kelp and VE.

2.7.1 Auxins

2.7.1.1 Nature of indole-3-acetic acid

Indole-3-acetic acid (Figure 2.6) is the most common auxin in plants.

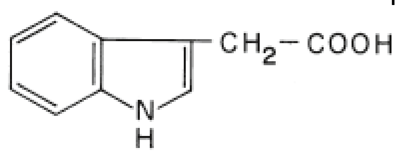


Figure 2-6 Indole-3-acetic acid (Davies 2010)

IAA precursors may also exhibit auxin activity, e.g. indole-acetaldehyde. IAA is also available as various conjugates such as indole-acetyl aspartate (Bandurski et al. 1995; Aloni et al. 2006). 4-chloro-IAA has also been identified in several plant species whilst some synthetic auxins are also used in commercial applications (Bandurski et al. 1995).

2.7.1.2 Sites of Biosynthesis and transport

Auxins are mainly synthesized from tryptophan or indole, mainly in young leaves, leaf primordia and in developing seeds. Auxin transport is from cell to cell, particularly in the vascular cambium and procambial strands. Transport to the root may also possibly involve the phloem (Aloni et al. 2003, 2004; Davies 2010).

2.7.1.3 Functions

Auxins, originating from young shoots, play an essential role in various aspects of root growth, differentiation and development (Jacobs 1952; Aloni et al. 2003, 2006). Auxins regulate the development of lateral and primary roots (Casimiro et al. 2001; Taiz & Zeiger 2002; Blilou et al. 2005), the quiescent centre, root apical meristem (Jiang & Feldman 2005), root cap and root vascular differentiation (Aloni 2004). The effects of auxins in plants are summarized in Table 2-1.

Table 2-1 Functions of auxins in plants (Davies 2010).

Function	**Mode of action**
Cell enlargement	Auxins stimulate cell enlargement and stem growth
Cell division	Auxins stimulate cell division in the cambium and work in combination with cytokinins (CKs)
Root initiation	Auxins stimulate root initiation on stem cuttings, the development of branch roots and the differentiation of roots
Vascular tissue differentiation	Auxins stimulate differentiation of xylem and phloem vessels
Apical dominance	The auxin supply from the apical bud represses the growth of lateral buds
Tropistic responses	Auxins regulate the tropistic response of roots and shoots to gravity and light
Leaf senescence	Auxins delay leaf senescence
Assimilate partitioning	Assimilate movement is enhanced towards an auxin source probably through an effect on phloem transport
Growth of flower parts	Stimulated by auxins Promotes femaleness in dioecious flowers
Flowering	Promotes flowering in Bromeliads
Fruit ripening	Auxins delay fruit ripening
Leaf and fruit abscission	Auxins may inhibit or promote leaf abscission depending on the timing and position of the source

In several situations, e.g. root growth, auxins, especially at high concentrations, are inhibitory to growth. Endogenous auxins also occur in kelp. Studies by Stirk et al. (2009) on two seaweed species, *Ulva fasciata* and *Dictyota humifusa* led to the detection of indole-3-acetamide (IAM) and IAA. Crouch et al. (1992) detected several auxins in the commercial kelp product Kelpak® by GC-MS namely indole-3-carboxylic acid, indole-3-acetic acid, N, N-dimethyltryptamine, isoindole, 1, 3-dione and indole-3-aldehyde. Similarly, Gressler et al. (2010) identified IAM and IAA as the chief auxins in several *Rhodophyta* seaweeds from Brazil. A bioactivity equivalent to $0.3mgL^{-1}$ was noted in Cytex, a seaweed concentrate prepared from *Fucaceae* seaweed of unknown species (Hong et al. 1995).

2.7.2 Cytokinins

2.7.2.1 Characteristics of cytokinins

Cytokinins (CKs) are signalling hormonal molecules that may play a crucial role in regulating cytokinesis, growth and development in plants (Aloni et al. 2006). They are adenine derivatives with an ability to induce cell division in plant tissues. In plants, the most common cytokinin base is zeatin (Figure 2.7). However, CKs are also available in the form of ribotides and ribosides.

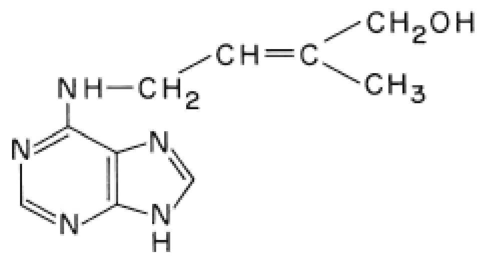


Figure 2-7 Zeatin (Davies 2010)

2.7.2.2 Sites of biosynthesis and transport

CK biosynthesis mainly occurs through biochemical modification of adenine. According to Miyawaki et al. (2004), the living cells in the roots and shoots are capable of generating CKs. However, the main site of CK synthesis is the root tip, particularly the root cap cells (Aloni et al. 2004, 2005). From the root cap, the CKs are transported upward through plasmodesmata in the meristematic and elongation zones (Kim & Zambryski 2005). CKs are also transported from differentiation zones through vessels of the xylem by the transpiration stream, mainly to developing organs with high transpiration rates (Emery & Atkins 2002).

2.7.2.3 Functions

CKs have contrary roles in roots and shoots; in roots they are negative regulators of growth and development (Werner at al. 2003) but in shoots CKs positively promote shoot growth and development (Howell et al. 2003). The functions of cytokinins are summarized in Table 2-2

Table 2-2 Functions of cytokinins in plants (Davies 2010)

Cell division	Endogenous CKs promote cell division in crown gall tumours on plants. Exogenous applications of CKs in the presence of auxin promote cell division in tissue culture.
Growth of lateral buds	Exogenous CK applications or the rise in CK levels in transgenic plants containing genes for enhanced CK synthesis may lead to release of lateral buds from apical dominance.
Delayed leaf senescence	CKs delay leaf senescence
Stomatal opening	CKs may improve stomatal opening in some plant species
Morphogenesis	CKs induce bud formation in moss and promote shoot initiation in crown gall and tissue culture
Leaf expansion	CKs promote leaf expansion by promoting cell enlargement. Through this mechanism, the total leaf area is modified to compensate for the extent of root growth since the amount of CKs reaching the shoot will reflect the extent of the root system.
Chloroplast development	Application of CKs promotes conversion of etioplasts into chloroplasts which may lead to an accumulation of chlorophyll
Shoot initiation	CKs promote shoot initiation

Seaweeds comprise of a significant amount of CKs which are thought to stimulate plant growth responses in plants to which seaweed concentrates have been applied (Papenfus et al. 2012). These include zeatin, dihydrozeatin, isopentenyladenosine and isopentenyladenine (Papenfus et al. 2012). Stirk et al. (2003) established cis-zeatin and isopentenyladenine as the principal

CKs in 31 seaweed species. Nonetheless dihydrozeatin-type CKs occurred at low concentrations in nine seaweeds species. Although dihydrozeatin, trans-zeatin and aromatic CKs occurred in smaller amounts in *Dictyota humifusa* and *Ulva fasciata*, the CKs riboside and ribotide chains were mainly found in these species (Stirk et al. 2009). In order to identify the existence of CKs in Chase organics seaweed concentrate, aqueous seaweed concentrate was added to the growing medium of *Atropa belladonna* (a cytokinin lacking strain). An enhancement of callus cell growth similar to a growth medium where kinetin has been added was observed (Brain et al. 1973). Through GC-MS and soybean callus bioassay, Tay et al. (1985) discovered zeatin, zeatin riboside and dihydrozeatin ribosides in a seaweed concentrate made from *Durvillia potatorum*. The overall CK content in the seaweed concentrate was 0.115 mg L^{-1}. Recent studies by Zhang et al. (2014) also indicated the presence of CKs in VE. Using LC-MS/MS, they screened for the various classes of PGRs (auxins, cytokinins, abscisic acid and gibberellins) in vermicompost tea. This investigation yielded the first mass spectrometric evidence for the presence of CKs in vermicompost tea specifically N^6-isopentenyladenine (iP), *trans*-Zeatin (*t*Z) and N^6-isopentenyladenosine. The three CKs were present at 3.33, 0.06 and 0.02 nmol L^{-1} respectively.

2.7.3 Exogenous application of auxins and cytokinins

The flower number of different crops was found to be positively responsive towards exogenous application of growth regulators (Mir et al. 2010). It was detected that treatment with kinetin along with P boosted the flower number of *Lycopersicon esculentum Mill* (tomato) (Mir et al. 2010). However, a clear decrease in flower number was prominent in P deficient plants. Foliar spray of NAA (100 and 200 mg/L) and N applied at 0, 60, 90 and 120 kg (N)ha^{-1} resulted in an increase in the leaf area index of field grown *Oryza sativa L.* (rice) (Grewal & Gill 1986). It was also detected that chlorophyll content of plants is affected by the application of plant growth regulators in conjunction with certain nutrients. An intensification of the chlorophyll content of sunflower (*Helianthus annuus* L.) was reported following the application of IAA with N (Mir et al. 2010). Meyyappan et al. (1991) remarked that joint application of NAA + Borax improved the overall chlorophyll content of *Arachis hypogea* L (peanut). Grewal and Gill (1986) detected that foliar application of NAA and N considerably boosted interception of photosynthetically active radiation in a field grown rice paddy.

2.7.4 Improved root growth due to application of auxins and cytokinins

PGRs, in particular, auxins control most of the features of root systems, including primary root growth and the formation of root hairs and lateral roots (Bradford & Trewavas 1994). Several plant species react to the exogenous application of auxins through generating great quantities of lateral roots. Proof of the essential role of auxins in the synthesis of lateral roots was established by Lopez-Bucio et al. (2003). In their experiment, application of some auxin-transport inhibitors N-(1-napthyl) phtalamic acid (NPA) and 2,3,5-triiodobenzoic acid (TIBA) restrained the development of proteoid or lateral roots in Lupin and Arabidopsis, respectively. It was concluded that auxins are necessary for lateral root development under P-limiting conditions. Furthermore, Arabidopsis plants growing under an inadequate P concentration (1 mM) had higher sensitivity to auxins in terms of the inhibition of primary root elongation and enhancement of lateral root density. This shows that variations in auxin sensitivity may play an essential part in the influence of P deprivation on root-system growth. On the other hand, cytokinins reduce lateral root initiation in low-P-grown plants. López-Bucio et al. (2003) showed that the rise in the ratio of shoot/root development that transpires in reaction to low P is coupled with a reduction in cytokinin concentration. Exogenous cytokinins suppressed the appearance of low-P-regulated genes such as *Arabidopsis thaliana* induced by phosphate starvation (AtIPS1), signifying that these hormones not only control root construction but likewise control further characteristics of the low-P rescue response (López-Bucio et al. 2003).

2.7.5 Other plant growth hormones in seaweed extracts and vermicast extracts

Even though the auxins and cytokinins are fairly accountable for the growth promoting responses following applications of commercial kelp, their effects do not completely explain all the observed effects due to kelp treatment. As a result of this, other researchers such as Papenfus (2011) have diverted their attention to other PGRs such as polyamines, abscisic acid, gibberellins and brassinosteroids. Polyamines are found in virtually all plant and animal cells and microorganisms, as well as eukaryotic algae and they are also known to induce growth promoting responses in plants. Stirk et al. (2014) analyzed some samples of Kelpak® harvested over a 2 year period and freshly harvested *E. maxima* through HPLC-MS/MS in an attempt to quantify other PGRs present in seaweed products. From the analysis, 18 gibberellins with concentration ranges from 187.54 to 565.96 $pgmL^{-1}$ were identified in the Kelpak® harvested over a 2 year period and in freshly harvested *E. maxima* as well as brassinosteroids such as brassinolide (BL) and castasterone (CS). However, the overall concentration of the biologically active gibberellins, that is GA1, GA3, GA4, GA5, GA6 and GA7, was below 3%. Kelpak® reportedly contains very high concentrations of GA13, although low concentrations were realized in *E. maxima*. Stirk et al. (2014) could not detect abscisic acid in *E. maxima*, but they managed to identify abscisic acid in Kelpak® falling in the range between 0.31 to 20.70 pg mL^{-1}. Similarly, Papenfus (2011) conducted an experiment using a HPLC to determine concentration of Polyamines in *E. maxima* and Kelpak®. They discovered putrescine levels falling between 15.98-54.46 µg.g-1, 6.01-40.46 µg.g-1 and 50.66-220.49 µg.g-1 dry weight in the stipe, fronds and seaweed concentrate, individually. Spermine levels stretched from 1.02-35.44 µg.g-1, 1.05-26.92 µg.g-1 and 7.28-118.52 µg.g-1 dry weight in the stipe, fronds and seaweed concentrate, respectively. It was therefore suggested that this mixture of PGRs available in Kelpak® may act hand in hand to bring about all the favourable physiological effects brought about by application of Kelpak® to plants (Stirk et al. 2014).

2.8 Improved plant growth and health due to application of seaweed extracts

2.8.1 Root development and mineral absorption

Seaweed extracts have been used in agriculture to improve root development in various crops. In a study by Van Staden et al. (1994) there was a significant increase in root growth and overall plant size when *Eucalyptus* seedlings received an early dose of 10% Kelpak® solution when compared to the control. These findings are consistent with what Van Staden et al. (1995) found, they also observed an increase in lateral root formation and plant size when one dose of 10% Kelpak® solution was applied to *Eucalyptus* seedlings. Jones and Van Staden (1997) noted a 70% increase in rooting when *Pinus patula* (pine) seedlings were treated with Kelpak®. Similarly Atzmon and Van Staden (1994) reported that the increase in shoot growth which was observed in pine seedlings was due to the substantial improvement in root length and root number after the application of Kelpak®. A significant increase in seedling growth was also observed in cabbage, when cabbage seedlings were dipped for five minutes in a 1:500 solution of SWEs before transplanting. The treated plants had significantly higher stem diameters at three to four weeks after transplanting compared to the control (Aldworth & Van Staden 1987). Kelpak® application also led to significant increases in shoot growth in pine seedlings. Whilst still on root development, Papenfus (2011) reported a 70 % improvement in percentage rooting in pine cuttings after the application of Kelpak®. In addition, Anisimov et al. (2013) conducted a study on the influence of liquid extracts obtained from the brown seaweeds: *Sargassum pallidum*, *Saccharina japonica*; green seaweeds: *Codium fragile* and *Ulva fenestrata* and red seaweeds: *Tichocarpus crinitus, Neorhodomela larix*, on root length of *Fagopyrum esculentum* seedlings, commonly known as buckwheat. Results obtained revealed that ultra-low and low concentrations (from 10^{-14} to10^{-3} g of dried seaweeds mL^{-1} distillated water) of SWEs induced root elongation in *Fagopyrum esculentum* seedlings (Anisimov et al. 2013). Since little work has been done to test the effect of commercial kelp on root development in rough lemon seedlings, this study will contribute to filling this gap in literature.

2.8.2 Enhanced chlorophyll content and photosynthesis due to application of seaweed extracts

Application of SWEs enhances leaf chlorophyll content (Blunden et al. 1996). The rise in chlorophyll content is due to a decline in chlorophyll degradation partly caused by cytokinins and betaines which may be present in the SWEs (Khan et al. 2009). According to Khan et al. (2009), glycine betaine from SWEs also reduces the loss of photosynthetic activity by hindering chlorophyll degradation under storage conditions in isolated chloroplasts. When tomato seedlings were treated with Kelpak® applied as a soil drench, Crouch & Van Staden (1992) observed an increase in root growth and photosynthetic accumulation efficiency in treated plants compared to the control plants.

2.8.3 Increased flowering, fruit set and crop yield due to application of seaweed extracts

Seaweed extracts have also been used to enhance flowering, fruit set and crop yield. Koo (1988) treated a citrus hybrid of Robinson and Osceola thrice a year for three years with seaweed based nutrient sprays. The citrus trees were treated at post-bloom, summer oil and 40 days after summer oil and a significant increase in fruit yields in two out of the three years was observed. In another study, Lourens (2010) observed a 16% increase in yield (kg $tree^{-1}$) when commercial kelp was applied on soft citrus in 5 trials in South Africa. An increase in shoot length, shoot number, leaf size and percentage budding of 12%, 14%, 18% and 12% compared to 10%, 11%, 13% and 0% for the control treatment respectively, was observed on Satsumas treated with Kelpak® in Japan (Lourens 2010).

