SEAWEED EXTRACT EFFECTS ON POTATO (*SOLANUM TUBEROSUM* 'BP1') AND GRAPE (*VITIS VINIFERA* VAR. SULTANA) PRODUCTION

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A thesis submitted in partial fulfilment of the requirements for the degree of Doctor Philosophiae: Biodiversity in the Department of Biodiversity and Conservation Biology (BCB), University of the Western Cape

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KEYWORDS

Antioxidants

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ABSTRACT

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ABSTRACT

Field trials were conducted to determine the effects of a locally produced seaweed extract product **(**Afrikelp® LG-1), on the growth parameters and yield of *Solanum tuberosum* 'BP1'potato crop and *Vitis vinifera* var. sultana grapes. The extrapolation of these results will assist local potato and grape farmers to produce quality crops with improved yields.

Field experiments were conducted at the Agricultural Research Council (ARC) Nietvoorbij site in Stellenbosch, South Africa. The experimental layout consisted of four randomised blocks, and four replicates were used per treatment. Various concentrations of seaweed extracts (0, 2, 3, 4, 5, 6, and 7 l/ha) was applied as soil drench and foliar sprays over two cropping seasons, namely 2010 and 2011 (potato), and 2011 and 2012 (sultana grapes).

The effects of drench and foliar application of a commercial seaweed extract on the leaf nutrient content, the antioxidant levels and food proximate values of the potato tubers and leaves were determined. The effects on chlorophyll, growth and yield were also examined. The effects of a seaweed extract on the growth and yield of sultana grapes, under soil drench and foliar applications were investigated.

Field trials showed that there was no difference in leaf nutrient content due to the method of application (drench or foliar). The leaf nutrient content of the mineral elements studied. N, P, K, Ca, Mg, Na, Mn, Cu, Zn and B increased with seaweed extract concentrations.

For antioxidant in 2010, the foliar method of seaweed extract application resulted in lower levels of ORAC (Oxygen Radical Absorbance Capacity) in the tuber over drench, thereby showing lowered plant oxidative stress. Higher antioxidants values of polyphenols and ORAC in 2011 were present at lower seaweed treatments and at the controls. Food proximate values (crude fibre and protein) were enhanced by foliar treatment in 2010 and 2011.

Potato leaf chlorophyll *a*, *b* and total chlorophyll levels increased with higher seaweed extract concentrations. Growth parameters (foliage fresh and dry mass, plant height, and tuber diameter) increased significantly with higher seaweed extract concentrations. The highest concentration of 7 l/ha had the most significant effect on total tuber yield in 2010 (125%) and 2011 (109%) respectively.

Field trials showed that for sultana grapes seaweed extract concentrations of 7 l/ha produced the highest results for all measured vine growth parameters (bunches per vine, bunch length and diameter). For total yield, at the 7 l/ha seaweed extract concentration, gave a commercially important increase of 355% in 2011, and 210% in 2012. The field trials showed that seaweed extract have the capability to promote growth and yield of potato and grape crops.

DECLARATION

I declare that "Seaweed extract effects on potato (*Solanum tuberosum* 'BP1') and grape (*Vitis vinifera* var. sultana) production" is my own work, that it has not been submitted before for any degree or assessment in any other university, and that all the sources I have used or quoted, have been indicated and acknowledged by means of complete references.

J.R.V. October October 2017

Signature

DEDICATION

I dedicate this doctoral thesis to

My wife,

Astrid October,

And my children,

Nina, Matthew and Amy.

Without their support, patience and understanding this achievement could not have been accomplished.

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External laboratories – BemLab for providing the soil and leaf mineral nutrient analysis.

LIST OF PAPERS TO BE SUBMITTED

1. **October, J.R.V.**; Raitt, L.M.; P.A. Ndakidemi, P.A. The effects of drench and foliar application of a commercial seaweed extract on the leaf nutrient content of potato, *Solanum tuberosum* 'BP1'

2. **October, J.R.V.**; Raitt, L.M.; P.A. Ndakidemi, P.A. The effects of drench and foliar applications of a seaweed extract on the antioxidant levels and food proximate values of the tubers and leaves of *Solanum tuberosum* 'BP1'

3. **October, J.R.V.**; Raitt, L.M.; P.A. Ndakidemi, P.A. The effects of soil drench and foliar application of a commercial seaweed extract on chlorophyll, growth and yield of *Solanum tuberosum* 'BP1'

4. **October, J.R.V.**; Raitt, L.M.; P.A. Ndakidemi, P.A. The effects of a commercial seaweed extract on the growth and yield of *Vitis vinifera* var. sultana grapes, under soil drench and foliar applications.

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LIST OF ABBREVIATIONS

PLANT GROWTH

Chl_a	Chlorophyll *a*
Chl_b	Chlorophyll *b*
Cmol	Centimoles
DAP	Days after planting
DW	Dry weight
FW	Fresh weight
HPLC-UV	High-performance liquid chromatography-Ultraviolet–Visible
HEU	Hot extraction unit
IAA	Indoleacetic acid (Chemical name of Auxin)
IBA	Indolebutyric acid
ICP-MS	Inductively coupled plasma-mass spectrometry
Kcal	Kilocalorie
KCL	Measurement of hydrogen ion concentration (pH)
LG	Liquid green
LSE	Liquid seaweed extracts
NO3-N	Nitrate as nitrogen
RCBD	Randomized block design
SE	Seaweed extracts
SLF	Seaweed liquid fertilizer
SOM	Soil organic matter
SWC	Seaweed concentrate
TSS	Total soluble solids
AOAC	Association of Official Analytical Chemists
FAO	Food and Agricultural Organization of United Nations
SCWG	Soil Classification Working Group

ANTIOXIDANTS

AA	Ascorbic acid
Ac	Anthocyanins
FRAP	Ferric reducing/antioxidant power
GAE	Gallic acid equivalent
HAT	Hydrogen atom transfer
ORAC	Oxygen radical absorbance capacity
P	Polyphenols
SET	Single electron transfer
TEAC	Trolox equivalent antioxidant capacity
UmolTE/g	Unit moles trolox equivalent per grams (weight of antioxidants)

CHAPTER 1: INTRODUCTION: SCOPE

CHAPTER 1

INTRODUCTION

1.1 Background

Potatoes are one of the most important food crops and were ranked fourth in the world after maize, wheat and rice, with a production of 329 million tons (FAO, 2009). As for the harvested area, potato ranked seventh after wheat, rice, maize, barley, sorghum and rapeseed worldwide. In terms of consumption, potato ranked third after rice and wheat. Interestingly, the importance of potato in the diet is higher in developed as compared to developing countries, accounting for 130 kcal per person per day for the developed world and for 41 kcal per person per day in the developing world (Burlingame *et al.*, 2009). In Europe, the per capita consumption reached 90 kg per year, whereas in developing countries, per capita consumption was smaller, reaching around 20 kg per year. Singh & Kaur (2009), reported that potatoes supply dietary fibre, carbohydrates, high-quality proteins, vitamins and minerals. According to the USDA National Nutrient Database, contents for potatoes are 2.4 g for dietary fiber, 15.7 g for carbohydrates, 1.7 g for protein content, 19.7 g for vitamin C, each per 100 g of white, raw potato (flesh and skin). As for the minerals, iron and zinc contents are around 0.52 mg and 0.29 mg per 100 g.

On the local front, in South Africa potatoes are a very important food commodity. It has therefore become important for all potato growers to produce successfully and profitably. Farmers, and in particular emerging farmers in South Africa, are faced with many challenges, particularly in coping with high fertilizer input costs and an increasingly competitive market. A successful and sustainable crop growing system therefore relies on an integrated management approach, which will include the implementation of appropriate plant feeding practices that result in the production of a quality crop, which then maximizes financial returns for the South African farmer (Venter, 2005). Within the South African context, the gross value of potato production accounted for about 43% of major vegetables, 15% of horticultural products and 4% of total agricultural production. On average domestic potato farmers harvested about 1.6 billion Rand's worth of potatoes each year. These domestic farmers comprised about 1 700 potato farmers (including approximately 400 seed growers) and 66 600 farm workers. Potatoes are grown year round owing to the country's unique geography and climate (Venter, 2005).

Viticulture is an agricultural activity extensively practiced in the Western Cape, South Africa, which is regarded as a coastal region. The *Vitis vinifera* var. sultana, table grape industry is one of the most important fruit growing sectors in this region and provides employment to a large sector of the Western Cape population (Dept. of Agriculture, Forestry & Fisheries, 2012).