Foliar application of Kelpak® resulted in 10% growth in harvestable fruits, 17% increase in fruit fresh weight and early flowering (Crouch & Van Staden 1992). The early flowering in plants treated with Kelpak® could be due to enhanced growth and development of the plants which enabled the plants to reach maturity early (Papenfus et al. 2012). This is quite important economically since plants with earlier fruit set usually fetch better market prices (Papenfus et al. 2012). In a similar, study a notable increase in culm diameter, grain and a reduction in lodging was observed when Kelpak® was applied to wheat (Nelson & Van Staden 1984a). Nelson and Van Staden (1984a) advocated that this increase in yield could be due to an increase in all cells, particularly the cells of the vascular bundles. Similarly, application of Kelpak® on barley resulted in a 50% rise in grain yield due to an upsurge in the total fertile spikelets per ear (Featonby-Smith & Van Staden 1987). A 50% increase in seed production following application of Kelpak® on marigold (*Tagetes patula*) seedlings was also noted compared to the control plants (Van Staden et al. 1994). In a similar study with a bean crop, a 56% increase in yield of soybean (*Glycine max*) was noted when soybean was treated with SWEs from the red seaweed, *Kappaphycus alvarezii* (Rathore et al. 2009). Likewise, foliar application of Kelpak® resulted in 24% increase overall dry mass of beans (*Phaseolus vulgaris*). A possible explanation for this increase in dry mass was the increase in rooting and fruit set (Featonby-Smith & Van Staden 1987).

2.9 Alleviation of abiotic stress in crop plants using seaweed extracts

2.9.1 Drought stress

It has been shown in earlier studies that SWEs positively affect crops' tolerance to drought stress and nutrient stress. Spann and Little (2011) did an experiment on Hamlin sweet orange trees grafted onto 'Swingle' citrumelo and 'Carrizo' Citrange. The container grown sweet orange trees were foliar sprayed with SWEs of *Ascophyllum nodosum* at 5 and 10 mL L^{-1} once every week. 50% of trees in the respective treatments were irrigated at 50% of evapotranspiration to induce drought stress, while the remaining 50% was fully irrigated (100% of ET). Although the drought stressed trees displayed a reduction in leaf photosynthesis and shoot growth they showed superior total plant growth and root growth comparative to the volume of water administered to them, thus improving plant water use efficiency. More total growth was observed in drought-stressed trees to which SWEs were administered compared to untreated drought-stressed trees for all rootstocks and it was also suggested that the ability of the SWEs to maintain plant growth in drought stress was not connected to photosynthesis (Spann & Little 2011). Nonetheless, these outcomes are an indication that SWEs may be a valuable means of increasing tolerance to drought stress of citrus trees grown in containers.

2.9.2 Nutrient deficiency

Nutrient deficiency on arable land is constantly a challenge for agronomists. Substantial sums of money are spent annually to increase the nutrient status of arable land. While synthetic fertilizers have injurious effects on the environment, there is currently nothing on the market which can substitute the application of fertilizers (Papenfus et al. 2012). However, since products such as commercial kelp have the ability to expand the plant's root system and its nutrient uptake they may possibly increase nutrient use efficiency of the plants and thus reduce the fertiliser amount required by the plant. In this regard, they can also relieve the stress experienced by nutrient-stressed plants and consequently lead to the saving of large amounts of money. After conducting an experiment with lettuce plants, Crouch et al. (1990) conveyed that Kelpak® treatment did not improve the yield of nutrient-stressed lettuce. In contrast, Beckett & Van Staden (1989) reported that application of Kelpak® as a root flush improved the yield of wheat getting a low nutrient amount (1% Hoagland's nutrient solution). A number of physiological responses due to applications of a commercial kelp product, Kelpak® and polyamines (PAs) on plant development were also studied in a sequence of pot trials by Papenfus (2011). Kelpak® considerably concealed the unfavourable effects induced by P and K deficiency and significantly enhanced the growth of K and P deficient okra seedlings. However, applications of PAs were unable to alleviate K and P deficiency but improved root growth considerably in seedlings getting a sufficient nutrient supply. Papenfus (2011) suggested that the additional PAs were responsible for supporting auxin-mediated root development.

Nelson & Van Staden (1984b) investigated the effects of seaweed concentrate on the growth of greenhouse cucumbers subjected to nutrient stress. When the nutrient-stressed cucumbers were treated with Kelpak®, higher yields were realized. The growth regulators contained by the seaweed concentrate led to an increase in the root: shoot ratio and overall dry mass of the cucumbers, accounting for the higher yields. Furthermore, root growth was enhanced to a greater extent as compared to shoot growth which increased total photosynthetic accumulation efficiency within the plants (Nelson & Van Staden 1984b). Cucumbers to which Kelpak® had been administered had higher levels of P, whilst a notable reduction in N levels was observed. To account for the loss of N, Nelson and Van Staden (1984b) proposed that the enlarged root growth drew greater amounts of N to the roots essential for incorporation into N containing compounds like cytokinins which are produced in the roots.

2.10 Alleviation of biotic stress in crop plants using seaweed extracts and vermicast extracts

Several reports have shown that SWEs boost plant defence against pests and diseases (Sangha et al. 2014). They promote plant health by influencing the rhizosphere community, for example SWEs were found to have an effect on the population of nematodes in the soil. There was a notable reduction in nematode infestation in plants treated with SWEs compared to control plants (Featonby-Smith & Van Staden 1983; Crouch & Van Staden 1993). Since treatment with SWEs did not affect the population of nematodes in the rhizosphere, it was suggested that application of SWEs imparts resistance to nematode attack by modifying the auxin: cytokinin ratio in the plant. Seaweeds and their extracts also contain plant defence elicitor molecules of plant defence against diseases such as oligosaccharides, polysaccharides, proteins, lipids and peptides (Khan et al. 2009) and mucilages which are able to elicit defence responses in plants.

Vermicompost extracts have been used widely by organic farmers and gardeners to stimulate plant growth by application as soil drenches and to suppress plant diseases by application to soils or as foliar sprays. In a laboratory and greenhouse experiment by Edwards et al. (2007) to determine the effects of vermicompost teas on nematodes, tomato plants were planted in soils which had been artificially inoculated with the root knot nematode, *Meloidogyne incognita* (a very serious pest of an extensive variety of crops). The comparative development of tomato plants in reaction to the nematode infestations are shown in Figure 2-8 and Figure 2-9. There was a considerable reduction in the number of root knot galls on the roots of tomato plants that were treated with vermicompost tea compared to the control. Additionally, all three dilutions of vermicast teas significantly suppressed aphid populations ($p<0.05$) and spider mite levels. Humus in VE poisons harmful bacteria and fungi from the soil thereby protecting plants (Sinha et al. 2010). As a result, the use of VE significantly lessens the requirement for chemical pesticides.

Figure 2-8 A comparison of tomato plants infested with *Meloidogyne hapla* and treated with vermicompost or thermophilic compost teas (Edwards et al. 2007).

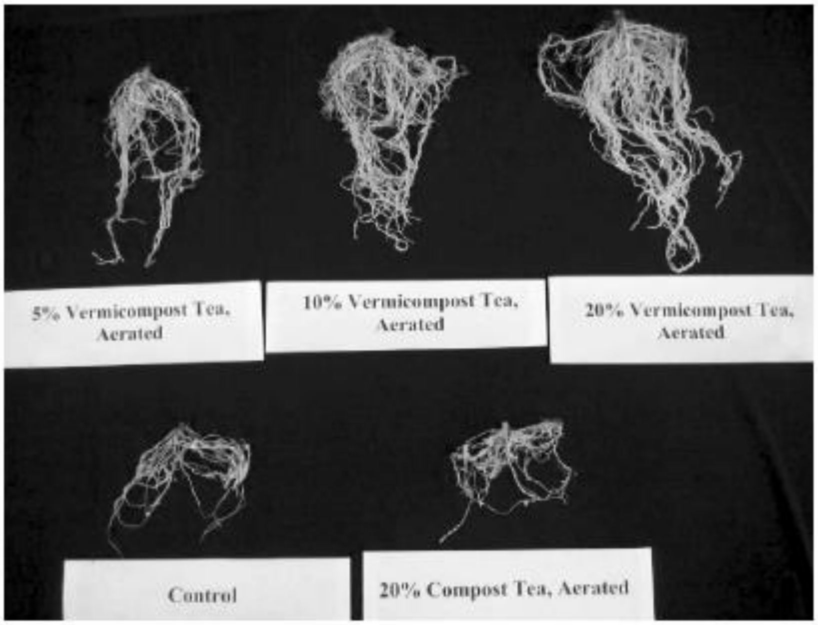


Figure 2-9 The roots of tomato plants infested with *Meloidogyne hapla* and treated with 5%, 10% and 20% aerated vermicompost teas or 20% thermophilic compost tea compared to those only treated with water (control) (Edwards et al. 2007).

2.11 Conclusion

SWEs and VE are sources of natural growth stimulants that can be used to improve plant growth in the horticultural and agricultural industries. These products enhance plant growth, nutrient uptake, yields and crop resistance to abiotic and biotic stresses. The increased nutrient uptake and resistance to pests and diseases is beneficial in that it lowers the levels of synthetic fertilisers and pesticides that are used. SWEs and VE also contain PGRs which are thought to be responsible for the growth promoting effects observed upon their application on plants. Although a lot of studies have been done on the use of SWEs and VE in other crops, additional research is needed on the role of SWEs and VE on the growth of rough lemon seedlings. With the rising alertness of the negative environmental impact of synthetic agrochemicals, the use of SWEs and VE could be a favourable option.

CHAPTER 3 Materials and methods

3.1 Site description

The experiment was carried out during 2015/2016 in a glasshouse at the Hatfield Experimental Farm, University of Pretoria located at 23◦ 45' S, 28◦ 16' E, and an altitude of 1372 m above sea level.

3.2 Experimental design and treatments

Rough lemon seeds were placed in beakers and imbibed for 1 hour in distilled water. The imbibed seeds were then planted into seedling trays (68cm x 35cm x 11cm), containing pre-moistened Hygrotech growing medium, before being covered with a thin layer of Hygrotech growing medium. Thereafter, the seedling trays were placed in a Conviron growth cabinet at a constant temperature of 25°C, 12 hours light, 12 hours dark and a light intensity of 400 µmol. m^{-2} s^{-1}. The seeds were left to germinate for approximately 21 days and watered using tap water. Upon the seedlings reaching a four-leaf stage (Figure 3-1 A), seedlings of a similar size were transplanted into plastic trays (28cm x 20 x 2.5cm) containing five holes of approximately 2 cm in diameter. Only two seedlings were used per tray. These trays were floated on the surface of water filled rectangular containers (26cm x 18cm x 13cm) (Figure 3-1 B). Each container contained 10 litres of full-strength Hoagland solution, designed for the hydroponic growing of rough lemon seedling, prepared according to the formula of Hoagland and Arnon (1938). The hydroponic containers were placed on continuously rotating round tables in a glasshouse, in a completely randomised experimental design, to ensure uniform growing conditions (Figure 3-1 B). Seaweed extracts, VE and plant hormone treatments were applied once in the first week and reapplied at fourteen-day intervals thereafter till the experiment was terminated (after 12 weeks).

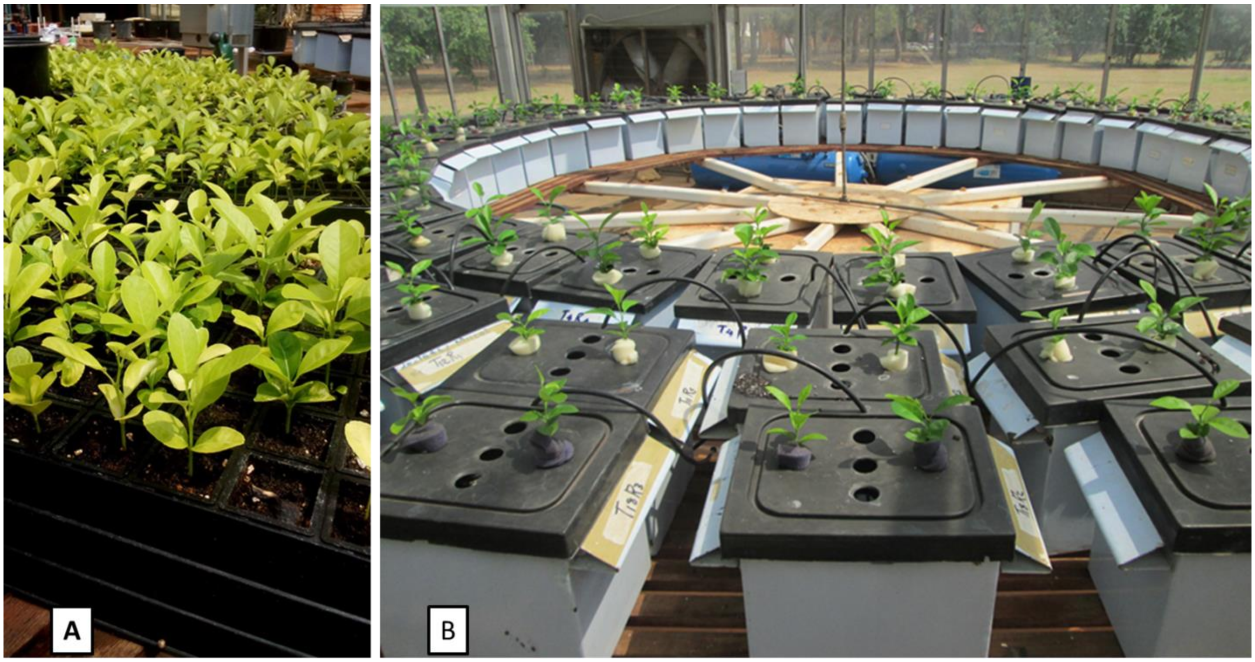


Figure 3-1 A-rough lemon seedlings at the four leaf stage; B-Hydroponic system and rough lemon seedlings on continuously rotating round tables in a glasshouse trial.

The treatments were Kelpak® (EM1) and Afrikelp® (EM2) (seaweed extracts made from the brown seaweed, *Ecklonia maxima*), vermicast extracts (VE) (all applied at 0.5, 1 and 2% dilution), indole-3-butyric acid (IBA) and naphthalene acetic acid (NAA) applied at 0.1, 0.2 and 2 mg L^{-1}. A combination of 0.05 mg L^{-1}IBA and 0.05 mg L^{-1} NAA was also applied. Each treatment was replicated five times (10 seedlings per treatment) with a total of 18 treatments. An air pump was used to continuously oxygenate the solution throughout the duration of the experiment.

3.3 Data Collection

3.3.1 Plant growth parameters

Weekly measurements of plant height, stem diameter, chlorophyll content and root length were done. Plant height and root length were measured using a graduated ruler. Stem diameter was measured with a Vernier calliper whilst chlorophyll content was measured using a chlorophyll SPAD meter. Root volume measurements were also taken every fortnight using the water displacement technique, care being taken to make sure that the plant roots were not injured in the process.

3.3.2 Plant dry matter

At the end of the experiment (after 12 weeks) each plant was separated into roots, shoots and leaves and weighed to determine the fresh mass. After determining the fresh mass, leaves were rinsed in 10% acetone, and then washed in deionised water. The different plant parts were bagged separately, dried at 60°C until constant weight (±three days) before determining dry mass.

3.4 Nutrient analysis

Dried leaf samples were ground into a fine powder using an electric grinder. For each ground sample, 0.5 g of material was weighed out and placed into a digestion tube before adding 6 ml HNO_3 (65%) to the tube. There were 16 digestion tubes per digestion cycle and at least 1 blank (containing all reagents excluding the sample) was used for each digestion cycle. The blank was necessary in order to pick up any deviations in reagents or equipment. After microwave digestion, the samples were then analysed using an ICP and the concentrations for the different elements were calculated for each sample. Nitrogen analyses were done by an AgriLASA accredited commercial laboratory.

3.5 Photosynthesis

Net CO_2 assimilation rate (A_{CO2}) was measured periodically during the experiment on mid-stem leaves using a portable photosynthesis system (LI-6400; LI-COR Inc.). The internal light source was maintained at the ambient PAR as determined with a LI-6400 light sensor. All measurements were done from 08:00 to 10:00am to avoid elevated afternoon temperatures and low humidity

3.6 Plant growth hormone analyses

Samples of Kelpak® (EM1), Afrikelp® (EM2) and vermicast extracts (VE) were sent for plant growth hormone analyses at Laboratory of Hormonal Regulations in Germany.

3.7 Sources of seaweed extracts, vermicast extracts and nutrient elements

3.7.1 Seaweed extracts

Seaweed extracts were obtained in the form of liquefied *E. maxima* containing its natural PGRs from two manufacturers; Afrikelp® (Cape Town, South Africa) and Kelpak® (Simons Town, South Africa). The SWEs were periodically added to the full-strength Hoagland solution at their respective concentrations during the trial. Throughout the following chapters, the names *E. maxima* (EM1) and *E. maxima* (EM2) will be used to refer to Kelpak® and Afrikelp® respectively.

3.7.2 Vermicast extracts

Liquid VE were obtained from a local vermiculture facility, Victus, located in Pretoria, South Africa. The VE were added to the Hoagland solution during the course of the trial at their respective concentrations.

3.7.3 Nutrient elements

Nutrient elements used to make the full-strength Hoagland solution were obtained from Merck and Sigma-Aldrich.

3.8 Data analysis

At the end of the trial, analysis of variance was carried out on all data using SAS (SAS Institute Inc., Cary, NC, and USA. 2002-2003). The relevant ANOVA tables may be obtained in the Appendix. Mean separation was done using Tukey’s test. The standard errors of means were calculated using Microsoft Office Excel. Except when indicated otherwise; all discussed treatments were significantly different at a probability level of 5%.

CHAPTER 4 Comparison of different Ecklonia maxima and vermicast extracts on plant growth

4.1 Abstract

Several reports have been made showing that seaweed extracts (SWEs) increase overall root growth and seedling establishment in a number of crops. Atzmon and Van Staden (1994) suggested that the observed increase in shoot growth in plants, to which Kelpak® has been administered, is due to the substantial improvement in root length and root number. The growth-promoting effects of SWEs and vermicast extracts (VE) have been widely attributed to the presence of plant growth regulators (PGRs), particularly the auxins and cytokinins. A hydroponic experiment was carried out at the University of Pretoria experimental farm in a glasshouse. Liquid SWEs (*Ecklonia maxima)* from different manufactures (EM1 & EM2) and commercially available VE were applied separately to rough lemon seedlings as a drench (at 0.5%, 1% and 2%), at 14 day intervals. Since it is widely reported that SWEs and VE contain PGRs, pure hormones were used to test if their effects would mimic some of the observed effect due to the application of EM1, EM2 and VE. NAA and IBA were separately applied at 0.1 mgL^{-1}, 0.2 mg L^{-1} and 2 mg L^{-1} with respect to the levels of auxins in a similar volume of the SWEs and VE that were tested. A combination of 0.05 mg L^{-1} NAA and 0.05 mg L^{-1} IBA was also applied to test if there was any synergistic growth promoting effect of the two auxins on rough lemon seedlings. Deionized water and full strength Hoagland solution were used as controls. A completely randomized design, with 5 replicates per treatment was used. Root volume, root length, stem diameter, plant height and chlorophyll content, were measured weekly for 12 weeks. At the end of the trial, the average root dry mass and shoot dry mass for each treatment was determined. Amongst the detected effects of applications of EM1, EM2 and VE are the development of a prolific root system. Seedlings treated with EM1, EM2 and VE had considerably greater root volume and root dry mass than the deionised water control, with the 2% VE resulting in the

highest increase in root volume, root dry mass and shoot dry mass compared to the other treatments. EM1 and EM2 applied at lower concentrations (0.5% and 1%) led to greater root growth (root length, root volume and root dry mass), whereas the higher concentration (2%) was detrimental to root development. Although the deionised water control significantly had the highest root length, this was at the expense of dry mass accumulation as it had the lowest root and shoot dry mass. For the EM1 treatments, when looking at the above-ground growth parameters, 1% EM1 induced the greatest increase in stem diameter. The 1% EM2 significantly had the highest plant height, followed by EM1 and VE applied at the same concentration. Whilst 0.5% EM1 induced the highest increase in chlorophyll content, the differences between the Hoagland solution and 2% EM1 were not significant. The chlorophyll content for deionised water control plants was significantly lower than all the other treatments. From the results obtained, it is clear that VE applied at 2% induce the best overall plant growth in rough lemon seedlings compared to 0.5% VE, 1% VE, EM1 and; EM1 and EM2 applied at similar (0.5%, 1% and 2%) dilutions. It is recommended to apply VE at 2% dilution to enhance growth of rough lemon seedlings although it is necessary to carry out several other experiments under field conditions to test if these results are reproducible.

4.2 Introduction

According to Steveni et al. (1992), application of SWEs to crop plants can increase plant growth and yield. Exogenous application of CKs to plants has been shown to mimic some of the reactions identified when SWEs are similarly applied. These CKs were reportedly most effective when applied at early growth stages. Cytokinins have been described as a “shoot factor” which is translocated from synthesis sites in the roots, to the shoots where they actively regulate protein levels in the leaves (Steveni et al. 1992). The occurrence of auxins and CKs in SWEs and VE potentially promotes an extensive root system, consequently setting a framework for enhanced plant growth. Auxins and CKs have antagonistic roles in root development (Casimiro et al. 2001; Guo et al. 2005; Woodward & Bartel 2005). In plant roots CKs suppress root development and reverse the auxin effect (Lloret & Casero 2002), whilst auxin application promotes formation of lateral roots and adventitious roots (Sorin et al. 2005). In a study by Lohar et al. (2004) the little CK content in CK-deficient transgenic plants which overexpress cytokinin oxidase/dehydrogenase (CKX) genes led to a bigger root meristem, enhanced root branching, formation of lateral roots closer to the root apical meristem and increased lateral root formation. A decrease in endogenous auxin concentrations induced by application of IAA transport inhibitors led to a reduction in adventitious roots formed by flooded plants (Visser et al. 1996). Torrey (1976) also illustrated that auxin-induced lateral root formation in isolated pea segments was increased by certain concentrations of kinetin (cytokinin). In a similar study, Finnie & Van Staden (1985) observed that at low concentrations of CKs (zeatin) (10^{-6} M and below) root growth was promoted whilst root growth was inhibited at levels above 10^{-6} M of zeatin. Presently, little has been done to study the effect of plant hormones on root development in citrus but a lot of work has been done using the model species *Arabidopsis thaliana* (Petricka et al. 2012).

Rose et al. (1991, 1997) and Jacobs et al. (2005) noted a positive connection between seedling root volume and field performance. In a study on ponderosa pine (*Pinus ponderosa* Dougl. ex Laws), seedlings with larger initial root volumes (> $7 cm^3$) had significantly higher survival two year after field planting than those with smaller root volumes (< $4.5 cm^3$) (Rose et al. 1991). Furthermore, seedlings with larger root volumes had greater plant heights after 2 years than those with smaller root volumes (10.3 vs $6.4 cm^3$) (Rose et al. 1991). In a similar experiment, using Douglas-fir seedlings (*Pseudotsuga menziesii* (Mirb.) Franco), the increase in plant height after a single growing season was significantly higher in seedlings with larger root volume (> 13 cm^3) than in those with smaller root volumes (< 9 cm^3) (Rose et al. 1991). It was evident that Douglas-fir seedlings with greater root volume are able to alleviate the effects of transplant shock except in cases of severe moisture stress (Haase and Rose, 1993). Under non-drought conditions, loblolly pine (*Pinus taeda* L.) seedlings with large root volumes had greater hydraulic conductivity than those with smaller root volumes (Carlson & Donald 1986). Hence it is needful to apply products which can potentially improve root volume such as SWEs and VE to enhance seedling vigour.

A poorly developed plant's root system can result in a reduction in water and nutrient absorption which can impede shoot growth. Suggestions have also been made that roots are involved in the synthesis of PGRs necessary for shoot growth (Kende & Zeevaart 1997). Some of these hormones include gibberellins, CKs and abscisic acid (Davies & Zhang 1991). Since shoots are dependent on roots for PGRs such as abscisic acid and CKs, damage to plant roots would ultimately interfere with the supply of these hormones to the shoots (Sinclair & Bartholomew 1944). Weston (1996) indicated that roots also produce flavonoids which have allelopathic effects and aid in protecting them against pests like nematodes and fungi. In certain plants, ammonia is converted to useful organic compounds in roots. Some proteins and peroxidases are also synthesised in the roots and transported to other parts in the xylem sap of several plant species (Biles & Abeles 1991). In a study done in tomatoes, Johnson et al. (1994) noted substantial absorption of carbon dioxide from the root medium coupled with transport of carbon compounds from roots to shoots. The experiment reported on in this chapter was conducted to determine if the SWEs EM1 and EM2; and VE would improve root growth and above-ground growth in rough lemon seedlings grown hydroponically. Since these products are reported to have auxins and CKs, tests were also done to determine whether the pure hormones (IBA and NAA) would increase root growth and above-ground growth in a similar manner as observed due to application of EM1, EM2 and VE when applied at same concentration of auxins.

4.3 Plant growth of hydroponically grown rough lemon seedlings due to application of seaweed and vermicast extracts

4.3.1 Average root volume in response to the application of seaweed and vermicast extracts

Amongst the observed effects of SWE (EM1 and EM2) and VE application is the development of a vigorous root system, especially with 1% SWEs and 2% VE applications. A negligible increment in root volume for the seedlings treated with deionised water was observed from day 0 (transplanting) to the termination day (84) and a constant root volume of 2.5 cm^3 was maintained Figure 4-1. For all the treatments, the seedlings followed the sigmoid growth curve where root volume (2.5 cm^3) remained constant from day 0 (transplanting day) to day 28 as the seedlings were acclimatising to the new environment (Figure 4-1). Thereafter, there was an exponential increase in root volume for each treatment from day 28 to day 56 and a decrease in growth rate from day 56 to day 84. In general, rough lemon seedlings treated with the SWEs EM1 and EM2; and VE had greater root volume than the deionised water (control) plants (Figure 4-1) due to a lot of fibrous roots formed (Figure 4-2).

Ecklonia maxima – Kelpak® (EM1)

Treatment with SWEs and VE resulted in a dose-dependent increase in root volume. Visible differences in root volume were only observed after day 28 of transplanting (Figure 4-1 C). From day 28 to day 56 seedlings treated with 1% EM1 increased in root volume by 0.5 cm^3 on average per day, thereafter the rate decreased to 0.23 cm^3 day^{-1} reaching a maximum volume of 23.5 cm^3. Similar growth rates were observed in 2% and 0.5% EM1 with higher growth rate of approximately 0.4 cm^3 day^{-1} from day 28 to day 56 and thereafter the rate decreased to 0.2 cm^3 day^{-1} reaching a final volume of 21 cm^3 and 19.6 cm^3 for 0.5% EM1 and 2% EM1 respectivelyFigure 4-1 C). Statistical analysis showed that for the kelp extracts 1% EM1 significantly produced the highest root volume, whilst0.5% EM1 and 2% EM1 were not significantly different from each other. The higher concentration (2% EM1) was inhibitory to root development. Application of EM1, at any of the three concentrations used led to a significantly higher root volume than the controls (Hoagland solution and deionised water).

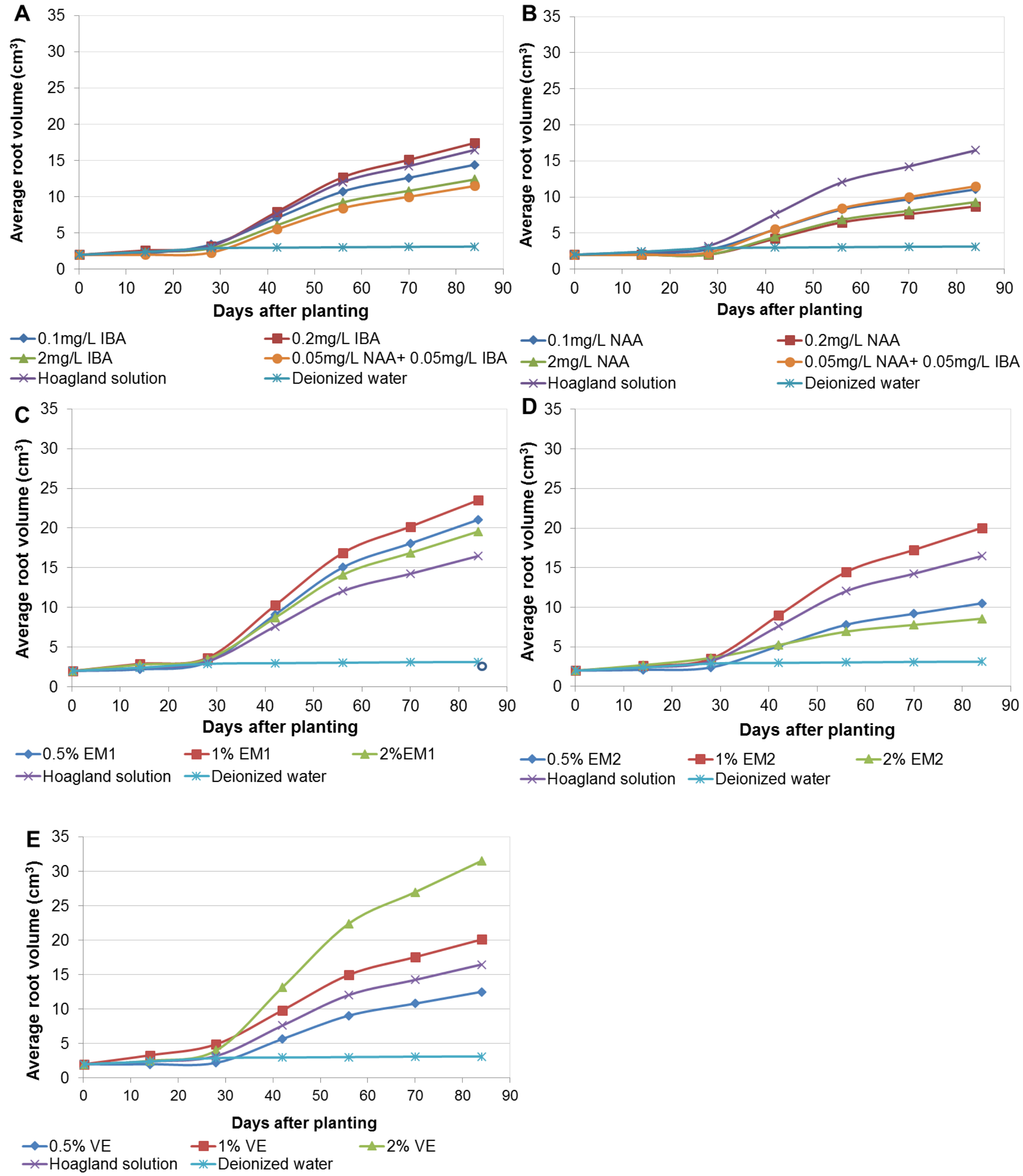
Ecklonia maxima – Afrikelp® (EM2)

In a similar manner to EM1, EM2 also led to a dose-dependent change in root volume. Visible differences in root volume were only observed after day 28 of transplanting. From day 28 to day 56 seedlings treated with 1% EM2 increased in root volume by 0.39 cm^3 on average per day, thereafter the rate decreased to 0.20 cm^3 day^{-1} reaching a maximum volume of 20 cm^3 which was significantly different from seedlings treated with 0.5 and 2% EM2. Unlike EM1, application of EM2 at a concentration of 0.5% and 2% led to a significantly lower root volume than the Hoagland solution control plants (Figure 4-1 D). Root volume for seedlings treated with Hoagland solution increased in volume at a constant rate of about 0.31 cm^3 day^{-1} from day 28 to 56 and there was a decline thereafter to 0.16 cm^3 day^{-1} from day 56 to day 84 reaching a maximum of 16.5 cm^3. As observed in EM1, seedlings treated with 2% EM2 resulted in significantly lower root volume and lower growth rate. Growth rate was initially 0.12 cm^3 day^{-1} from day 28 to 56 and thereafter reduced to 0.06 cm^3 day^{-1} reaching a final volume of 8.6 cm^3 (Figure 4-1 D)

Vermicast extracts

By visual inspection, seedlings treated with 2% VE had higher root numbers and generally better root development than the other treatments (Figure 4-2). A direct relationship was observed between the concentration of VE and the root volume. An increase in VE concentration resulted in an increase in the root volume. Of all the treatments, the highest root volume was observed with 2% VE (Figure 4-1 E). From day 28 to day 56 seedlings treated with

2% VE increased in root volume by 0.7 cm^3 on average per day, thereafter the rate decreased to 0.33 cm^3 day^{-1} reaching a maximum volume of 31.5 cm^3. Seedlings treated with 1% VE increased in root volume by 0.36 cm^3 day^{-1} from day 28 to day 56 and thereafter, there was a decline to 0.18 cm^3 day^{-1}, reaching a final volume of 20.1 cm^3. Seedlings treated with Hoagland solution performed better than those treated with 0.5% VE which had a final root volume of 12.5 cm^3.



EM1= *Ecklonia maxima* - (Kelpak®), EM2 = *Ecklonia maxima* - (Afrikelp®), VE=Vermicast extracts, HS = Hoagland solution and DW = deionised water, IBA = Indole butyric acid and NAA = naphthalene acetic acid.

Figure 4-1 Root volume of hydroponically grown rough lemon seedlings as affected by (A) NAA, (B) IBA, (C) EM1, (D) EM2 and (E) VE. NB: Root volume and root length results for IBA and NAA will be discussed in the next section.