The South African table grape export industry is situated in mild Mediterranean and arid subtropical climates. More than 80% of table grape production in South Africa occurs in the Western Cape region. Other production areas include the Northern Cape, Eastern Cape, Limpopo, Free State and Mpumalanga. Internationally, table grapes are among the most traded fruit types in the world (Dept. of Agriculture, Forestry & Fisheries, 2012). In the table grape industry, berry quality and certain growth characteristics plays an important role for marketing table grapes. In the local table grape industry crop quality is measured in terms of seediness, appearance, eating quality characteristics, which include sweetness, firmness, texture and flavour. The appearance of bunches is important for marketing table grapes: this includes size, diameter and length (Clingeleffer, 1985).

On the global front there is an increasing trend among consumers to use healthier foods. The challenge of global warming, the resulting change in climate and the way it will affect agricultural production in future confronts all crop producers. This challenge presents an opportunity for agriculturist to produce healthier crops using innovative feeding practices (André, *et al*., 2007, and FAO, 2009). In many countries such as Europe, the USA, and Japan, this has led to an increasing use of organically produced crops. This is mainly due to the perception that crops produced by "conventional" agricultural practices using artificial fertilizers, pesticides, and herbicides have long-term injurious effects on the health of the consumer. Organic agriculture relies on natural products and processes to grow crops, while at the same time improving soil quality, controlling pests and promoting biodiversity. It uses the maintenance of sustainable ecosystems (people, plants, animals and soil) as the guiding principle for production. The term organic does not refer to the quality of the food, but rather the process by which that food is produced (Alleman, 2003).

Marine algae are used in agricultural and horticultural crop production as a source of organic fertilizer, and beneficial effects, in terms of plant growth, enhancement of yield and quality have been reported (Blunden, 1991; Crouch and Van Staden, 1994). Liquid seaweed extracts (LSE) have gained importance as foliar sprays for many crops including various grasses, cereals, flowers and vegetable species (Crouch and Van Staden, 1994).

The use of organic based bio stimulants on potatoes and grapes is a standard practice in many horticultural and agricultural crop regimes. Seaweed extracts are often regarded as soft or natural products that can enhance crop growth and development (Norrie and Hiltz, 1999). For example a marine algae extract from *Ascophyllum nodosum*, has often been applied as foliar and root drench bio stimulant or supplemental fertilizer, and has been recognised source of mineral nutrients (Senn, 1987).

In South Africa many farmers are aware of the benefits associated with commercial seaweed products, but their use is limited as a result of limited research on the possible benefits they have on specific crops under local growing conditions. This study will therefore evaluate the use of commercial seaweed product (AfriKelp® LG-1) locally produced for agriculture and horticulture sectors. It is produced from the marine algae, *Ecklonia maxima* (Osbeck) Papenfuss, the South African giant brown seaweed, which grows in the clean southern oceans. Its manufacturing involves a cool fragmentation technology process. As this method differs from other seaweed technologies, there may be differences in its hormonal efficacy (www.Afrikelp.com/product/, 2010).

The local seaweed extract product (Afrikelp® LG-1) contains various amounts of auxin, cytokinin, gibberellins, and abscisic acid, compounds which may be absorbed by the plant. The products composition includes naturally occurring bio stimulants derived predominantly from the auxin plant hormone group, which are present in a special ratio of high auxin and low cytokinin levels that stimulate the growth of crops. When it is applied to the leaves, the auxins are transported down to the root tips and cause the roots to grow. In the root meristem, the plant produces cytokinins, which are transported upwards and initiate corresponding shoot growth (www.Afrikelp.com/product/, 2010).

The local seaweed extract product is thus reputed to stimulate the plant to produce its own plant hormones to put it into balance again. The end result means more roots, a stronger and healthier plant that can take up more available nutrients and defend itself better against biotic and abiotic stress factors, resulting in higher yield and quality crops (www.Afrikelp.com/product/, 2010).

The primary purpose of this study was to ascertain the extent to which commercial seaweed extract (Afrikelp® LG-1), produced from marine algae, *Ecklonia maxima* (Osbeck) Papenfuss, affected specific growth parameters of *Solanum tuberosum* 'BP1' (potato) and *Vitus vinifera* var. sultana (table grape). A comparative analysis of both crops was conducted over a two year period and compared with a control.

Application methods (foliar and soil drench) and the effects of various seaweed concentrations were studied. The potato crops were analyzed for the following variables: Leaf nutrient content of nitrogen, phosphorous, potassium, calcium, magnesium, sodium, manganese, iron, copper, zinc, and boron. Antioxidant levels for the tubers (ascorbic acid, polyphenols and ORAC) and for the leaves (polyphenols and ORAC) were measured. Food proximate analysis for protein and crude fibre were conducted on the tubers and leaves. Potato leaf chlorophyll *a*, *b* and total chlorophyll values were calculated. Growth parameters foliage fresh and dry mass, plant height, tuber diameter and total yield were evaluated.

The effects of a local seaweed extracts were measured on the physical growth parameters of *Vitis vinifera* var. sultana grapes, for number of bunches, length of bunches, diameter of bunches and total yield.

The seaweed extract (Afrikelp® LG-1) was evaluated under local agricultural conditions, which are experienced by local potato and grape farmers. The results will thus be applicable under local farming practices.

In a South African context, the outcomes of this study will indicate whether a local seaweed extract has the ability to increase the yield capacity of potato farmers and grape producers. This may have potential social benefits, and contribute toward employment opportunities and economic improvement in the Western Cape. The majority of potato and grape farming areas are located in close proximity to communities that are economically marginalized. New technologies and products to help grow horticulture, agriculture and viticulture sectors will create new income opportunities in rural settlements, a region hard-hit by poverty in South Africa.

1.2 Problem Statement and Research Motivation

Field trials were conducted to determine the effects of the commercial seaweed extract (Afrikelp ® LG-1) on the growth and yield of *Solanum tuberosum* 'BP1' and *Vitus vinifera* var. sultana grown under foliar and soil drench application over a two year period.

On a local front there are many farmers utilizing the seaweed product across the farming sector in the Western Cape. The majority of these farmers are growing crops such as potatoes, BP1 and sultana table grapes. Potato BP1 cultivar grows well in most soil types, but in certain potato growing regions of South Africa, and in particular the Sandveld region in the Western Cape, farmers generally grow potatoes in soils that are sandy and as a result require significant inputs of fertilizers and organic matter. As a result of the challenging farming environment substantial costs are incurred by the local farmers to condition soils ensuring that they are drained, friable, have the ideal pH, and are rich in mineral nutrients and organic materials. By adopting farming practices, which achieve these conditions, the farmer can produce high yields of tubers with excellent culinary and processing quality. The local seaweed extract contain significant amounts of auxins and cytokinins. When applied to crops, which are growing in nutrient deficient soils with poor soil structure, and exposed to high daytime temperatures, the seaweed extracts are reputed to stimulate root growth then foliage growth and ultimately bring into balance root and foliage growth of the plant. This process results in a higher yield and a better quality crop. The organic nature of the seaweed helps promote soil microbes (www.Afrikelp.com/product/, 2010).

Currently there are no records of field trials of the effects of the local seaweed extract (Afrikelp® LG-1) on potato BP1 and sultana grapes. This report is based on trials conducted on field grown potato and sultana crops, and unlike previous research work, which was done under greenhouse conditions or on commercial observations, which is information mainly gathered by farmers.

In practical terms, the results identified those areas of potato and sultana production, influenced by Afrikelp® LG-1, and provided greater information for farmers who ultimately seek to enhance quality of their crop by using an organic base plant food. This ultimately will translate to a healthier food product for the consumer and greater financial return for the local farmers.

1.3 The objectives of the study

The Problem Statement is expressed in the form of appropriate researchable objectives in order to facilitate management of the problem as a whole, which can be categorized into the following:

1.3.1. The first objective was to investigate the effects of drench and foliar application of a local commercial seaweed extract on the leaf nutrient content of potato *Solanum tuberosum* ‘BP1’ over two growing seasons in the field.

1.3.2 The second objective was to explain the effects of drench and foliar applications of a local commercial seaweed extracts on oxidative stress reduction as recorded by reduced antioxidant levels, and on food proximate values of the tubers and the leaves of *Solanum tuberosum*, BP1 over two growing seasons in the field.

1.3.3 The third objective was to determine the effects of foliar and soil drench application of a local commercial seaweed extract on chlorophyll content, growth and yield of *Solanum tuberosum* 'BP1' over two growing seasons in the field.

1.3.4 The fourth objective was to ascertain the effects of foliar and soil drench application of a local commercial seaweed extract on the growth and yield of *Vitis vinifera* var. sultana grapes, over two growing seasons in the field.

1.4 The thesis hypotheses

1.4.1 There will be a direct relationship between potato leaf nutrient content, seaweed extract treatment and method of application.

1.4.2 For potato antioxidants the potato plants grown using seaweed extracts would show an inverse relationship with antioxidant levels (ascorbic acid, polyphenols, and ORAC), and a direct correlation between food proximate values (crude fiber and protein) and seaweed extract treatment.

1.4.3 There will be a direct relationship between the potato leaf chlorophyll content, growth, total yield, seaweed extract treatment and method of application.

1.4.4 For sultana grape parameters and total yield there will be a direct relationship to the seaweed extract treatment.

1.5 Delineation of the problem

1.5.1 The crops selected for this study will be limited to the potato (*Solanum tuberosum* 'BP1') and the grape crop (*Vitis vinifera* var. sultana). Other crop plants will be excluded.

1.6 The assumptions

1.6.1 The commercial seaweed extract liquid (Afrikelp® LG-1) supplied by the company Afrikelp for the first and second year crop growth will be true to its packaging label, be identical in its composition and have the same chemical properties.

1.6.2 Information related to the research site, which will be provided by the Agricultural Research Council (ARC), such as weather conditions, rain fall figures and soil types, have been validated and authenticated by the ARC.

1.6.3 Potato tubers *Solanum tuberosum* 'BP1' cultivar supplied by a local agricultural supplier, Kaap Agri for the first and second year growing season will be identical and true to the packaging information.

1.6.4 The horticultural practices, research methodologies, methods of data collection and pre and post-harvesting techniques used in both growing seasons are consistently the same and applied across both growing seasons.

1.7 Brief overview of the chapters

The thesis consists of seven chapters.

1.7.1 Chapter one presents a general introduction, problem statement and motivation, sub-problems, objectives of the study, research hypothesis, delimitations and assumptions.

1.7.2 Chapter two presents an overview of the literature of all the research focus areas pertaining to this study.

1.7.3 Chapter three of the thesis is the first research paper. It provides evidence of the effects of varying concentrations of commercial seaweed extracts (Afrikelp® LG-1) on the leaf nutrient content of *Solanum tuberosum* ‘BP1’ grown under foliar and soil drench feeding methods, conducted over a period of two growing seasons. The results will report whether seaweed extract influences leaf nutrient content of nitrogen, phosphorous, potassium, calcium, magnesium, sodium, manganese, iron, copper, zinc and boron. It will emphasize whether leaf nutrient content of these nutrients are significantly influenced by the application of seaweed extracts and methods (drench and foliar), when compared to the control.

1.7.4 Chapter four of the thesis outlines the second research paper. This chapter revolves around the effects of seaweed extracts (Afrikelp® LG-1) applied by means of foliar and drench methods on the antioxidant levels and food proximate values in the potato crop. This experiment evaluates, whether the seaweed extract has the ability to reduce the oxidative stress as recorded by reduced antioxidant levels of ascorbic acid, polyphenols, and ORAC in the potato BP1 tubers and leaves over two growing seasons. Food proximate components of protein and crude fibre will be assessed.

1.7.5 Chapter five introduces the third research paper. This chapter deals with a series of comparative plant growth parameter experiments, conducted over two years, to determine the how seaweed extract (Afrikelp® LG-1) applied by drench and foliar application affects the chlorophyll content, growth parameters and total yield of *Solanum tuberosum* ‘BP1’. The results of this chapter will emphasise the seaweed concentrations and application methods that have the most profound influence on potato growth and yield.

1.7.7 Chapter six provides a narration of the fourth research paper and gives an account of the effects of seaweed extracts on *Vitis vinifera* var. sultana grapes. In the table grape industry, certain physical growth characteristics of the grapes plays important role for marketing table grapes. Growth parameters tested over a two year growth season were namely, number of bunches, length of bunches, diameter of bunches and total yield. The results of the treated crops will be compared with the control crops.

1.7.8 Chapter seven reflects on all previous experimental chapters. It highlights the correlations and possible relationships that exists between the chapters. It deals with the treatment of the hypothesis, and is a general discussion and conclusion of all the experimental work done in the study. Final recommendations are made to local potato and grape farmers.

1.8 Brief overview of the statistical procedures

1.8.1 The experimental data collected was analyzed using STATISTICA Software (StatSoft, 2013) and Two-Factor analysis of variance (ANOVA) was performed. The Fisher's least significant difference (LSD) was used to compare treatment means at $P \leq 0.05$, level of significance (Steel and Torrie, 1980).

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CHAPTER 2: LITERATURE REVIEW:

Seaweed products and crop production

CHAPTER 2

LITERATURE REVIEW

This chapter covers research that has been conducted on the use of liquid seaweed extracts for plant growth. It identifies opportunities for further research on a local South African commercial seaweed product (Afrikelp® LG-1). The following topics are under review: History of seaweed use and the South African overview, Chemical composition, Application techniques, Leaf nutrient concentration, Plant chemical composition (antioxidants, food proximate values, chlorophyll content), Plant growth and yield, Potatoes, Grapes, and Conclusion.

2.1 A historical perspective of seaweed utilization and the South African overview.

There are reports of the use of marine algae as fertilizers, which date back to the ancient Greeks and Vikings (Crouch, 1990). Historical records indicated that seaweeds were originally used as an animal fodder and soil conditioner (Stephenson, 1974). In the 12th Century the large brown algae were used for manure along the coastal areas of France, Ireland, Scotland and Normandy (Booth, 1964). More recently the largest application of seaweed was along the cost of Brittany (Chapman and Chapman, 1980). By the 17th century, the first industry involving seaweeds, the kelp trade, had developed (Crouch, 1990). The algae of Europe used for the kelp trade were species of *Laminaria, Fucus* and *Ascophyllum nodosum* (L.) Le Jolis. These seaweeds provided a source of soda (*Fucus* and *Ascophyllum*), iodine (*Laminaria*) and later, in the 19th century products such as ammonia and potash (Senn and Kingman, 1978). In order for seaweeds to be utilized as an economic manure for inland farmers, seaweeds had to be treated. This resulted in the granting of the first patent in 1856 for dried seaweed manure. In 1912 the first alkaline liquid seaweed extract (Penkals) was patented (Crouch, 1990).

In the 1880's the global agricultural sector favoured chemical fertilizers as a crop fertilizer, rather seaweed. Seaweed as a form of soil enrichment started to decrease in popularity. In recent times, the detrimental effect of synthetic products upon the environment became apparent and natural bio-degradable fertilizers received renewed attention. The marketing of 'Biomisation Fluid' or 'Baby Bio', and 'Biohumus' followed shortly afterwards (Stephenson, 1974).

Studies conducted by Booth (1969) highlighted the importance of other seaweed products, which became available in the 1960's. Commercial products such as Marinure, Sea Born, Seahorse, Sea Magic, 3 (S.M.3), Trident and a product made by Algae Produckter A/S, and the advantages of these products for plants were promoted in the agriculture industries. The manufacturing process was critical to the quality of the seaweed product, and included high pressure alkaline extraction of large brown seaweeds. The application of several seaweed products by foliar spraying resulted in worldwide reports of the improvements in plant vigour and yield. This resulted in a sharp global increase in seaweed research and attempts were made to establish the regulatory action of the different seaweed products (Booth, 1969).