EM1= *Ecklonia maxima - (Kelpak®),* EM2 = *Ecklonia maxima - (Afrikelp®),* VE=Vermicast extracts, HS = Hoagland solution and DW = deionised water.

Figure 4-2 Root growth due to application of EM1, EM2 and VE.

4.3.2 Average root length in response to the application of seaweed and vermicast extracts

For all the treatments the seedlings followed a sigmoid growth curve. Root length increased exponentially from day 0 to day 28, thereafter there was a decrease in growth rate from day 28 to day 56 and increase in growth rate from day 56 to day 84 for EM1 and VE, but not for EM2. It was noted that maximum root lengths were observed in seedlings treated only with deionised water up to day 63, afterwards, significant root growth was observed with other treatments (Figure 4-3). Rapid root growth with the deionised water treatment was at the expense of root volume, the absence of nutrients resulted in fewer thinner roots, which elongated exponentially scavenging for nutrients. The roots were long and not fibrous, while the other treated plants developed a fibrous and well developed root system.

Ecklonia maxima – Kelpak® (EM1)

Changes in root length were observed on day 7 after transplanting, seedling root length increased linearly. From day 0 to day 28, seedlings treated with 0.5% EM1 increased in root length by 0.25 cm on average per day. After that, the rate decreased to 0.14 cm day^{-1} for the period of 28 – 56 days and maximum growth rate (0.53 cm day^{-1}) was observed from day 56 to day 84, reaching a length of 32.6 cm. The lowest growth rate for this treatment was observed in seedlings treated with 1% EM1, from day 0 – 28 root length increased by 0.05 cm day^{-1} , the growth rate further increased to 0.15 cm day^{-1} from day 28 - 56 and a further increase was observed from day 56 – 84 reaching a final length of 20 cm (Figure 4-3 C). Statistical analysis showed that 0.5% EM1 significantly produced the highest root length amongst the seedlings which were treated with 0.5%EM1, 1% EM1 and 2% EM1, which was not significantly different from the seedlings treated with 1% EM1. In comparison, seedlings treated with EM1 had significantly lower root length than the two controls (deionised water and Hoagland solution).

Ecklonia maxima – Afrikelp® (EM2)

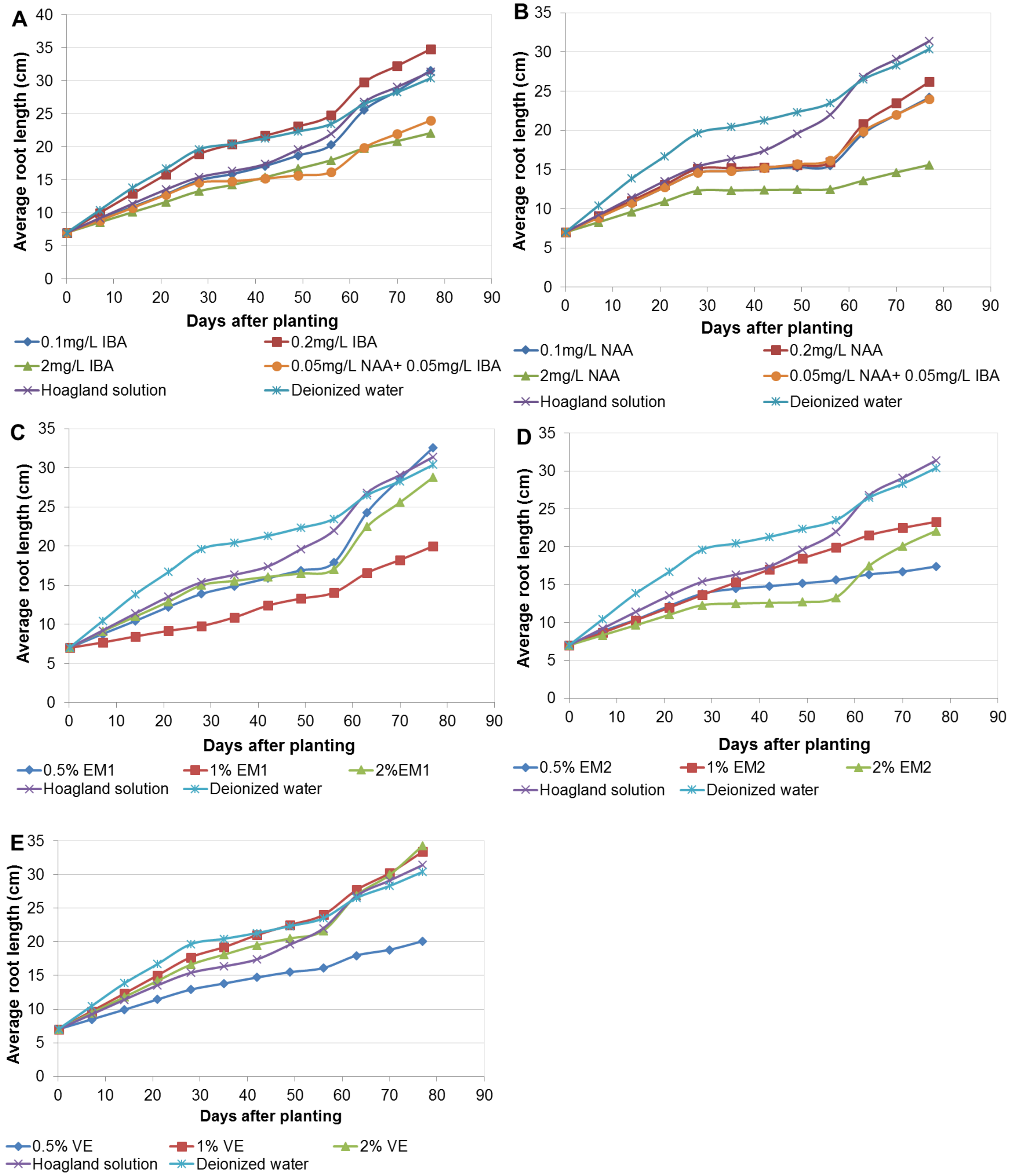
For the whole period of the experiment the controls had significantly higher root lengths than the EM2 treatments (Figure 4-1 D). Of the three treatments 0.5%, 1% and 2% EM2, seedlings treated with 1% EM2 resulted in significantly the highest root length, whereas the seedlings treated with 0.5 and 2% EM2 were not significantly different from each other (Figure 4-1 D). From day 0 – 28 root length of seedlings treated with 1% EM2 increased by 0.24 cm day^{-1}, then dropped to 0.22 cm day^{-1} from day 28 - 56 with a further decrease in growth rate to 0.12 cm day^{-1} resulting in a final root length of 23.3 cm (Figure 4-3 D). Seedlings treated with 2% EM2 and 0.5% EM2 had a final root length of 22.1 cm and 17.4 cm respectively.

Vermicast extracts

Similar to what was observed with the root volumes for seedlings that were treated with 0.5% VE, 1% VE, and 2% VE; there was a direct relationship between the concentration of VE and the root length whereby an increase in VE concentration resulted in an increase in root length. Statistical analysis showed that 2% VE significantly produced the highest root length which was not significantly different from root lengths of the seedlings that were treated with 1% VE. From day 0 to day 28 seedlings treated with 2% VE increased in root length by 0.34 cm on average per day. Thereafter the rate decreased to 0.18 cm day^{-1} from day 28 – 56, followed by a maximum growth rate of 0.45 cm day^{-1} (day 56-84) reaching a maximum root length of 34.3 cm (Figure 4-3 E). Least root lengths were observed in seedlings treated with 0.5% VE throughout the experimental period.

Plant growth regulators (IBA and NAA)

The results observed with the SWEs and VE treatments were also consistent with the observed effects when PGRs (IBA and NAA) were applied. Root development due to application of IBA was dose-dependent. When IBA was applied at lower concentrations (0.1 and 0.2 mg L^{-1}), it stimulated more growth (root length, root volume and root dry mass) whilst the higher concentration (2 mg L^{-1}) was inhibitory to root development (Figure 4-1). The maximum root volume due to IBA treatment was observed with 0.2 mg L^{-1} IBA, followed by 0.1 mg L^{-1} IBA, although the results from the two concentrations were not significantly different from each other Figure 4-1. From Figure 4-1 A, the optimum concentration of IBA for a higher root volume is 0.2 mg L^{-1} IBA, which is consistent with the concentration of auxins in 1% EM1 and 1% EM2. This serves as an indication that the increased root volume due to application of the SWEs may be due to the action of auxins such as IBA and NAA. A similar trend in root development was also observed with NAA, whereby there was more root growth (root length, root volume and root dry mass) at 0.1 and 0.2 mg L^{-1} NAA, whilst there was a notable decrease in root development at higher concentration of NAA (2 mg L^{-1}) (Figure 4-3B and Figure 4-1B). The highest root volume was observed with the 0.1 mg L^{-1} NAA treatment although this was not significantly different from the combination of 0.05 mg L^{-1} NAA with 0.05 mg L^{-1} IBA. Although the 0.1 mg L^{-1} and 0.2 mg L^{-1} NAA and IBA treatments respectively resulted in an increase in root volume, the average increase was notably lower than that of the SWEs when applied at a similar concentration of auxins. This indicates that, the SWEs contain a mixture of PGRs other than auxins and CKs, which act synergistically to enhance root development.



EM1= *Ecklonia maxima* - (Kelpak®), EM2 = *Ecklonia maxima* - (Afrikelp®), VE=Vermicast extracts, HS = Hoagland solution and DW = deionised water, IBA = Indole butyric acid and NAA = naphthalene acetic acid.

Figure 4-3 Root length of hydroponically grown rough lemon seedlings as affected by (A) NAA, (B) IBA, (C) EM1, (D) EM2 and (E) VE

4.3.3 Stem diameter response to the application of seaweed and vermicast extracts

The steady increase in stem diameter for all the treatments are characterised by constant growth rates (linear) and can be categorised into three phases viz. day 0 – 42, day 42 – 56 and day 56 – 70 as shown in Figure 4-4. A similar sigmoid growth curve as observed in root volume and root lengths was observed for all treatments, with a lag phase (cell differentiation) and a rapid growth phase (cell elongation) (Figure 4-4). In general the controls had lower stem diameter compared to all the treatments. Plants treated with deionised water had a final stem diameter of 0.1 cm and the seedlings treated with Hoagland solution had a final stem diameter of 0.4 cm.

Ecklonia maxima – Kelpak ® (EM1)

Stem diameter increased at a steady rate of 0.002 cm on average per day for all the EM1 treatments for the first lag, thereafter there was an exponential increase in stem diameter by a rate of 0.03 cm day^{-1} for both 1% and 2% EM1 and 0.04 cm day^{-1} for 0.5% EM1 (Figure 4-4 A). In the last lag, the highest growth rate was observed in 1% EM1 of 0.02 cm day^{-1} resulting in maximum stem diameter of 0.9 cm which was not significantly different from the seedlings treated with 0.5% EM 1 (Figure 4-4 A).

Ecklonia maxima – Afrikelp® (EM2)

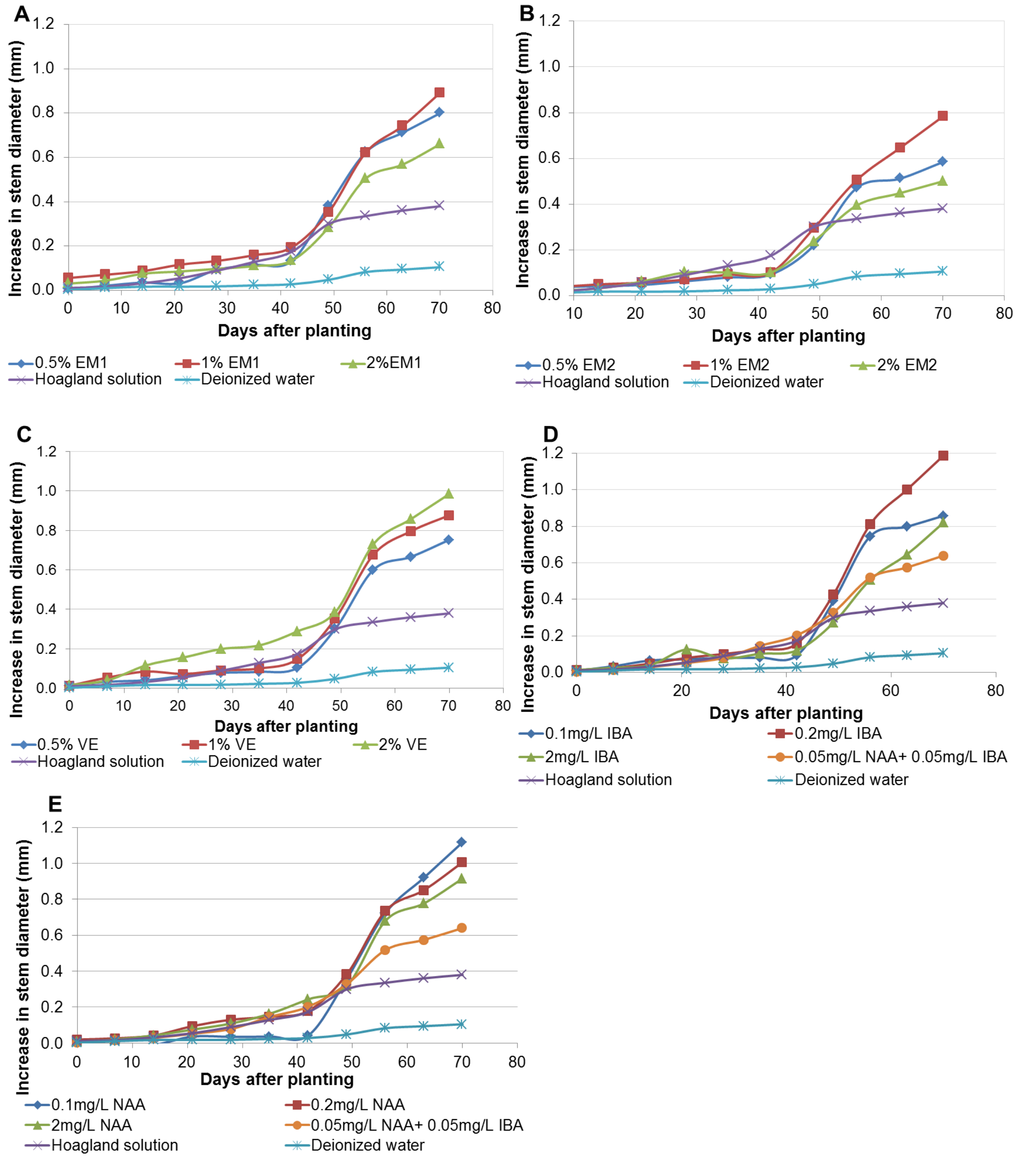
Similar to the seedlings treated with EM1, stem diameter increased at a steady rate of 0.002 cm on average per day for all the EM2 treatments for the first lag, subsequently, there was an exponential increase in stem diameter by a rate of 0.03 cm day^{-1} for both 1 and 2% EM2 and 0.04 cm day^{-1} for 0.5% EM 2 (Figure 4-4 B). In the last lag, the highest growth rate was observed in 1% EM1 of 0.02 cm day^{-1} resulting in maximum stem diameter of 0.8 cm (Figure 4-4 B).

Vermicast extracts

For all the treatments maximum stem growth rate of 0.007 cm day^{-1} was observed in seedlings which were treated with 2% VE for the first lag, then the stem diameter increased exponentially at a rate of 0.03 cm day^{-1} (Figure 4-4 C). In the last lag highest growth rate was observed in 2% VE of 0.02 cm day^{-1} resulting in maximum stem diameter of 1 cm (Figure 4-4 C). As observed in root volume of the seedlings treated with VE, the stem diameter also increased with the increase in the concentration of the VE.

Plant Hormones

It was also intriguing to note that the same trend as observed with root volume and root length for IBA was observed for stem diameters, where the highest increase in stem diameter was recorded for 0.2 mg L^{-1}IBA (Figure 4-4 D). This was followed by 0.1 mg L^{-1}IBA, with the least increase in stem diameter being recorded for 2 mg L^{-1}IBA. On the other hand, the highest increase in stem diameter for NAA was recorded with 0.1 mg L^{-1}NAA, followed by 0.2 mg L^{-1}NAA, with the least increase in stem diameter being observed with the 2 mg L^{-1}NAA (Figure 4-4 E). In both cases, the combination of 0.05 mg L^{-1}IBA and 0.05 mg L^{-1}NAA did not perform better than all three concentrations of IBA and NAA used. Both IBA and NAA, and their combination significantly performed better than both Hoagland solution and deionized water controls as they had higher stem diameters compared to the controls.



EM1= *Ecklonia maxima* - (Kelpak®), EM2 = *Ecklonia maxima* - (Afrikelp®), VE=Vermicast extracts, HS = Hoagland solution and DW = deionised water, IBA = Indole butyric acid and NAA = naphthalene acetic acid.