The South African seaweed industry has been in existence for over sixty years. The seaweeds that form the basis of the current industry are the kelps (*Ecklonia maxima* and to a lesser extent *Laminaria pallida*) and red seaweeds of the genus *Gelidium* (Anderson and Rothman, 2013). The commercial exploitation of seaweeds in South Africa was based largely on beach-cast collecting and cutting of kelp. Harvesting of *Ecklonia maxima* (Osbeck) Papenfuss and *Laminaria pallida* Greville ex J. Agardh started in the

1940's as a result of the scarcity of kelp during the Second World War (McHugh, 1987). When supplies of agar from Japan became unavailable, various potential resources were identified. However, commercial exploitation in South Africa only began in the early 1950s (McHugh, 1987), followed by hand-picking of *Gelidium* sp. in the Eastern Cape since 1957. Most of the South Africa harvest was shipped to Europe, North America, and Asia for alginate extraction (Anderson *et al.*, 1989). South African kelps yielded alginate concentrations of between 22% and 40% (Anderson *et al.*, 1989). Trade figures showed that powdered kelp was also exported to Japan for use in formulated fish-feed (Zhang *et al.*, 2004). Since 1975, wet kelp has been harvested from along the west coast solely for the production of commercial product Kelpak®, which is a plant-growth stimulant and soil conditioner (Khan *et al.*, 2009). Similar harvesting of wet kelp in smaller quantities started in 1979 on the west coast and later on the south coast for the production of Afrikelp®, which is a plant-growth stimulant. This harvesting continues today (Anderson *et al.*, 1989, 2003; Robertson-Anderson *et al.*, 2006; Troell *et al.*, 2006).

This review is focused on commercial species and those with commercial potential. It evaluates a local commercial seaweed extract, produced from *Ecklonia maxima* (Osbeck) Papenfuss in South Africa by a cool fragmentation technology.

Due to the detrimental effects of synthetic plant food products upon the environment, attention was given to natural bio-degradable plant fertilizers, which resulted in the manufacture of commercial seaweed products such as Marinure, Sea Born, Seahorse, Sea Magic, 3 (S.M.3), and Trident. In South Africa the seaweeds that forms the basis of the seaweed industry are the kelps *Ecklonia maxima, Laminaria pallida,* and genus *Gelidium,* and the commercial products manufactured were mainly Kelpak® and Afrikelp®.This research will focus on a locally produced seaweed extract (Afrikelp®

LG-1) and the impacts it has on *Solanum tuberosum* 'BP1'potato, and *Vitis vinifera* var. sultana grapes.

2.2 Chemical composition

Nelson (1985) discussed the importance of the process in the manufacture of liquid extracts of seaweed. Several processes are used including: stirring macerated seaweed in a vat containing hot water; acidic or alkaline hydrolysis with or without steam; and the pressure burst technique. In the latter method, employed in South Africa, a liquid seaweed concentrate is produced without resorting to chemical or heat extraction. The use of pressure to break down the structural components of the cell allowed the release of practically all the seaweed's essential constituents, including plant growth regulators.

As an animal feed seaweed supplements protein intake, being similar in protein content to good quality hay (Black, 1955; Beleau *et al.*, 1975). Seaweed as an animal fodder is valuable as a source of trace elements (Thivy, 1961 and Jensen, 1971), vitamins and vitamin precursors including carotenoids and xanthophylls (Chapman & Chapman, 1980). This made seaweed a valuable food supplement on mineral deficient pasture land. Booth (1966) indicated that many of the observed effects of seaweed products can be ascribed to plant growth hormones, which are constituents of seaweed, in particular cytokinins. In Booth's study commercial seaweed products were sourced internationally and analyzed.

Stephenson (1968) recognized that marine algae contain all major plant nutrients and trace elements, as did Booth (1964), Yamamoto and Ishibashi (1972), Yamamoto *et al.*, (1979). At least seventeen of the common amino acids occur in the macro algae, of which at least aspartic acid, glutamic acid and alanine are present in commercially important species (Huve and Pelligrini, 1969). Alginic acid, laminarin and mannitol are also important components, which represented half of the total carbohydrate content of commercial seaweed preparations. Seaweed contains a wide variety of vitamins, which may be utilized by crops. Vitamins C, B (thiamine), B2 (riboflavin), B12, D, E, K, niacin, pantothenic, folic and folinic acids occur in algae (Yamamoto and Ishibashi, 1972). The effects of liquid seaweed extracts on plant growth have shown that they contain cytokinins, auxin and abscisic acid like growth promoting substances (Mooney and Van Staden, 1986).

In related studies (El-Migeed *et al.*, 2004; El-Moniem and Abd-Allah 2008) it was noted that the increase in plant yield and general healthy characteristics of the plant could be due to several important facts, such as seaweed extracts containing macro, micronutrients and organic matter like amino acids, and these factors having the potential to improve nutritional status, vegetative growth and yield quality.

Seaweed extracts contain vitamins B1, B2, C, E, auxin, GAs, and ABA-like growth substances (Stephenson, 1968; Munda and Gubensek, 1975; Abetz, 1980; Finnie and Van Staden, 1985; Moor and Van Staden, 1986). Enhanced plant growth due to enhanced soil conditions was attributed to seaweed application to the soil, which increased trace element supply to the plant (Francki, 1960). Low rates of seaweed extract could also promote plant growth significantly (Crouch and Van Staden, 1992, 1993). In further research it was suggested, that depending on the species of seaweed, organic compounds entering into the soil by means of seaweed drench application, rather than mineral elements applied onto the plant directly, were more relevant and therefore responsible for improved growth. It has been demonstrated that seaweed products contain phytohormones, and the stimulating effects of seaweed extracts may be attributed to these components, especially cytokinins (Brian *et al.*, 1973; Blunden and Widgoose, 1977; Finnie and Van Staden, 1985; Crouch and Van Staden, 1993).

Data gathered from previous investigations, which described and highlighted the most effective methods of seaweed application, revealed that seaweed concentrates, depending on the method of application, caused many beneficial effects on plants as they contain growth promoting hormones (IAA and IBA, Cytokinins) trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins and amino acids (Challen and Hemingway, 1965).

The method employed in South Africa to produce liquid seaweed extracts (Afrikelp® LG-1) exclude the addition of chemicals and heat. It involves the use of pressure to break down the structural components of the cell to release the essential growth promoting components. Important chemical and nutrient constituents of seaweeds identified, include a wide spectrum of plant beneficial amino acids and proteins, vitamins, growth hormones, trace elements, organic and inorganic mineral components.

2.3 Application techniques

According to Crouch (1990) the utilization of natural, unprocessed seaweed in agriculture was limited to coastal areas where it was commonly used as an animal fodder for livestock farming, and as a soil additive by crop farmers. Seaweed treatment of crops has grown in popularity and led to the development of many processed seaweed products. These products may be categorized into three groups: large volume soil supplement meal; for blending into defined rooting media for glasshouse crops and applied as a liquid or powder extracts, and concentrates employed as root dips, soil drenches and as foliar sprays applied as a fertigation or chemigation application, which involved an injection of fertilizers, soil amendments, and other water-soluble products into an irrigation system (Booth, 1969; Senn, 1987; Metting, Rayburn and Reynaud, 1988). The application of seaweed by means of soil drench and foliar sprays have emerged as a reliable techniques for promoting and sustaining plant growth (Senn, 1987).

In trials conducted by Booth (1966) and Bokil *et al*. (1974) commercial liquid seaweed extracts were evaluated. These products included Maxicrop, Algifert , Goemar , Kelpak, Seaspray, Seasol, SM3, Cytex and Seacrop. These products were successfully used as foliar sprays for several crops and applied as fertigation and chemigation applications. In this study it was observed that the value of seaweeds as fertilizers was not due to nitrogen, phosphorus and potash content, but because of the presence of trace elements, metabolites, and the foliar method of application. This study reported that aqueous extract of *Sargassum wightii* when applied as a foliar spray on *Zizyphus mauritiana* showed an increased yield and quality of fruits. A similar study conducted by Rama Rao (1991) which utilized seaweed extracts proved the importance of trace elements and metabolites and showed how these elements can improve plant growth. The growth promoting effects of seaweed liquid fertilizer (*Enteromorpha intestinalis*) was tested on a sesame crop and showed similar positive results (Gandhiyappan and Perumal, 2001). The influences of foliar method of application on crop growth were further highlighted by Temple (1989) where foliar applications increased harvestable bean yields by an average of 25%. Staked tomato yields increased by up to 99%, a result supported by Csizinszky (1984). Passam *et al.*, (1995) reported that early yield of greenhouse cucumber, and greenhouse tomato total fruit fresh weights increased by 17% due to foliar application. Crouch and Van Staden (1992), illustrated that foliar method of application had an effect on the final outcome.