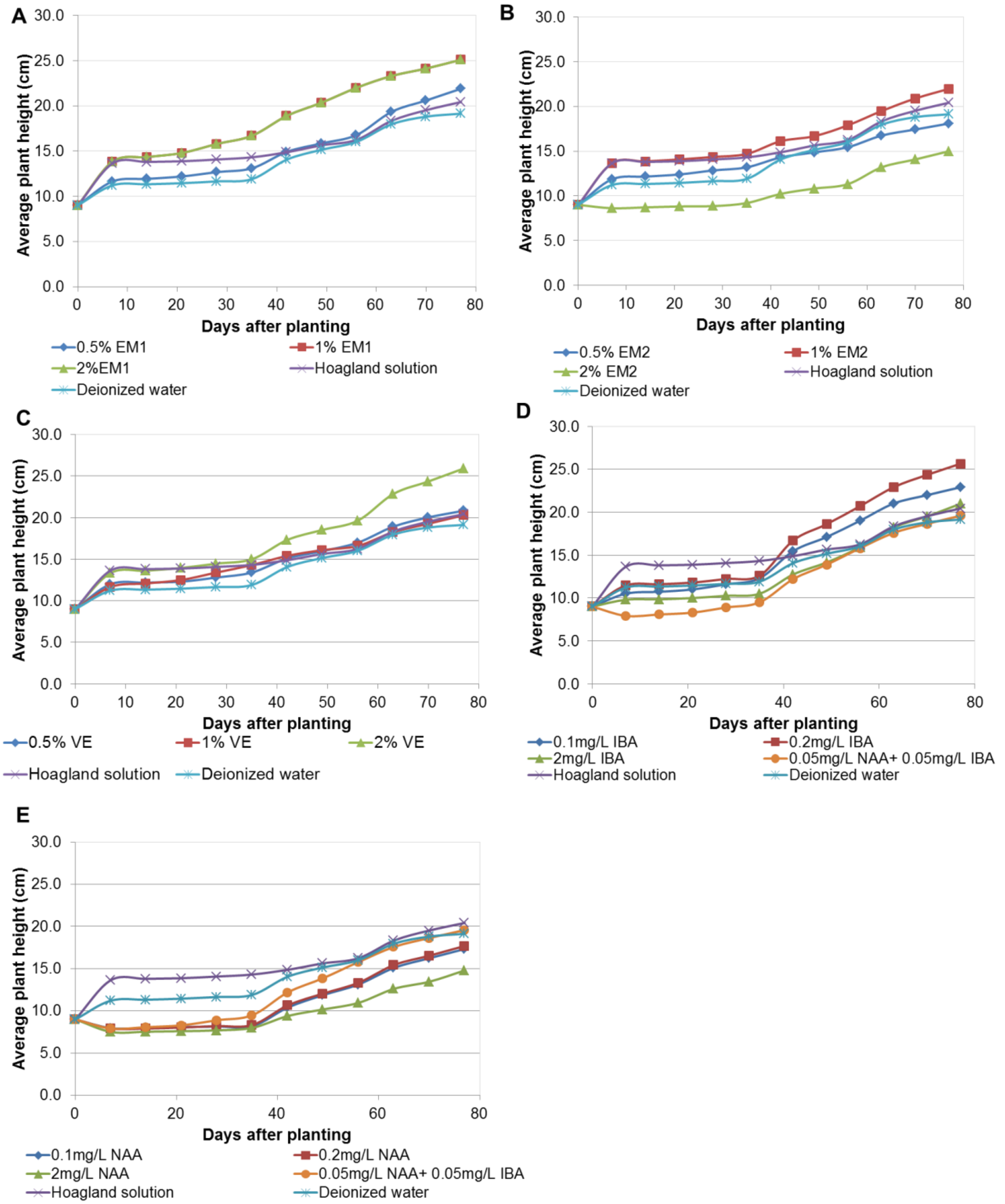
Figure 4-4 Stem diameter of hydroponically grown rough lemon seedlings as affected by (A) EM1, (B) EM2, (C) VE, (D) IBA and (E) NAA.

4.3.4 Plant height of hydroponically grown rough lemon seedlings as affected by seaweed and vermicast extracts

A sigmoid growth curve as observed in root volume and root lengths was observed for all treatments, with the lag phase and the rapid growth phase. In general the deionised water and Hoagland solution controls had lower plant height compared to all the kelp and VE treatments (Figure 4-5). Plants treated with the Hoagland solution had a final plant height of 20.7 cm and the seedlings treated with deionized water, had a final plant height of 19.0 cm. For the EM1 treatments it was found that the highest significant plant height (25.1 cm) was observed with the 1% treatment, but there were no significant differences in plant height between seedlings treated with 1% EM1 and 2% EM1 which also had a final plant height of 25.1cm (Figure 4-5 A). The lowest plant height (21.8 cm) for EM1 was observed with the 0.5% EM1 treatment. On the other hand, the highest significant plant heights for EM2 (22.0 cm) treatments were observed with the 1% EM2 treatment. This was followed by the 0.5% EM2 treatment, which had an average final plant height of 18.1 cm. The 2% EM2 treatment had detrimental effects on plant growth which resulted in very stunted plants with an average final plant height of 15.0 cm which was significantly lower than the controls. When compared to the Hoagland solution and deionized water control, both 0.5% EM2 and 2% EM2 did not perform better than the controls. For VE, the highest significant plant height (25.9 cm) was observed with the 2% VE treatment. There were no significant differences in plant heights between the 0.5% VE and 1% VE treatments which had final plant heights of 20.8 cm and 20.3 cm respectively. Both, 0.5% VE and 1% VE treatments were not significantly different from the deionized water and Hoagland solution controls.

When looking at the pure plant hormones, the highest significant final plant height (25.6 cm) for IBA was observed with the 0.2 mg L^{-1} IBA treatment followed by 0.1 mg L^{-1} IBA, which had a final plant height of 22.9 cm. The lowest final plant height (21.0 cm) was observed with the 2 mg

L^{-1} IBA. There were no significant differences in plant heights between the combination of 0.05 mg L^{-1} IBA and 0.05 mg L^{-1} NAA (19.6 cm) and deionized water and Hoagland solution controls (Figure 4-5 D). Results for NAA showed a different trend whereby the highest plant heights (19.6 cm) were observed with the combination of 0.05 mg L^{-1} IBA and 0.05 mg mg L^{-1} NAA followed by 0.2 mg L^{-1} NAA and 0.1 mg L^{-1} NAA which had plant heights of 17.3 cm and 17.7 cm respectively. These differences were not statistically different. The lowest plant height (14.8 cm) was observed with 0.2 mg L^{-1} NAA (Figure 4-5 E).

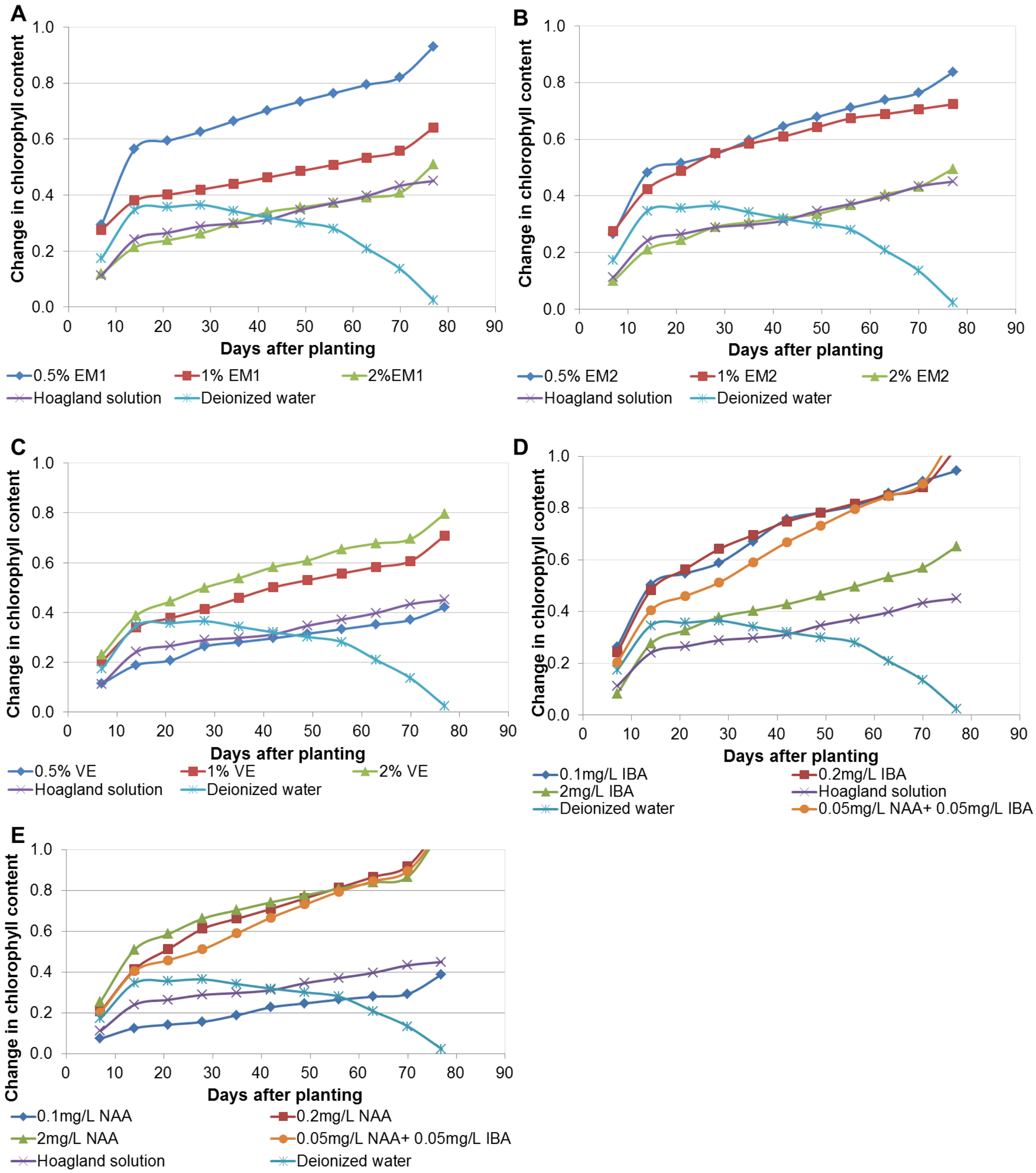


EM1= *Ecklonia maxima* - (Kelpak®), EM2 = *Ecklonia maxima* - (Afrikelp®), VE=Vermicast extracts, HS = Hoagland solution and DW = deionised water, IBA = Indole butyric acid and NAA = naphthalene acetic acid.

Figure 4-5 Plant heights of hydroponically grown rough lemon seedlings as affected by (A) EM1, (B) EM2, (C) VE, (D) IBA and (E) NAA.

4.3.5 Chlorophyll content of rough lemon seedlings in response to seaweed and vermicast extracts

For all the treatments, an increase in concentration of EM1, EM2 and VE resulted in an increase in the chlorophyll content (Figure 4-6 A, B and C). A sharp increase in leaf chlorophyll content was observed from day 7 to day 14, thereafter the chlorophyll content increased at a steady rate to day 77 (Figure 4-6 A, Figure 4-6 B and Figure 4-6 C). For EM1 and EM2, the highest chlorophyll content was observed with the 0.5% treatment (Figure 4-6 A and Figure 4-6 B), whilst the highest chlorophyll content for VE was observed with the 2% VE. However, with the deionised water a decrease in the chlorophyll content was observed after day 7 with very low chlorophyll contents recorded for this control. The rapid increase in chlorophyll content which was observed in seedlings treated with deionised water could have been as a result of the use of mineral nutrients in reserve, thereafter a breakdown of chlorophyll yielded low values of the chlorophyll content leading to leaf chlorosis. Whilst the 2 mgL^{-1} IBA significantly had the lowest chlorophyll content compared to the other treatments, there were no significant differences in chlorophyll content between 0.1 mg L^{-1} IBA, 0.2 mg L^{-1} IBA and the combination of 0.05 mg L^{-1} IBA and 0.05 mg L^{-1} NAA (Figure 4-6 D). For NAA, the lowest chlorophyll content was observed with the 0.1 mg L^{-1} NAA. There were no significant differences in chlorophyll content between 0.2 mg L^{-1} NAA, 2 mg L^{-1} NAA and he combination of 0.05 mg L^{-1} IBA and 0.05 mg L^{-1} NAA (Figure 4-6 E). Results obtained showed that application of SWEs and VE to hydroponically grown rough lemon seedlings significantly enhanced the leaf chlorophyll content compared to control plants. The increased chlorophyll content can possibly be due to a reduction in leaf chlorophyll degradation (Tuchy, Chowańska, and Chojnacka 2013). A study by Lötze and Hoffman (2016) showed that the SWEs, Kelpak® had significantly higher levels of nutrients such as Ca, Mg and K than Afrikelp® and Basfoliar® Kelp; whilst significantly higher levels of N and P were found in Afrikelp® and Basfoliar® Kelp than in Kelpak®. Hence there is also a possible contribution of mineral nutrients such as Mg and N found in EM1, EM2 and VE which play a role in chlorophyll formation (Lötze & Hoffman 2016).

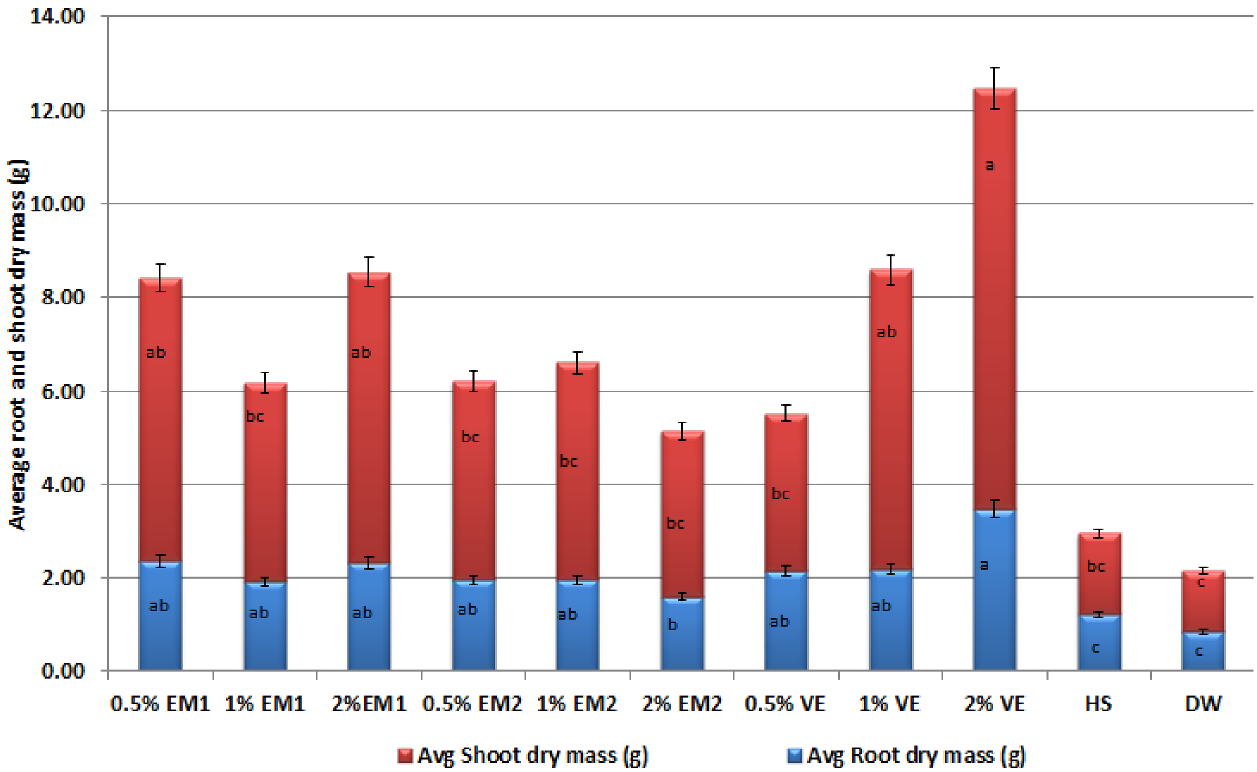


EM1= *Ecklonia maxima - (*Kelpak®), EM2 = *Ecklonia maxima* - (Afrikelp®), VE=Vermicast extracts, HS = Hoagland solution and DW = deionised water, IBA = Indole butyric acid and NAA = naphthalene acetic acid.

Figure 4-6 Change in chlorophyll content of hydroponically grown rough lemon seedlings as affected by (A) EM1, (B) EM2, (C) VE, (D) IBA and (E) NAA.

4.3.6 Average shoot and root dry masses of rough lemon seedlings in response to seaweed and vermicast extracts

For the VE treatments, an increase in concentration of VE resulted in an increase in the root and shoot dry masses, with the highest shoot dry mass being observed in the 2% VE treatment (Figure 4-7). There were no significant differences in root dry masses between the 0.5% and 1% VE treatments. For EM1 treatments, the highest root dry mass were observed with the 1% EM1 treatment, but it was not significantly different from the 0.5% and 2% EM1 treatments (Figure 4-7). Whilst the highest root dry mass for EM2 treatments was observed with 1% EM2, it was also not significantly different from the 0.5% and 1% EM2 treatments (Figure 4-7). The lowest root dry mass was recorded for the deionised water treatment owing to the low root development as a result of nutrient stress. When all the treatments were compared, the 2% VE treatment significantly had the highest shoot dry mass, whilst the deionised water control had significantly the lowest shoot dry mass. There were no significant differences in shoot dry masses for EM1 and EM2 applied at 0.5% and 2% levels.



Bars of the same colour with the same letter are not significantly different from each other (P≤0.05) according to Tukey's Studentized Range (HSD) test. EM1= *Ecklonia maxima* (Kelpak®), EM2 = *Ecklonia maxima* (Afrikelp®), VE=Vermicast extracts, HS = Hoagland solution and DW = deionised water, IBA = Indole butyric acid and NAA = naphthalene acetic acid.

Figure 4-7 Average shoot and root dry masses of seedlings as affected by EM1, EM2 and VE

4.4 Plant growth hormones detected in seaweed extracts and vermicast extracts

Results from the Liquid chromatography mass-spectrometry (LCMS) analyses indicated that the SWEs (EM1 and EM2) and VE contained different levels of PGRs (Table 4-1).When compared to EM1 and EM2, the two samples of VE had approximately ten times the levels of NAA (830 and 583 pmol/ml respectively) although the levels of IBA were roughly the same in all three products (Table 4-1). It is most likely that the action of NAA was mostly responsible for the high root dry mass and root volume observed with the 2%VE treatment. Some studies in literature have also shown that auxins and polyamines (PAs) work in synergy to enhance root growth (Papenfus, 2011). From Table 4-1, the levels of PAs in EM2 (0.1 pmol/ml) were generally lower compared to EM1 and VE as also confirmed in Lötze and Hoffman (2016). This could have been a contributing factor to the poor root development observed with EM2. The highest levels of other PGRs such as salicylic acid (SA) and benzyl-adenine (BzA) were detected in EM1 (Table 4-1).

Table 4-1 Phytohormones detected in EM1, EM2 and VE through Liquid chromatography-mass spectrometry (LCMS).

	Sample Number	PA	NAA	IBA	SA	BzA	JA
VE	1 (pmol/ml)	0.1	830	411.8	49.9	2679.3	4.1
VE	2 (pmol/ml)	0.2	583	393.3	56.2	1373.3	2.7
100% EM1	3 (pmol/ml)	0.1	54.5	354.5	71.2	3526.3	2.6
100% EM1	4 (pmol/ml)	0.2	47.5	439.3	74.4	4061.9	3.9
100% EM2	5 (pmol/ml)	0.1	24.8	632.9	43	1881.3	3.2
100% EM2	6 (pmol/ml)	0.1	36.2	491.1	55	2090.3	4.5

EM1= *Ecklonia maxima - (*Kelpak®), EM2 = *Ecklonia maxima* - (Afrikelp®), VE=Vermicast extracts, PA = Polyamines, NAA = Naphthalene acetic acid, IBA = Indole butyric acid, SA = Salicylic acid, BzA = Benzyl-adenine and JA = Jasmonic acid

4.5 Discussion and conclusion

Hydroponically growth rough lemon seedlings were treated with 0.5%, 1% and 2% of SWEs and VE and plant growth was monitored. Although no published literature is available to show the effects of SWEs and VE in rough lemon seedlings, several studies have been done in other crops. In a study by Steveni et al. (1992), hydroponically grown barley seedlings were treated with 1:3000 dilution of a SWE (Maxicrop). A 56 - 63% increase in plant growth was detected. In another study, the SWE Kelpak® was applied as a soil drench or to the foliage every fortnight at 5% dilution in Swiss chard plants and there was a remarkable improvement in plant growth and chlorophyll content (Arthur et al. 2013). When 1L of 0.5% Kelpak® drench was also applied annually per tree, followed by five 0.2% Kelpak® sprays per season, there was an 80% increase in shoot mass in nectarine trees (Kelp products (Pty) Ltd., n.d.). Studies done in tomato plants have also revealed that a 1:500 dilution of Kelpak® 66 applied as a root flush at transplanting led to improved root growth and a reduction in infestation by root-knot nematodes (Featonby-Smith & Van Staden 1983).

The experimental results showed that SWEs induced significantly higher root volume and root length compared to the pure hormones (IBA and NAA). Even though auxins such as IBA and NAA are the main PGRs responsible for stimulating root growth (Hopkins and Huner, 2004), SWEs and VE contain other PGRs, such as polyamines (PAs), mineral nutrients and amino acids (Lötze & Hoffman 2016) which can lead to better root development in plants treated with SWEs and VE compared to those treated with the auxins (IBA and NAA). From this experiment, high concentrations of IBA and NAA (2 mg L^{-1}) were inhibitory to root development as indicated by the lower root volume and root length for this treatment. The optimum concentration for root development for IBA was the 0.2 mg L^{-1} IBA treatment, whilst there were no significant differences in root volume between 0.1mg L^{-1} NAA and the combination of 0.05 mg L^{-1} IBA and 0.05 mg L^{-1} NAA. The pure hormones, IBA and NAA when applied at 0.5%, 1% and 2 mg L^{-1} induced significantly greater root development compared to the deionised water control. However, the root volume was significantly lower than that for the Hoagland solution control for NAA at all three concentrations. Although the deionised water control had greater root length compared to the other treatments, it was clear that the roots were poorly developed as shown by the low root volume. As the trend for the change in root volume for both NAA and IBA is similar to that for EM1 EM2 and VE, it is highly probable that the auxins in the SWEs and VE act synergistically to promote root development. In terms of above-ground growth parameters, there were no significant differences in plant height for all dilutions used for EM1 and EM2; whist the 1% dilution induced the highest stem diameter for both SWEs. Both EM1 and EM2 at 0.5% significantly enhanced the highest chlorophyll content. For the vermicast extracts, the 2% dilution induced the highest above-ground growth parameters compared to the other dilutions. In terms of above-ground growth parameters, 0.2 mg L^{-1} IBA induced the highest increase in chlorophyll content, stem diameter and plant height compared to the other treatments. Higher concentrations of IBA (2 mg L^{-1}) were detrimental to above-ground plant growth as shown by the results. While 0.2mgL^{-1}NAA induced better chlorophyll content and stem diameter than 0.2 mg L^{-1}NAA, the differences were not significant. In general, the 2 mg L^{-1} NAA treated led to a reduction in above-ground plant growth.

In light of the root growth and shoot growth results, the recommended treatment would be the 2% VE as supported by the results indicating that it had the highest significant root volume, root dry mass, plant height, stem diameter, chlorophyll content and shoot dry mass compared to the other treatments. Although 0.5% EM1 had the highest chlorophyll content, the best dilution for optimum plant growth for EM1 would be 1% dilution as it had the highest root volume, stem diameter and plant height compared to the other treatments. For EM2, the best dilution would be 1% EM2 as it showed superior plant growth compared to the other dilutions for the root growth parameters and above-ground parameters measured. However, it is highly recommended to do more field trials in order to ensure that results are reproducible under field conditions.

CHAPTER 5 Photosynthetic response curves, carboxylation efficiency and nutrient uptake in response to seaweed and vermicast extracts

5.1 Abstract

Previous studies have indicated that citrus trees typically have very low photosynthetic rates ranging between 6-12 µmol $m^{-2}s^{-1}$ which is much lower than photosynthetic rates for most woody perennial plants (Kriedemann 1971). The apparent low photosynthetic rates in citrus species could be one of the main factors limiting their productivity. The response of net assimilation rate of CO_2 to leaf internal CO_2 concentration was measured weekly in hydroponically grown rough lemon seedlings to which EM1, EM2 and VE were applied. Since there is a positive correlation between leaf N and photosynthesis, an accumulation of total N per unit leaf area due to improved nutrient uptake is expected to increase the quantity of soluble proteins, which are involved in the Calvin cycle. As shown in Chapter 4, SWEs and VE have an impact on root development. Since roots are responsible for the uptake of water and nutrients, a hydroponic experiment was carried out in a glasshouse to determine if SWEs and VE can affect nutrient uptake and carboxylation efficiencies of rough lemon seedlings. SWEs (EM1 and EM2) and VE were applied at 0.5%, 1% and 2% dilution with respect to the contents of each hydroponic tank and replaced every 14 days. Rough lemon seedlings treated with EM1, EM2 and VE had significantly greater nutrient levels and carboxylation efficiencies than the control, with the 2% VE having the highest N, P, K and Ca levels than other treatments. EM applied at 1% stimulated more carboxylation efficiency, whilst the higher concentration (2%) negatively affected carboxylation efficiency. For EM2, there were no significant differences in carboxylation efficiencies between the 1% and 2% EM2 treatments. Although the 1% VE had the highest carboxylation efficiency, this was not significantly different from the 2% VE treatment. Even

though the 1%VE had statistically similar carboxylation efficiency as the 2%VE treatment, it is recommended to apply VE at 2%, as this also resulted in the greatest nutrient uptake compared to the other treatments. However, additional experiments would need to be carried out under field conditions to test reproducibility of results.

5.2 Introduction

The major source of plant nutrition is the fixation of carbon dioxide (CO_2) from the atmosphere into simple sugars through the use of sunlight energy during photosynthesis. Most of dry matter accumulation by plants, and nearly every process required in crop growth and production, rely on assimilates produced during photosynthesis (De Vries 1972). Photosynthesis can be categorized into three main parts (Figure 5-1) namely; photo and cellular respiration, electron transfer rate and incorporation of CO_2 into carbohydrates and the efficiency with which Rubisco catalyses the carboxylation (carboxylation efficiency) of ribulose-1,5-bisphosphate (RuBP). The net assimilation rate of CO_2 (An) can be expressed as a function of internal leaf CO_2 concentration (A/Ci curves). The initial slope of the A/Ci curve represents the carboxylation efficiency which is the CO_2-limited region or RUBP-saturated region. In this region RUBP is present in saturating quantities whereas the rate of functioning of RUBP is limited by levels of CO_2. At a saturating photosynthetically active radiation (PAR), the initial slopes indicate the leaf's carboxylation capacity.

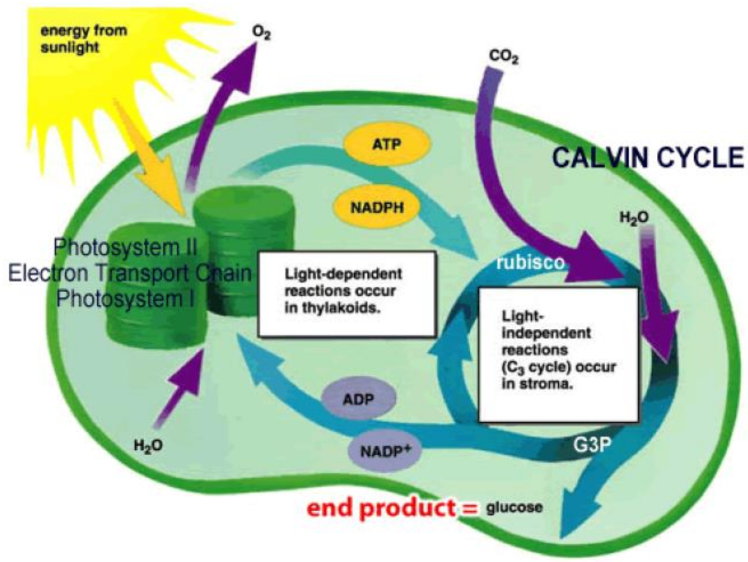


Figure 5-1 The three main stages of photosynthesis (http://slideplayer.com/slide/9022816/)

Evergreen fruit trees comprise of a number of economically important species like *Citrus spp.* These are grown mostly in areas with subtropical and tropical climates. Citrus leaves are relatively thick broad leaves consisting of a minor portion of leaf volume containing intercellular air space. On their adaxial surface is a shiny waxy cuticle and the stomata are usually located on the abaxial surface (Goldschimidt & Koch 1996). Citrus leaves also act as carbon storage organs and have low rates of assimilates export, which results in negative feedback to decrease photosynthesis (Syvertsen & Lloyd 1994). Leaves of citrus trees have relatively low maximum CO_2 assimilation rates (normally <12 µmol m^{-2} s^{-1}) compared with other C-3 plants which has assimilation rates ranging between 20-30 µmol m^{-2} s^{-1} (Kriedemann 1971; Syvertsen & Lloyd 1994). The mechanisms behind the low ACO_2 in citrus trees are not well understood, but it is believed that the low ACO_2 is one of the main causes which can limit plant growth and productivity in citrus trees (Goldschmidt 1999). Hence, it is needful to determine the significance of factors limiting ACO_2 like source-sink balance, CO_2 supply, temperature, light and certain production practices such as the application of SWEs and VE, which can potentially improve uptake of essential nutrients like N. From a report by Evans (1989), thylakoid protein is directly proportional to chlorophyll content. Photosynthetic capacity of leaves is also related to the N content mostly due to the fact that the proteins utilised in the Calvin cycle and thylakoids signify the bulk of leaf N (Evans 1989). There is also a strong linear correlation between both RUBP carboxylase and chlorophyll and N content. With an accumulation of total N per unit leaf area, the fraction of soluble proteins some of which are involved in the Calvin cycle increases. Previous studies have also shown that auxins can have an effect on photosynthesis. In a study done in apples, IAA and cytokinin levels increased to their peak levels before bloom and decreased as shoot growth stopped. IAA has been shown to stimulate photosynthesis in a number of plants including radish (Joann et al. 1984). Since SWEs and VE contain auxins, it is possible that they can have a positive impact on photosynthesis in rough lemon seedlings which can result in better dry mass accumulation and better productivity.

Plants also require 17 essential mineral nutrients comprising of the micronutrients (Mn, Bo, Cu, Fe, Zn, Mo…) and macronutrients (Ca, Mg, N, P and K). Nitrogen plays a critical role in photosynthesis, differentiation, cell division, chlorophyll content, growth, anthocyanin production, electron transport rate, rubisco activity, photosynthetic rate and is a major component of the proteins needed for various metabolic processes that occur during plant growth (Novoa and Loomis, 1981; Guidi et al. 1998; Jain et al. 1999). According to Wittenmayer and Merbach (2005) P also plays an essential role in improving shoot canopy, axillary bud growth and leaf expansion, which leads to an improved photosynthetic surface area and carbohydrate utilization. Potassium is also important for many physiological processes required for plant growth, such as maintenance of plant water balance and during protein synthesis (Fenn, 1940). Nutrient deficiency on arable land is continuously a challenge for agriculturalists. Large amounts of money are spent every year to increase the nutrient status of arable land. Although synthetic fertilizers have negative effects on the environment, there is presently nothing in the market which can substitute the application of fertilizers (Papenfus, 2011). However, since organic products such as SWE and VE have the capacity to improve the plant's root system and its nutrient harnessing ability they have the potential to reduce the fertilizer requirement of plants, resulting in the saving of a great deal of money. The aims of this section were (i) to evaluate the effect of SWEs and VE on nutrient uptake and; (ii) to determine if SWEs and VE induced any differences in CO_2 assimilation rates and carboxylation efficiencies.

5.3 Results

5.3.1 Nutrient uptake and fertiliser use efficiency

In general seedlings treated with SWEs and VE resulted in an increase in nutrient uptake. As expected, the concentration of all the nutrients tested (N, Ca, P and K), were the lowest in seedlings grown with deionised water (control) (Figure 5-2).