Literature cited by Blunden and Gordon (1986), Metting *et al.* (1988), Temple and Bomke (1988) emphasized the benefits of seaweeds as sources of organic matter and fertilizer nutrients for the soil and plants. The effectiveness of seaweeds as an organic soil application subsequently led to seaweeds being used as soil conditioners in the horticultural and agricultural sectors. In a report released by the FAO (2006), 15 million metric tons of seaweed products were produced annually, and a considerable portion of this was utilized to amend the soil's physical, textural and nutrient capabilities. Aitken and Senn (1965), and Crouch (1990) mentioned the importance of seaweed being applied as an organic soil amendment. In their report it was emphasized that seaweeds added to the soil transformed the soil to a balanced pH and made available a prominent presence of essential soil minerals.

Quastel and Webley (1947) illustrated the importance of seaweeds as a form of organic application and their soil conditioning properties, which are attributed to the alginic acid in seaweeds, which comprises about one-third of their carbohydrate content. The report highlighted the importance of alginates in seaweeds, which enhanced the crumb structure and moisture retaining characteristics of light soils, and this has the ability to ameliorate the sticky nature of clay soils. The three species most commonly applied as soil organic conditioners are *Ascophyllum nodosum* (L.) Le Jolis, *Ecklonia maxima* (Osbeck) Papenf. and *Fucus vesiculosis* (L.) C. Agardh. Less commonly employed species are *Laminaria* and *Sargassum* species (Blunden *et al.*, 1968; Blunden and Wildgoose, 1977).

The application methods of seaweeds, utilized for plant growth were categorized into the following: Applied as an organic soil conditioner or as an amendment, added to the soil as a powder or liquid; for blending (raw ground particles) as a rooting media; employed as a liquid root dip; and applied as a soil drench and foliar spray. The techniques of application involved fertigation or chemigation application. The products utilized included various commercial products, mainly used as foliar sprays for several crops grown under greenhouse conditions. This research will focus on a locally produced seaweed extract (Afrikelp® LG-1), which will be evaluated under local conditions for the most effective method of application on potato and sultana crops.

2.4 Plant chemical composition

In addition to oxygen, carbon dioxide and water, Marschner, (1995) and Mengel *et al.* (2001), emphasized that plants require at least fourteen mineral elements for adequate nutrition. Deficiency in any one of these mineral elements would reduce the plant growth and crop yields. Plants acquired their mineral elements from the soil solution. Six mineral elements, nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg) and sulphur (S), are required in large amounts, whilst chlorine (Cl), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), nickel (Ni) and molybdenum (Mo) are required in smaller amounts.

2.4.1 Effects on leaf nutrient concentration

In research conducted by Thomas (1996) where seaweed (Sea Buckthorn) was applied as soil amendment, the effects on growth responses of the herbaceous Rosemary plant was investigated. It was found that herbaceous plants grown on seaweed treated soil had a high response to Sea Buckthorn as compared to that grown on untreated soil since the former plant, exhibited more improved nutritional content of macro and micro elements in the leaves and other plant organs. In this study it is reported that Seaweed extracts contain cytokinins, which induced the physiological activities (for instance activating some enzymes that are involved in photosynthesis) and due to this, increased the total chlorophyll in the plant. This positively reflected on the activity of photosynthesis and the synthesized materials which positively reflected on shoots characteristics. It was observed that the increase in shoot characteristics was due to the macronutrients present in seaweed extracts. Attememe (2009), also experimented with seaweed extracts and Rosemary, *Rosmarinus officinalis.* In this report it identified that seaweed extracts had significant macronutrients, which played an essential role in plant nutrition, especially increasing the nitrogen, potassium and phosphorous content in the leaves, which had an enhancing effect on chlorophyll pigmentation and plant photosynthesis.

Gollan and Wright (2006), reported that seaweed extracts from *Caulerpa taxifolia* have the ability to increase shoot characteristics in plants, due to the auxin content in the seaweed extracts. This resulted in an increased mineral content in the leaves, and had an effective role in leaf cell division and enlargement, which resulted in an increase in shoot growth, leaf area, photosynthesis, plant dry weight and ultimately yield.

Kowalski *et al.* (1999), described the positive affection of seaweed extracts on plant growth and yield by using Kalpak extracts on potato plant. Here the Kalpak significantly affected shoot growth and leaf content of nutrient minerals and increased quantitative and quality yield traits. Also, Jensen (2004), reported that seaweed extracts contain various micro nutrients in addition to macro elements and contain Auxins, Gibberellins' and Cytokinins, and when sprayed on plants lead to increase root growth ability, leaf nutrient concentration, stem thickness and promoted growth. Eris *et al.* (2008), showed that seaweed extract *Ascophyllum nodosum* when sprayed on pepper plants led to increase growth, yield and concentration of nutrient elements in the leaves.

Various researchers (Marschner, 1995; Mengel *et al.*, 2001; Karley and White, 2009; Miller *et al.*, 2009; Miwa *et al.*, 2009; Puig and Pen˜arrubia, 2009; White and Broadley, 2009) reported on the uptake of mineral elements by plant roots and leaves from the soil and their subsequent distribution within the plant. The studies undertaken by these researcher's highlighted factors which influenced the plants ability to increase leaf nutrient content. These factors included organic and inorganic soil additives, as well as a wide spectrum of liquid foliar plant foods. Turan and Köse (2004) reported on the effects of seaweed extracts in lettuce plants and the subsequent increase of Ca, Mo, Na, Cd and Al ion concentration in the plants roots, which resulted in an enhanced mineral concentration of Mg, K and Ca in leaves of lettuce. Mancuso *et al.* (2006), reported that the presence of marine bioactive substances in seaweed extract improved nutrient content of N, P, K and Mg in the roots and leaves of grapevines and cucumbers. Furthermore, it was observed that leaf stomata uptake efficiency improved in the treated plants compared to non-treated plants.

A field experiment conducted by Leindah Devi and Mani (2014) on sugarcane was conducted to evaluate the effects of seaweed saps of *Kappaphycus alverizii* and *Gracilaria* spp. They were tested at different concentrations of litres per hectare (2.5, 5.0, 6.25, 7.5 and 10) on nutrient content in cane, nutrient content in leaves and nutrient uptake by sugarcane. Significant improvement were observed in increasing nutrient content in cane, nutrient content in leaves and uptake by sugarcane at the 10 l/ha concentration of seaweed saps. Hence, the study found that 10 l/ha seaweed saps to be the recommended dose to improve the nutrient content in the leaves, in the cane and increase nutrient uptake when compared to the other concentrations.

Rathore, *et al.*, (2009) investigated the effects of foliar applications of different concentrations seaweed extracts of *Kappaphycus alvarezii* on leaf nutrient content of soybean [*Glycine max* (L.) Merr.]. The use of the seaweed extract significantly increased leaf mineral content of N, P, S and K.

Studies conducted on various species of seaweed products have shown that seaweed extracts contained major and minor nutrients, and have been reported to stimulate the growth and yield of various plants (Rama Rao, 1991), develop tolerance to environment stress (Zhang and Schmidt, 2000; Zhang *et al.,*2003), increased leaf nutrient content from the soil (Verkleij, 1992; Turan and Köse, 2004). Liquid seaweed extracts tested on various crops showed an increased uptake of essential inorganic constituents from the soil to the leaves (Senn *et al.* 1961).

In studies undertaken by Button and Noyes, (1964); Beckett and Van Staden, (1990) and Yan, (1993), they reported that liquid seaweed led to enhanced nutrient concentration, regulated plant growth substances, increased chlorophyll content, promoted protein synthesis and cell division, stimulated root and shoot growth and improved seed germination.

In a study conducted by Gugino *et al*. (2009), seaweed products were applied during soil preparation for crop cultivation. The research outcomes indicated that seaweed had the ability to positively change important soil indicators, such as soil pH, cation exchange capacity (CEC) and increase nutrient levels in the soil rhizosphere. The results reported that on the basis of these improved soil factors, these conditions were the main factors that contributed to the health of the plant, increased leaf nutrient content and ultimately increased total yield.

Allen, *et al.* (2001), highlighted the advantage that seaweed products have due to their high organic matter content, in that they promoted plant health by positively affecting the population of the rhizosphere microbial community around the roots of the plant. The relationship between the plant and the microbial community was therefore mutually enhanced and this relationship assisted with an improved uptake of soil nutrients by the roots and enhanced accumulation of minerals in the leaves.

The N, P, K, total salts and micronutrients availability in unprocessed seaweeds have been positively linked to increasing nutrient concentration in various organs of the plant, especially the leaves (Stephenson, 1974; Senn and Kingman, 1978).