Ecklonia maxima – Kelpak® (EM1)

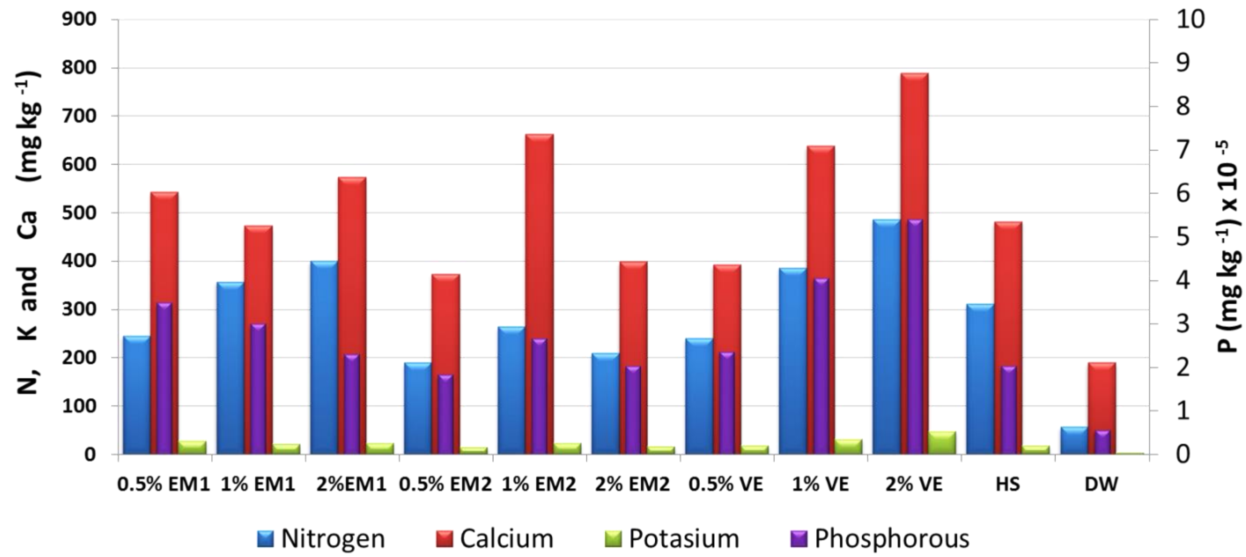
An increase in the concentration of EM1 resulted in an increase in the uptake of N. Nitrogen analyses showed that, only the 0.5% EM1 treated seedlings had N concentration (245 mg kg^{-1}) lower than the Hoagland solution control seedlings (312 mg kg^{-1}). Maximum concentration (400 mg kg^{-1}) of N was observed with 2% EM1 (Figure 5-2). The trend was not the same for P and K accumulation, where an increase in EM1 concentration resulted in a decrease in the concentration of these two nutrients. Maximum concentrations of P and K (3.5x 10^{-5} mg kg^{-1} P, 27.9 mg kg^{-1} K) were observed in seedlings treated with 0.5% EM1 and the lowest concentration of P and K (2.3 $x10^{-5}$ mg kg^{-1} P, 24.0 mg kg^{-1} K) in seedlings treated with 2% EM1 (Figure 5-2). This was, however, higher than values obtained for both controls. Maximum Ca concentration was observed in 2% EM1 (573.7 mg kg^{-1}) and the lowest concentration (473.1 mg kg^{-1}) in seedlings treated with 1% EM1.

Ecklonia maxima – Afrikelp® (EM2)

For the seedlings treated with EM2, remarkably similar trends were followed in concentration of the four nutrients (N, K, Ca and P) with maximum concentrations (264.5 mg kg^{-1} N, 663.3 mg kg^{-1} Ca, 24.3 mg kg^{-1} K and $2.66x10^{-5}$ mg kg^{-1} P) being observed in seedlings treated with 1% EM2 (Figure 5-2). The lowest concentration (189.5 mg kg^{-1} N, 373.6 mg kg^{-1} Ca, 14.6 mg kg^{-1} K and $1.83x10^{-5}$ mg kg^{-1} P) of all four nutrients was observed in 0.5% EM2. The seedlings treated with Hoagland solution had a higher concentration (312.0 mg kg^{-1}) of N than all the EM2 treatments (Figure 5-2).

Vermicast extracts

In a similar trend to what was observed with other growth parameters, seedlings that were treated with vermicast extracts displayed a direct relationship between the concentration of VE and the nutrient content. An increase in VE concentration resulted in increase in concentration of all the four nutrients. Of all the treatments, maximum (486.7 mg kg^{-1} N, 789.4 mg kg^{-1} Ca, 47.7 mg kg^{-1} K and $5.4x10^{-5}$ mg kg^{-1} P) nutrient accumulation was observed with the 2% VE (Figure 5-2). Amongst the VE treatments the lowest concentration (240.5 mg kg^{-1} N, 391.7 mg kg^{-1} Ca, 18.8 mg kg^{-1} K and $2.35x10^{-5}$ mg kg^{-1} P) of nutrients was observed in 0.5% VE treated seedlings (Figure 5-2).



EM1= *Ecklonia maxima* - *(*Kelpak®), EM2 = *Ecklonia maxima* - (Afrikelp®), VE=Vermicast extracts, HS = Hoagland solution and DW = deionised water.

Figure 5-2 Nutrient uptake due to the application of seaweed and vermicast extracts

5.2.1 Photosynthetic output

Daily averages for photosynthetic output for three selected days (28, 29 January and 11 February 2016) are shown in Figure 5-3; the deionised control seedlings yielded the lowest photosynthetic output. Throughout the experimental period similar trends were observed for all the treatments.

Ecklonia maxima – Kelpak® (EM1)

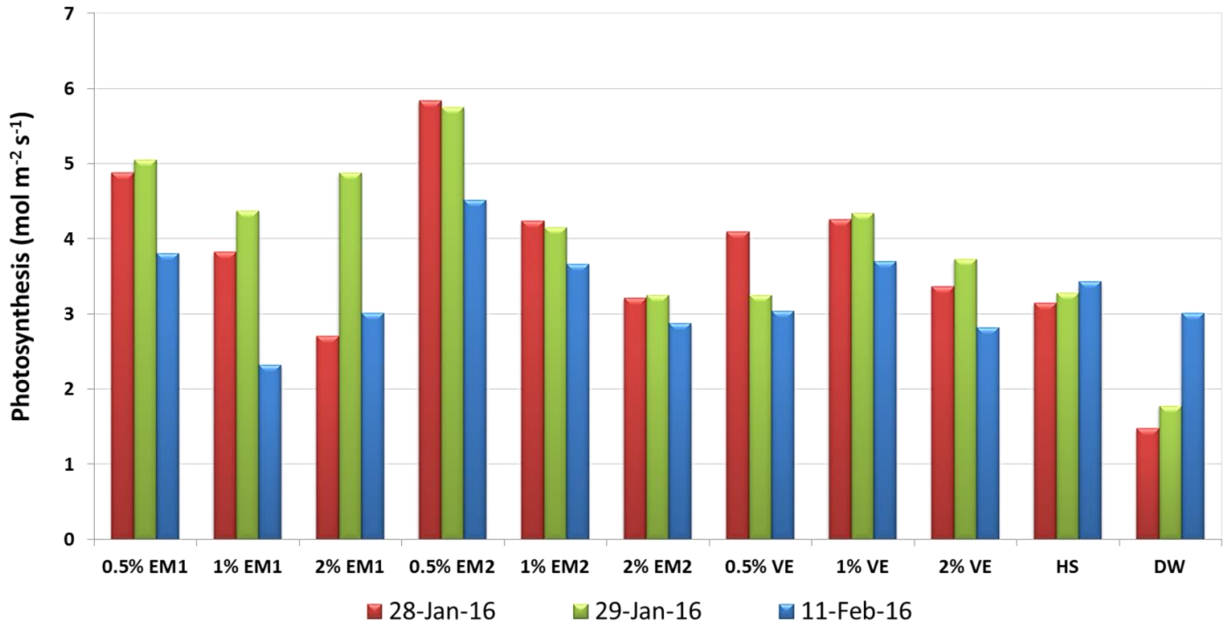
In general, for the seedlings, which were treated with EM1, photosynthetic output decreased as the concentration of EM1 increased except for the 2% EM1 measurements made on 29 Jan and 11 Feb, which were higher than the 1% EM1 measurements. The highest average photosynthetic output (4.59 mol m^{-2} s^{-1}) measured between 8am to 10:00am, was measured in seedlings treated with 0.5% EM1 for all the measurement days followed by the seedlings treated with 1% EM1 (3.54 mol m^{-2} s^{-1}) (Figure 5-3) The lowest photosynthetic output (3.51 mol m^{-2} s^{-1}) was reported for 2% EM1 treated seedlings which was lower than the control seedlings treated with Hoagland solution only (Figure 5-4). Photosynthetic output for the seedlings treated with either 0.5 or 1% EM1 was higher than the controls.

Ecklonia maxima – Afrikelp® (EM2)

It was fascinating to note that EM2 treated seedlings behaved the same way as the seedlings treated with EM1. Photosynthetic output similarly decreased with an increase in concentration. Maximum photosynthetic output for all the days of measurements were measured in seedlings, which were treated with 0.5% EM2 (Figure 5-3). As observed with seedlings treated with EM1, seedlings treated with 0.5% (5.38 mol m^{-2} s^{-1}) and 1% EM2 (4.03 mol m^{-2} s^{-1}) yielded higher photosynthetic output than all the controls. The output of seedlings treated with 2% EM2 (3.12 mol m^{-2} s^{-1}) were slightly lower than that of the seedlings grown in Hoagland solution but higher than those grown in deionized water. It was also noted that, the seedlings which were treated with 0.5% EM2 had the highest chlorophyll content, suggesting a relationship between chlorophyll content and photosynthetic capacity, this relationship was also observed in 0.5% EM1.

Vermicast extracts

Seedlings treated with vermicast extracts did not follow the same general trend as those treated with EM1 and EM2. Seedlings treated with 1% VE (4.11 mol m^{-2} s^{-1}) yielded high photosynthetic output, which was higher than both controls (Figure 5-3). Whereas seedlings treated with 0.5% VE (3.47 mol m^{-2} s^{-1}) and 2% VE (3.31 mol m^{-2} s^{-1}) yielded nearly the same photosynthetic output as the plants treated with Hoagland solution, but were higher than that of the deionised water control (Figure 5-3).



EM1= *Ecklonia maxima* - (Kelpak®), EM2 = *Ecklonia maxima* - (Afrikelp®), VE=Vermicast extracts, HS = Hoagland solution and DW = deionised water.

Figure 5-3 Daily average photosynthetic output due to the application of seaweed and vermicast extracts.

5.2.2 Photosynthetic (A/Ci) response curves and carboxylation efficiency

The response of assimilation rate (A) to intercellular carbon dioxide concentration (Ci) is parabolic and decreases with the accumulation of carbohydrates (Baldocchi and Amthor, 2001). The A/Ci curves can be categorised into the Ribulose bisphosphate carboxylase oxygenase (Rubisco) limited phase and Ribulose bisphosphate (RuBP) limited phase. The CO_2 concentration that results in zero net photosynthesis is called the CO_2 compensation point. At this point, leaf internal CO_2 is so low that it restricts photosynthesis to the extent that gross photosynthesis is exactly in balance with leaf respiration, including photorespiration. The CO_2 compensation point for all the seedlings in different treatments was slightly lower than 100 µmol CO_2 mol^{-1} (Figure 5-4). For all the treatments, photosynthesis increased with the increase in intercellular carbon dioxide (Ci) reaching a maximum between 300 - 400 µmol CO_2 mol^{-1} (Figure

5-4) intercellular carbon dioxide, which was similar to other findings in *Citrus sinensis* (Vu & Yelenovsky, 1988). During electron transport limitation, the RuBP limited phase, CO_2 uptake still increases because CO_2 out-competes O_2 for the available RuBP, but during the triose limitation phase, photosynthesis is no longer CO_2 dependent (Von Caemmerer, 2000). The results obtained with lemon under unlimited light conditions showed maximum values of around 9 µmol m^{-2} s^{-1}. This value is very low as net CO_2 assimilation rates of citrus under non-stressed conditions are generally low unlike other woody perennials (Downton et al. 1987; Kriedemann 1971). Optimum leaf photosynthetic rates of field-grown citrus trees as indicated by Kriedemann (1968; 1971) under optimum growing conditions range between 6-11 µmol m^{-2} s^{-1}.

Ecklonia maxima – Kelpak® (EM1)

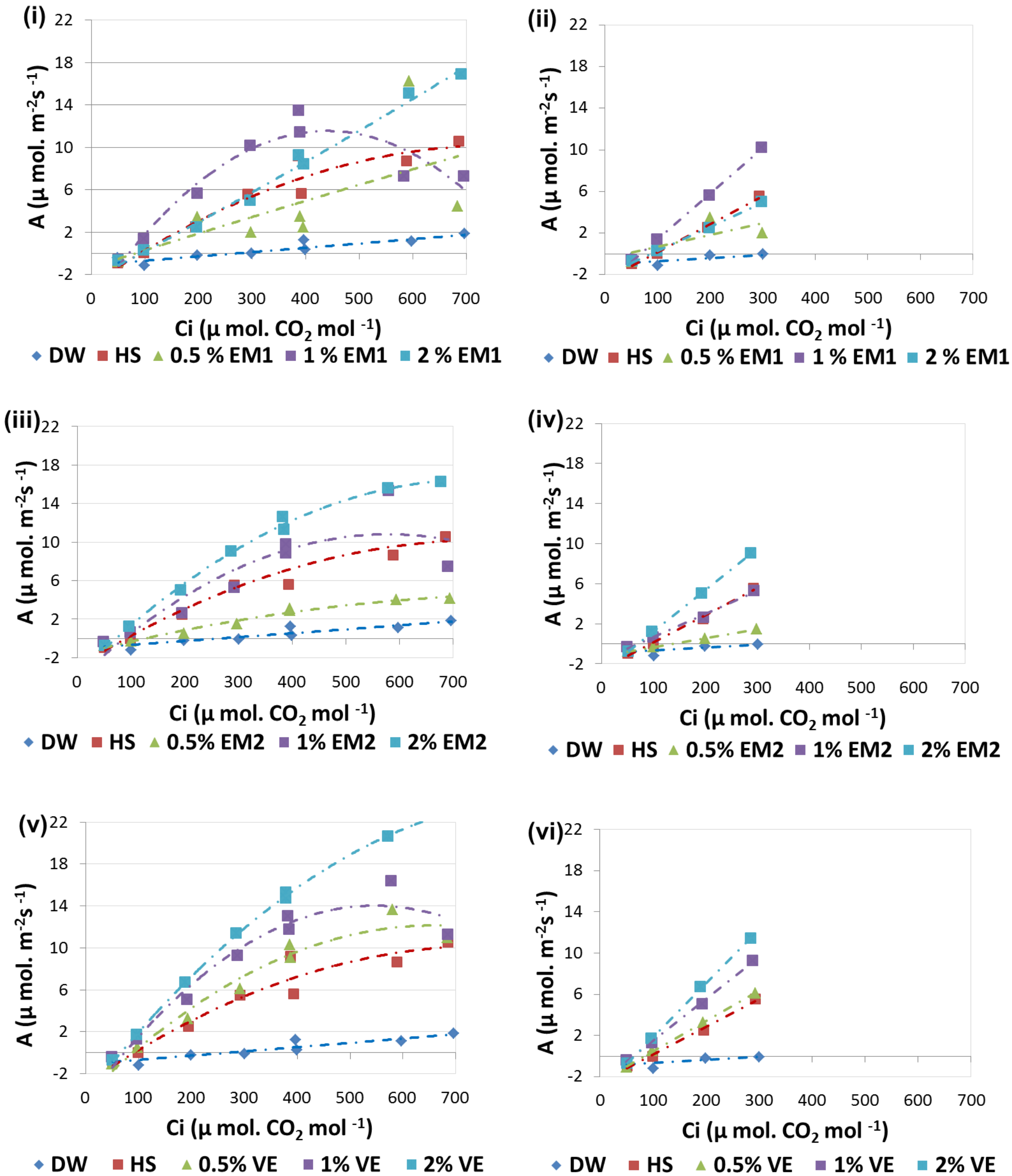
For the seedlings treated with EM1 the rate of CO_2 assimilation was significantly higher in seedlings treated with 1% EM1, which was not significantly different from the seedlings treated with 1% and 2% VE (Figure 5-4). The carboxylation efficiency ($\Delta A/\Delta Ci$) (0.03) which is a linear function from 0-300 µmol CO_2 mol^{-1} was significantly higher in seedlings treated with 1% EM1, which translate that the rate of the manufacturing of carbon skeletons were slightly higher in the seedlings treated with 1% EM1 (Figure 5-5). The rate of assimilation was higher for both 1% EM1 and 2% EM1 treatments than for the controls. Seedlings treated with 0.5% EM1 had lower values than the seedlings treated with the Hoagland solution but was higher than the deionised water control.

Ecklonia maxima – Afrikelp® (EM2)

For the seedlings treated with EM2 the rate of assimilation was significantly higher in seedlings treated with 2% EM2, which was not significantly different from the seedlings treated with 1% EM2 (Figure 5-4). The carboxylation efficiency ($\Delta A/\Delta Ci$) (0.025) for both groups of seedlings treated with 1% and 2% EM2 was not significantly different (Figure 5-5). Seedlings treated with 0.5% EM2 yielded the lowest rate of assimilation (4.16 µmol CO_2 mol^{-1}) and carboxylation efficiency (0.011) which was significantly lower than the Hoagland solution control, but significantly higher than the seedlings treated with deionised water (Figure 5-4 and Figure 5-5).