The results of various researchers (Beckett and van Staden 1989; Hankins and Hockey 1990; Blunden 1991; Norrie and Keathley 2006), revealed a wide range of beneficial effects of seaweed extract applications on plants. These results included early seed germination and establishment, increased leaf nutrient content, improved crop performance and yield.

There are certain factors that influence leaf nutrient content in plants. These factors may be influenced by the addition of organic and inorganic soil amendment products, a wide-spectrum of liquid foliar plant foods, methods of application, and organic and inorganic fertilizers. Seaweed extracts contains marine bio active substances. Depending on the crop, seaweed product, dosages, method and frequency of application, a variety of essential plant minerals may stimulate an effect on leaf nutrient concentration. It will be evaluated whether a seaweed extract (Afrikelp® LG-1) may enhance leaf nutrient concentration in the potato plant.

2.5 Antioxidants

A study conducted by Verkleij (1992), it demonstrated the correlation between liquid seaweed extracts, adverse growing conditions and plant antioxidant levels. The results show that due to the liquid seaweed extracts and their high levels of macro and micro-nutrients they had the ability to promote quality growth characteristics in plants, and in so doing strengthened the crops growth ability against adverse growing conditions. This reduced plant stress and ultimately lowered the formation of oxidative stress and reduced the formation of antioxidant properties in the plant.

Nabati *et al.* (1994), illustrated the various beneficial effects seaweed extracts have on plant growth and development. In this study they provide evidence that seaweed extracts enhanced plant resistance to diseases (Featonby-Smith and Van Staden, 1983), and environmental stresses such as drought and salinity, thereby lowering oxidative stress and reducing the formation of antioxidants.

Fletcher *et al.* (1988), reported that under drought stress, seaweed extracts, which contained cytokinins, reduced the stress conditions within the plant. Jewer and Incoll (1980), that a range of naturally occurring and synthetic cytokinins can enhance plant performance such as leaf stomatal aperture, transpiration, protein formation (polysomes/ribosomes), flower formation, and have a reduction effect on antioxidant accumulation.

Various researchers (Gamble and Burke, 1984; Senaratna *et al.*, 1984; Wise and Naylor, 1987; Smirnoff and Colombe, 1988; Dhindsa, 1991; Seel *et al.*, 1992; Smirnoff, 1995; Doulis, 1994), exposed various ornamental and agricultural crops to a wide spectrum of inhospitable growing conditions. The results proved that plants that encounter adverse environmental circumstances, such as reduced soil minerals, absence of fertilizers, reduce water, high temperatures, unproductive soils, air pollution and harmful herbicides had a greater probability to produce oxidative stress. This oxidative stress was characterized by excess accumulation of reactive oxygen radicals, which were the requirements for antioxidants to form, and include polyphenols, ORAC and ascorbic acid.

In a study undertaken by Stuhlfanth *et al.* (1990) and Bowler (1992), it was noted that antioxidant activities are significantly elevated in the plant when exposed to stressful conditions. The results showed correlations between levels of plant antioxidants and how this relates to various environmental stress. Ultimately the results attempt to establish the optimum capacity of the plant to tolerate stress.

Shirochenkova (1985), found that when turnip greens were subjected to dry (and thus sunnier and warmer) conditions, they increased the Ascorbic Acid (AA) content in the crop. Similarly, Reder *et al.* (1943), provided evidence that onions, and black currant grown in hot, dry conditions contained more than twice the ascorbic acid of those grown in ideal to wet conditions. Stocker (1960), had reported an enhancement in ascorbic acid content of plants which were subjected to severe water shortage stress. Previous research results therefore indicated that, under dry conditions, a slight to high increase in the total ascorbic acid concentration have occurred. Stocker further illustrated that there was an extraordinary upsurge in the total ascorbic acid content along with abnormally high oxidation-reduction ratio and very high level of respiration under extreme drought conditions, suggesting a complete breakdown of the plant metabolism, and thereby negatively impacting on overall plant growth and yield. In a study conducted by Chinoy (1969), similar results were noted. Mukherjee and Choudhuri (1983), noted that AA increased significantly in the plant under water shortage stress conditions.

Sexton and Woulhouse (1984), observed that seaweed extracts containing cytokinin or cytokinin-like growth regulators had the ability to inhibit the activity of free radical groups, hydrogen peroxide and superoxide, which are the major factors, which inherently reduce photosynthesis and result in chlorophyll degradation in plants.

A study conducted by Wegener and Jansen (2013), concentrated on the effect of drought and wounding stress as two of the major environmental stresses for plants in agriculture, and which stimulated antioxidant properties in potato tuber. In their work, anthocyanins (polyphenols) values were recorded for one yellow fleshed commercial potato cultivar (cv. Agave) and two purple breeding potato clones (St 89403 and St 3792) grown under control conditions and drought stress conditions. Anthocyanins occur in vegetative organs such as roots, stems, axillary buds, leaves, stolon's, and tubers. They are part of the antioxidant system in plants and is a bioactive plant polyphenol and its presence in plants increases under stressful environmental conditions (Hooper and Cassidy, 2006; Hatier and Gould, 2009). The outcome of this study indicated that the radical scavenging capacity of anthocyanins pigments, under drought conditions and when compared with other plant vitamins, was greater than vitamins E and C. The two clones St 89403 and St 3792 revealed high amounts of Anthocyanins (Polyphenols) in drought stressed tubers. The clone St 3792 exhibited similar amounts of Anthocyanins in both tuber types, while in drought stressed tubers, it had higher Anthocyanin pigment content than the control tubers.

2.5.1 Food proximate values

In trials associated with liquid seaweed extracts (SLE), conducted by researchers Arthur, *et al.* (2003) and Zodape, *et al.* (2008), on peppers, and Zodape, *et al.* (2010), on mung beans, it was evident that the application of seaweed extract significantly increased pepper yield, pod weight and improved nutritional levels such as protein and carbohydrates of seeds.

Sivasankar and Oaks (1996), and Stitt (1999), evaluated the effects of seaweed liquid extract (SLE) on the protein activity and its formation in bean plants. The seaweed extracts were obtained from two macroalgae species *Ficus spiralis* and *Ulva rigida* and were applied by foliar spray or incorporated to the medium culture. The protein content in plants was significantly enhanced by both treatments of *Fucus spiralis* extract and

Ulva rigida extract compared to the control plants. When SLE were incorporated into the medium culture, it was observed that the protein content in bean plants increased for all treatments comparatively to the control.

Different seaweed extracts have shown to have a strengthening ability on plants against adverse growing conditions. This ultimately reduced plant oxidative stress and had a lowering effect on antioxidant formation. Certain nutritional levels (food proximate values) in crops were promoted by the use of certain seaweed extracts. Depending on the methods of seaweed application, plant type, and the seaweed extracts, this study will evaluate whether a local seaweed extract (Afrikelp® LG-1) have an influence on lowering oxidative stress and thereby reducing antioxidants, and increasing food proximate values in the potato plant.

2.6 Chlorophyll

In an investigation undertaking by Blunden *et al.* (1997b), various seaweed extract products were tested on plant growth. The seaweed extracts were found to contain significant amounts of cytokinins, auxins and betaines. Results reported that the presence of these growth promoting components resulted in an increase in the rate of photosynthesis, which promoted apical vegetative cell division, proliferated early stages of growth along with early induction of flower formation. Studies conducted by Schwab and Raab (2004), revealed that enhanced chlorophyll concentration in the leaves simultaneously occurred with proliferation of vegetative growth. Ghurbat (2013), conducted a greenhouse trial on the effect of liquid seaweed extracts Seamino on plant height and chlorophyll content of pepper *Habanero* cv. The results indicated that the highest average of plant height and chlorophyll content resulted from spraying with a concentration of a Seamino. Plant height and chlorophyll showed increasing averages of 95.11cm and 59.67% as compared with the lower averages of 84.89 cm and 56.56% at control treatments.

Whapham *et al.* (1992), reported in a study that the application of seaweed extracts to the soil or to the foliage of tomato plants produced leaves, which after 34 days of testing, were visually greener than those of the control. The possible role of betaines in producing this result was considered and the effect on leaf chlorophyll content was further investigated using a cucumber bioassay procedure devised for cytokinins (Fletcher, 1982). It was observed that the seaweed extract increased the chlorophyll levels of the cucumber cotyledons. The betaines in the seaweed extract, when tested separately, produced significantly chlorophyll concentrations in the cotyledons and 'peaks' of activity were observed for each betaine. It can be concluded that the effects of enhancing chlorophyll levels produced by the seaweed extract were due to the betaines it contained (Whapham *et al.*, 1993).