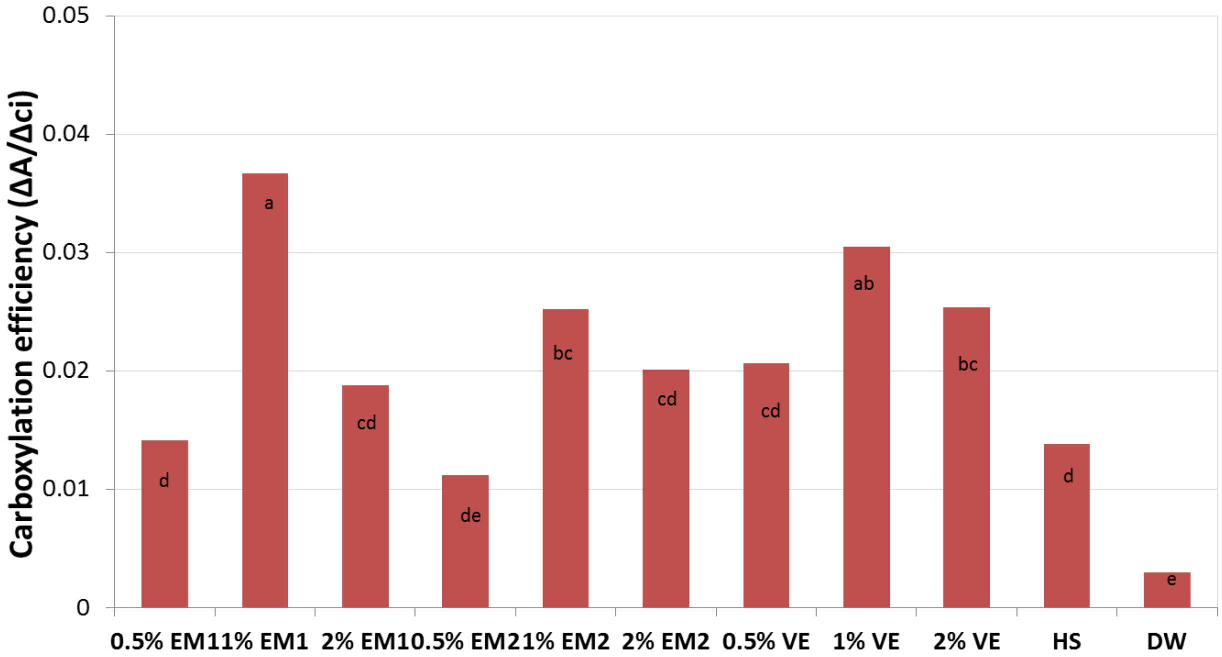
Vermicast extracts

The seedlings treated with vermicast extracts yielded higher assimilations rates and carboxylation efficiency than the seedlings treated with Hoagland solution and deionised water (Figure 5-4 and Figure 5-5). The rate of carbon dioxide assimilation into carbon skeletons was significantly higher in seedlings treated with 1% VE which was not significantly different from the seedlings treated with 2% VE. The carboxylation efficiency ($\Delta A/\Delta Ci$) (0.033) for both seedlings treated with 1% VE and 2% VE (0.032) was not significantly different (Figure 5-5). Seedlings treated with 0.5% VE yielded the lowest rate of assimilation (13.6 µmol CO_2 mol^{-1}) and carboxylation efficiency (0.017) compared to other treatments.



EM1= *Ecklonia maxima - Kelpak*®, EM2 = *Ecklonia maxima - Afrikelp*®, VE=Vermicast extracts, HS = Hoagland solution and DW = deionised water.

Figure 5-4 Net photosynthesis rate (A) versus intercellular CO_2 mole fraction (*Ci)* for EM1 (i), EM2 (iii) and VE (v) and; carboxylation efficiencies for EM1 (ii), EM2 (iv) and VE (vi)



Means followed by the same letter were not significantly different (*P* at 0.05) according to Tukey's Studentized Range test. EM1= *Ecklonia maxima* - (Kelpak®), EM2 = *Ecklonia maxima* - (Afrikelp®), VE=Vermicast extracts, HS = Hoagland solution and DW = deionised water.

Figure 5-5 Carboxylation efficiency of the seedlings treated with seaweed and vermicast extracts.

5.3 Discussion and summary

One of the aims of this chapter was to determine whether the SWEs (EM1 and EM2) and VE were able to improve nutrient uptake in hydroponically grown rough lemon seedlings. The main reason behind this was to see if the improved root development due to application of SWEs and VE would lead to better nutrient uptake since a better root system provides a greater absorptive root surface for water and nutrient uptake. By visual analysis, it was clear that seedlings treated with SWEs and VE did not show any signs of nutrient deficiency and appeared healthier compared to the deionised water control plants, which showed signs of leaf necrosis and yellowing (Figure 4-2). EM1 increased N and Ca levels in rough lemon seedlings whereas the levels of P and K decreased. For EM2, the 1% dilution was the optimum dilution for nutrient uptake as it displayed the highest level of all four nutrients analysed for N, P, K and Ca. On the other hand, the 2% VE treatment was deemed optimum for nutrient uptake as it also exhibited the highest concentration of all four nutrients. It is likely that the increase in N concentration could be due to the N-containing plant hormones, such as auxins and cytokinins in the SWEs and VE absorbed by the plants. The nutrients available in SWEs and VE are generally too low to have a significant impact on the nutrient levels detected in the plants. As a result of this SWEs and VE should not be applied to plants as a source of nutrients such as N and K. It is suggested that the SWEs and VE should be applied as agricultural biostimulants in a specific fertilizing regime in order to enhance nutrient uptake. This could in turn lead to a decrease in fertilizing costs and a subsequent reduction of input costs.

Another aim of this chapter was to determine if SWEs and VE had an effect on the assimilation rate when applied at different dilutions. In general, all the seedlings treated with different EM1, EM2 and VE treatments had higher carboxylation efficiencies and photosynthetic outputs than the seedlings grown in deionised water. The lower levels of photosynthesis and lower carboxylation efficiencies observed in the seedlings grown in deionised water were as a result of leaf necrosis and chlorosis as also observed by Habermann et al. (2003). Due to chlorosis, the leaves in the deionised water had an impaired ability to capture photosynthetically active radiation required for photosynthesis.

CHAPTER 6 General conclusions and future recommendations

A number of reports showed the positive effect of seaweed extracts (SWEs) and vermicast extracts (VE) on plant growth of different agricultural crops but very few studies have been done in citrus. Therefore, the effect of *Ecklonia maxima* – Kelpak® (EM1), *Ecklonia maxima* – Afrikelp® (EM2) and VE on growth of rough lemon seedlings was investigated. From the results obtained, it is clear that application of SWEs and VE have positive, neutral and inhibitory effects on plant growth, depending on the concentration applied. It was confirmed that the SWEs and VE are capable of enhancing root development, above-ground plant growth (chapter 4), photosynthetic capacity and nutrient uptake (chapter 5) in rough lemon seedlings. By enhancing root development, there was a significant increase in nutrient uptake as shown by increased levels of N, Ca, K and P in all plants treated with EM1, EM2 and VE compared to the deionised water control plants (chapter 5). Coupled with an increase in photosynthetic capacity (chapter 5), there was a notable increase in dry matter accumulation in all the plants treated with EM1, EM2 and VE (chapter 4).

When all the treatments of EM1, EM2 and VE were compared, the 2% VE treatment had the highest root volume, root and shoot dry masses, plant heights, chlorophyll content, nutrient content (chapter 4) and relatively high carboxylation efficiency (chapter 5) compared to the other treatments. However, as there was no decrease in plant growth when the concentration of VE was increased to 2%, this made it hard to conclusively pin point the optimum concentration of VE for plant growth. It is therefore needful to conduct more trials to test the effect of higher concentrations of VE and to identify at what level of VE plant growth would be inhibited. While, EM1 enhanced above-ground and root growth of rough lemon seedlings, grown hydroponically at 0.5% and 1%, the above-ground and root growth of rough lemon seedlings decreased at higher concentrations of EM1 (2%) (chapter 4). A similar trend was also observed with EM2, where the higher concentrations (2%) were inhibitory to root growth and above-ground plant growth. The optimum dilution for optimum plant growth for EM1 would be 1% dilution as it had the highest root volume, stem diameter, plant height and photosynthetic capacity compared to the other treatments. For EM2, the optimum dilution would be 1% as it showed better plant growth compared to the other dilutions. When EM1 and EM2 were compared, it was clear that EM1 performed better in terms of above-ground and below ground growth parameters compared to EM2. As mentioned earlier (chapter1), EM1 is manufactured using the cell burst method (CBM) whilst EM2 is manufactured using the cold micronization process CMP. This difference in manufacturing processes could result in a difference in concentrations of the growth promoting constituents of these two products, ultimately causing the observed difference in growth promoting effects. Some lab analyses that were conducted revealed a discrepancy in active principles in the SWEs and VE. The Liquid chromatography mass-spectrometry (LCMS) analysis of EM1, EM2 and VE revealed that these products have different levels of auxins (indole-butyric acid - IBA and naphthalene acetic acid - NAA), cytokinins (benzyl adenine - BzA) and other plant growth regulators which are responsible for the variation in their effects on growth of rough lemon seedlings (Table 4-1, chapter 4). From Table 4-1, it can be seen that the two samples of VE had more than ten times the levels of NAA compared to EM1 and EM2 whilst the levels of IBA were nearly the same in all three biostimulants. Since NAA is well-known for its root-promoting ability, it is most likely that the high root volume and root dry mass was due to the action of NAA. According to Papenfus (2011), auxins work synergistically with polyamines (PA) to promote root growth. The levels of PA in EM2 (0.1 pmol ml^{-1}) were lower compared to EM1 and VE and this may have contributed to the poor root development observed with EM2 (chapter 4). The different types of PGR conjugates which comprise the SWEs and VE are also essential, since some of them are more resistant to degradation and more physiologically active compared to others (Sanderson & Jameson 1985). The various conjugates in SWEs and VE stimulate different amounts of biological activity (Martin et al. 2001).

Since auxins are known to stimulate the formation of new meristematic tissue (Papenfus et al. 2012), they play an active role in root development. When EM1, EM2 and VE were compared to the plant growth regulators (PGRs; IBA and NAA), there was generally more root development in the plants treated with SWEs and VE than the plants treated with pure PGRs. The possible reason for this inconsistency is that the SWEs and VE contain a mixture of plant growth hormones, other than the auxins and cytokinins which are also involved in moderating plant development. A synergistic relationship was also observed when a combination of 0.05 mg L^{-1} IBA and 0.05 mg L^{-1} NAA was applied where this combination yielded nearly the same root development as the 0.1 mg L^{-1} NAA treatment. The SWEs and VE led to a significant increase in nutrient uptake and photosynthetic capacity compared to untreated plants.

From this study, it is clear that agricultural biostimulants such as SWEs and VE enhance growth and performance of plants which can result in reduction of quantities of fertilizer used, subsequently reducing input costs. Different SWEs (EM1 and EM2) and VE as presently used by the horticultural industry were compared and this will facilitate the citrus industry to selectively capitalize in the most efficient, cost effective product for growing quality citrus nursery seedlings with well developed, efficient root systems. Future research prospects will aim to investigate the possibility of using SWEs and VE for root recovery of nematode and *Phytophthora spp*-infested citrus trees as they are highly susceptible to nematode and *Phytophthora spp* attacks. The promising role of SWEs and VE in alleviating *Phytophthora spp.* infection in citrus plants through improved root growth and plant vigour will also lead to an increase in productivity which will result in improved and greater yields in the long-run. Since SWEs and VE are organic in nature, they are environmentally friendly and can play a critical role in integrated pest and disease management.

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Appendix

Chlorophyll content

The GLM Procedure

Dependent Variable: v1 Chlorophyll content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	97733.1379	4653.9589	185.44	<.0001
Error	1298	32575.9120	25.0970		
Corrected Total	1319	130309.0499			

R-Square	Coeff Var	Root MSE	v1 Mean
0.750010	8.644139	5.009691	57.95477

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	10	43816.97302	4381.69730	174.59	<.0001
date	11	53916.16493	4901.46954	195.30	<.0001

Stem diameter

The GLM Procedure

Dependent Variable: v1 Stem diameter

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	789.5158012	37.5959905	246.19	<.0001
Error	1298	198.2164106	0.1527091		
Corrected Total	1319	987.7322118			

R-Square	Coeff Var	Root MSE	v1 Mean
0.799322	11.66777	0.390780	3.349227

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	10	254.9289585	25.4928958	166.94	<.0001
date	11	534.5868427	48.5988039	318.24	<.0001

Plant height

The GLM Procedure

Dependent Variable: v1 Plant height

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	19863.37723	945.87511	132.67	<.0001
Error	1298	9254.14774	7.12954		
Corrected Total	1319	29117.52497			

R-Square	Coeff Var	Root MSE	v1 Mean
0.682179	17.15849	2.670121	15.56152

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	10	6393.77880	639.37788	89.68	<.0001
date	11	13469.59842	1224.50895	171.75	<.0001

Root length

The GLM Procedure

Dependent Variable: v1 Root length

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	21	53656.68289	2555.08014	375.32	<.0001
Error	1298	8836.47892	6.80776		
Corrected Total	1319	62493.16181			

R-Square	Coeff Var	Root MSE	v1 Mean
0.858601	15.97903	2.609169	16.32871

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	10	9441.86989	944.18699	138.69	<.0001
date	11	44214.81299	4019.52845	590.43	<.0001

Root volume

The GLM Procedure

Dependent Variable: v1 Root volume

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	16	33193.23665	2074.57729	195.71	<.0001
Error	753	7981.96288	10.60022		
Corrected Total	769	41175.19953			

R-Square	Coeff Var	Root MSE	v1 Mean
0.806146	38.03848	3.255797	8.559221

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	10	7793.18639	779.31864	73.52	<.0001
date	6	25400.05026	4233.34171	399.36	<.0001

Root dry mass

The GLM Procedure

Dependent Variable: v1 Root dry mass

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	38.3527564	3.8352756	3.24	0.0012
Error	99	117.3312800	1.1851644		
Corrected Total	109	155.6840364			

R-Square	Coeff Var	Root MSE	v1 Mean
0.246350	53.58022	1.088653	2.031818

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	10	38.35275636	3.83527564	3.24	0.0012

Shoot dry mass

The GLM Procedure

Dependent Variable: v1 Shoot dry mass

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	383.501740	38.350174	5.11	<.0001
Error	99	743.145220	7.506517		
Corrected Total	109	1126.646960			

R-Square	Coeff Var	Root MSE	v1 Mean
0.340392	51.28795	2.739802	5.342000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	10	383.5017400	38.3501740	5.11	<.0001

Nitrogen content for pure hormones (IBA and NAA)

The GLM Procedure

Dependent Variable: v1 N content for pure hormones

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	29.51312484	2.95131248	12.36	<.0001
Error	44	10.50500960	0.23875022		
Corrected Total	54	40.01813444			

R-Square	Coeff Var	Root MSE	v1 Mean
0.737494	11.20087	0.488621	4.362345

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	10	29.51312484	2.95131248	12.36	<.0001

Nitrogen content for EM1, EM2 and VE

The GLM Procedure

Dependent Variable: v1 N content for pure hormones

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	10	29.51312484	2.95131248	12.36	<.0001
Error	44	10.50500960	0.23875022		
Corrected Total	54	40.01813444			

R-Square	Coeff Var	Root MSE	v1 Mean
0.737494	11.20087	0.488621	4.362345

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	10	29.51312484	2.95131248	12.36	<.0001

Ca, K and P content for EM1, EM2 and VE

The GLM Procedure

Dependent Variable: v1 K content

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	31	177366073.5	5721486.2	21.36	<.0001
Error	128	34293737.7	267919.8		
Corrected Total	159	211659811.2			

R-Square	Coeff Var	Root MSE	v1 Mean
0.837977	13.56559	517.6097	3815.607

Source	DF	Type I SS	Mean Square	F Value	Pr > F
treatment	31	177366073.5	5721486.2	21.36	<.0001

Carboxylation efficiencies

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.001416	10	0.000142	3.054516	0.046357	2.978237
Within Groups	0.000464	10	4.64E-05			
Total	0.001879	20				

Source of Variation	SS	df	MS	F	P-value	F crit
Between Groups	0.001416	10	0.000142	3.054516	0.046357	2.978237
Within Groups	0.000464	10	4.64E-05			
Total	0.001879	20				