In a research conducted by Blunden *et al.* (1997a), trials were conducted on dwarf French bean plants, barley, wheat and tomato plants using a brown marine alga *Ascophyllum nodosum.* Applications were made to the soil or to the foliage and the chlorophyll levels of the leaves were measured. The treated plants had significantly higher contents of leaf chlorophyll than the control plants. In all instances, soil application of the seaweed extract resulted in higher chlorophyll contents of the treated plants. In the cases of barley, tomato and wheat plants, foliar application of the seaweed extract resulted in significantly higher leaf chlorophyll contents of the treated plants compared to the control.

2.6.1 Plant growth and yield

The application of seaweed extract for optimum crop growth and total yield was of great importance due to it containing high levels of organic matter, micro elements, vitamins, fatty acids and growth regulators such as auxins, cytokinin and gibberellins. This was shown in a study conducted by Crouch and Van Staden (1994), where the quality and yield of tomatoes were enhanced by seaweed extract application. The beneficial effect of seaweed extract application on total yield was as a result of mineral components in seaweed that worked synergistically at different concentrations (Fornes *et al.*, 2002). Seaweeds are a known source of plant growth regulators (Jameson, 1994) such as organic osmolites (e.g. betaines), amino-acids, mineral nutrients, vitamin and vitamin precursors (Blunden *et al.*, 1985; Berlyn and Russo, 1990). In particular, seaweed contains kahydrin, alginic acid and betaines which synergistically contribute to the efficacy of the liquid extract formulation and thereby effectively supporting crop growth and total yield (Vernieri *et al.*, 2006).

An experimental trial conducted by Ghurbat (2013), which determined the effect of Seamino and ascorbic acid on growth, yield and fruit quality of pepper *Habanero* cv., experimental results showed that vegetative growth characteristics (plant height, fruit weight, fruit diameter, chlorophyll, vitamin C, and yield) in all fruits harvested significantly benefited from the various treatments of Seamino and ascorbic acid.

Crouch and Van Staden (1992), demonstrated that commercial seaweed concentrate prepared from *Eklonia maxima* (Osbek) Papenfuss, applied as a soil drench or foliar spray had significantly improved the growth and yield of tomato plants. Seaweed products were sprayed onto plants in its seedling stage and showed profound result on the success of seedling establishment (shoots, roots fresh and dry weights, leaf surface area were measured) and ultimately the final growth and yield of the fruit (weight and size). In this study it was demonstrated that pre-harvest spraying with liquid seaweed extracts had positively influenced final growth of the fruit.

In a similar study Crouch and Van Staden (2005), found that spraying tomato plants with concentrated seaweed extracts gave increased fruit numbers by 10% and fruit weight by 15% when compared with the control. Similarly, the results of a study conducted by Saravanan *et al.* (2003), reported that the number of fruits per plant, fruit yield per plant and fruit yield per plot was significantly increased as a result of the application of 750 ppm of chlormequat and 1680 ppm of seaweed extract to tomato plants. Sethi and Adhikary (2008), conducted a study where yield parameters such as fruit length and fruit weight in pepper crops were measured. The results reported that foliar spray of seaweed liquid extracts increased fruit length and weight, leave numbers, leaf area, and dry weight. Al-Saaberi (2005), reported that an increase in fruit weight of lettuce, *Lactuca Sativa,* due to increasing liquid seaweed extracts, consequently resulted in improved plant physiological activities such as photosynthesis and plant nutrition, which were the provision and the reasons of increasing fruit weight.

Metting *et al.* (1990), has recorded that the application of commercial seaweed has many different beneficial effects for crops treated with seaweed extracts. These benefits were further substantiated in separate studies by Senn *et al.,* (1961), and Blunden (1972), and included increased crop yields, improved seed germination, increased resistance of plants to frost (Senn *et al.,* 1961), fungal attack and insect attack (Stephenson, 1966), and improve storage quality of fruit (Skelton and Senn, 1969).

In a study conducted by Blunden (1972), it recorded that potato plants treated with commercial seaweed product S.M.3, which is an aqueous extract of species of

Laminareaceae and *Fucaceae*, produced an increased average of between 18% and 34% tuber yields over the control. Furthermore, it showed that the potatoes from the treated plants, when compared with the control, were more even in size (diameter and length).

A potato trial conducted by Blunden and Wildgoose (1977), the results indicated that a commercial seaweed extract S.M.3 applied as a foliar spray, produced a significant increase in the yield of *Solanum tuberosum*, var. King Edward. An increase in the yield was achieved with the S.M.3 seaweed extract (containing cytokinin content equivalent to kinetin) when applied at a rate of 1.4 g/ha. The results suggested that the beneficial effects from the use of the seaweed extracts was due to its higher cytokinin content. Furthermore, an application of kinetin at a rate equivalent to 2.8 g/ha produced highly significant increases in the yield of potatoes of both King Edward and Pentland Dell varieties. In this study the potatoes harvested from the plots treated with either kinetin or with seaweed extract were more uniform in size than those from the control plots. The results indicated that the major effect of seaweed S.M.3 extracts on tuber yield became apparent late in the development of the plant. An increase in the tuber weights was apparent. The results showed that foliar application of the seaweed extract produced significant increases in the yields of King Edward potatoes of 1000 and 1250 kg/ha over the control crop.

Plant growth responses to seaweed application were further substantiated in studies done by Turan and Köse (2004), on grapevine, and Mancuso *et al.* (2006), and Rathore *et al*. (2009), on soybean. The results showed that there were profound increases in yield and growth parameters with application of seaweed extract for these studies. In the study conducted by Rathore *et al.* (2009), which tested the effects of foliar applications of different concentrations of seaweed extract (prepared from

Kappaphycus alvarezii) on the growth and yield of soybean [*Glycine max* (L.) Merr.]. The results showed that the use of seaweed extract increased all the growth parameters measured for soybean. In general, a gradual increase in plant height was observed with increasing seaweed extract application. Numbers of plants per square meter increased significantly compared to the control. Number of pods per plant, test weight, and number of grains per pod, were significantly improved by foliar applications of seaweed extract concentration.

Temple and Bomke, (1989), applied seaweed extracts (SE) to plants. It was reported that SE stimulated shoot growth and branching, increased root growth and lateral root development. In a study conducted by Weil *et al.* (2003); Gugino *et al*. (2009), it reported on the positive influences of seaweed on soil properties and how it can improve growth and yield. They reported on the correlation between improved soil quality and increased yield. Enhanced soil factors created by seaweed extracts included factors such as: optimum soil pH, availability of primary nutrients, and levels of active carbon, which supported the correlation with improved crop productivity and total yield.

Seaweeds extracts are a known source of beneficial plant growth regulators and thereby enhances leaf chlorophyll, crop growth and total yield. Commercial seaweed extracts have shown to have a wide-spectrum of useful results on plant vegetative growth parameters. Depending on the seaweed type, methods of application (foliar and drench), timing of the applications (pre-plant soil treatment, post emergent stage, seedling stage, juvenile stage, foliage application), dosages and frequency, seaweed extracts have enhanced crop growth. Liquid seaweed extract (Afrikelp® LG-1) will be evaluated for its impact on leaf chlorophyll content, growth parameters and total yield of *Solanum tuberosum* ‘BP1’potato crops.

2.7 Potatoes

A field trial was conducted by Haider *et al.* (2012), to investigate the effect of foliar application of seaweed extract "Primo" as an organic bio-stimulant on potato *Solanum tuberosum* cv. Sante. Foliar application of seaweed extract was carried out at different growth stages of the crop. Control plants were sprayed with water without seaweed extract. The results illustrated that a significant improvement in growth, yield and tuber quality of potato was observed where treatment was applied. The highest tuber yield was recorded with applications of seaweed extract at 30 and 60 day interval after planting. The treatment also improved nitrogen, total soluble solids and protein contents of the potato tubers. The results of the study concluded a positive response of potato plant growth and yield to the foliar application of seaweed extract.

Two years field studies were conducted by Prajapati *et al*, (2016), to evaluate the effect of seaweed extracts of *Kappaphycus alvarezii* (K sap) and *Gracilaria edulis* (G sap) on growth and yield of potato *Solanum tuberosum* Linn. Results indicated that application of 10% K sap and 10% G sap markedly improved the growth attributes like plant height, number of stems and tubers per hill of potato crop over the control. Increment in marketable and total tuber yield of potato by application of 10% G sap and 10%K sap over the control were 19.81% and 17.18%, 11.04% and 6.90%, respectively. Relative reduction in non-marketable tuber and damage tuber yields under the influence of 10% G sap and 10% K sap when compared to control were 39.94% and 34.37%,35.50% and 34.91%, respectively.

In a study conducted by Torres *et al.* (2004), it highlighted the influence of higher nutrient contents and how this is represented in the quality of the potato tuber. It mentions the high level of nitrogen in the tuber, and it being one of the most important macronutrients, required by the plant and how it influences the productivity of the plant and tuber quality**.** Whangchai *et al.* (2001), further illustrated the connection between higher nutrient contents and tuber quality with reference to potassium, nitrogen and phosphorous. It was found that potassium has a positive correlation with storage life of potato tubers**.** Potassium contents were found to increase in the tubers of all treatments were seaweed extracts were applied. Potassium has a stimulatory effect on flower formation and ultimately tuber formation in the soil. Furthermore an increase in nitrogen contents was observed in potato tubers treated with seaweed extract. Seaweed extracts increased phosphorous contents in the tuber, which had a stimulatory effect on root mass in the potato plant, thereby increasing nutrient uptake and tuber yield.

In summary the application of seaweed extracts to potato plants have increased growth parameters, mineral nutrient concentration, yield, and tuber quality. In response to the use and application of a local commercial product (Afrikelp® LG-1) the present study will evaluate whether the findings are in line with previous research work.

2.8 Grapes

In commercial grape growing, quality attributes of grapes are improved using management techniques such as girdling, topping, pinching, cluster thinning, trimming, sprinkler cooling and spraying of different growth regulators and bio-stimulants (Winkler *et al.*, 1974). In research conducted by Norrie *et al*. (2002), it explored the benefits of seaweed extract used for grapes. The results reported an increase in grape berry size, length of bunches and total yield. Avenant and Avenant (2006), utilized seaweed extracts for grapes. Berry colour and size is an important characteristic since it determines visual acceptability and size contributes to the yield. With regard to the improvement of grape colour, and yield, Redglobe grapes treated with *Ecklonia maxima* extract solution at 12 mm and 16 mm berry size, which were applied by means of drench and foliar application, produced a higher yield of bunches with a darker colour. Kok *et al.* (2010), conducted a similar study, which reported on the use of seaweed extracts and grapes. Their study utilized the seaweed extract *Ascophyllum nodosum*, which were applied by means of foliar application at 15 cm to 20 cm shoot/vine length, pre-bloom and at pea berry size. The results reported an improvement in berry brightness, redness, number of grape bunches, diameter and yield of cv. Trakya Ilkeren grapes (*Vitis vinifera* L.). Norrie *et al.*, (2002), further documented the importance of seaweeds utilized for grapes. In this study it documented that several spray applications of seaweed extract improved berry firmness, number of bunches, diameter of bunches, and yield of Sultanina. This is substantiated by related research conducted by Norrie and Keathley (2006), which demonstrated that supplementary foliar sprays with seaweed products have the potential to improve berry size, quality and yield.

With increasing energy prices and an elevated awareness of environmental issues such as excessive fertilizer, herbicide, and pesticide use, it is important to improve fertilizer and chemical efficiency and to find alternative methods to improve crop yields (Metting *et al.*, 1990). As a result, utilization from algae as bio-fertilizers in agriculture and treatment of seaweed extract as an organic bio-stimulant is becoming an accepted practice in horticulture (Verkleij, 1992), and also viticulture (Turan and Köse, 2004; Mancuso *et al.*, 2006), due to its beneficial effects.

Seaweed extract in viticulture has produced useful effects that indicates seaweed extracts are an alternative to chemical fertilizers. Growing of table grapes requires viticulture management techniques that overcome the low quality characteristics in the berry. Depending on the seaweed extract, dosages, method and frequency of application, seaweed extracts had various effects on the vine, berry quality, and yield A local seaweed extract product (Afrikelp® LG-1) will be tested on *Vitis vinifera* var. sultana grapes for its effect on physical growth characteristics and total yield.

2.9 Conclusion

The literature cited indicated that various seaweed extracts tested on different crops, the optimum application dosages and application methods were determined. Opportunities exist to conduct a field trial to evaluate the most effective dosages, application techniques (foliar or drench application), for a commercial liquid seaweed extract Afrikelp® LG-1 derived from *Ecklonia maxima* on crops *Solanum tuberosum* 'BP1' potato crop and *Vitis vinifera* var. sultana. On the basis of dosages and application methods it's unknown how this product simultaneously affects leaf nutrient content, lowering of plant oxidative stress, and a reduction in antioxidants, food proximate values, chlorophyll content, growth parameters and total yield in *Solanum tuberosum* BP1. Furthermore, no clear evidence exist with respect to the effects of Afrikelp® LG-1 on the physical growth parameters of *Vitis vinifera* var. sultana, grown in open ground. Previous research was mainly being conducted under greenhouse conditions over one growing season. The possibility of Afrikelp® LG-1 being tested for its effect on *Solanum tuberosum* BP1 and *Vitis vinifera* var. sultana, exposed to field conditions, over a period of two growing seasons remains unassessed.

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CHAPTER 3:

The effects of drench and foliar application of a commercial seaweed extract on the leaf nutrient content of potato, *Solanum tuberosum* 'BP1'

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The effects of drench and foliar application of a commercial seaweed extract on the leaf nutrient content of potato, *Solanum tuberosum* 'BP1'

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Abstract

A field experiment was conducted to quantify the effects of a commercial seaweed extract (Afrikelp® LG-1) on the leaf nutrient content of the poatao plant, *Solanum tuberosum* 'BP1' under soil drench and foliar application over two growing seasons, namely 2010 and 2011. Field experiments was conducted at the Agricultural Research Council (ARC) Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa during the late autumn for both years. The experimental design followed a randomized complete block design in a factorial arrangement of seven treatments (0, 2, 3, 4, 5, 6, 7 l/ha) and two application methods replicated four times. At 60 days after planting (DAP), *Solanum tuberosum* 'BP1' cultivar, leaves were sampled for nutrient analysis.

The results for the 2010 and 2011 seasons showed that there was no difference in the leaf nutrient content due to the method of application. N, P, K, Ca, Mg, Na, Mn, Cu, Zn and B increased with treatment concentrations, with minor exceptions (Cu in 2010 and Fe in 2011).

Keywords: *Commercial seaweed extracts, Leaf nutrient content, Potatoes, Solanum tuberosum 'BP1', Drench and foliar application.*

3.1 Introduction

In addition to oxygen, carbon dioxide and water, plants rely on at least fourteen mineral elements, mainly N, P, K, Ca, Mg, S, Cl, B, Fe, Mn, Cu, Zn, Ni and Mo for adequate functioning. Deficiency in anyone of these nutrient elements in the plant reduces plant growth and negatively affect optimal functioning and total yield. Plants generally acquire there mineral elements from the soil solution (Marschner, 1995; Mengel *et al.,* 2001). Six mineral elements namely, nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), and sulphur (S), are required in large amounts, whilst chlorine (Cl), boron (B), iron (Fe), manganese (Mn), copper (Cu), zinc (Zn), nickel (Ni) and molybdenum (Mo), are required in smaller amounts (MacNicol and Beckett, 1985; Brown *et al.,* 1987; Marschner 1995; Mengel *et al.,* 2001; White *et al.,* 2004 and Pilon-Smits *et al.,* 2009).

A study conducted by Torres *et al.* (2004), highlighted the influence of higher nutrient contents and how this is represented in the quality of the potato tuber. It mentions the high level of nitrogen in the tuber, and this being one of the most important macronutrients, required by the plant that influences the productivity of the plant and tuber quality**.** Whangchai *et al.* (2001), further illustrated the connection between higher nutrient contents and tuber quality with reference to potassium, nitrogen and phosphorous. They found that potassium had a positive correlation with the storage life of potato tubers**.** Potassium contents were found to increase in the tubers of all seaweed extract treatments. Potassium had a stimulatory effect on flower formation and ultimately tuber formation in the soil. Furthermore an increase in nitrogen contents was observed in potato tubers treated with seaweed extract. Seaweed extracts increased phosphorous contents in the tuber, which had a stimulatory effect on root mass in the potato plant, thereby increasing nutrient uptake and tuber yield.

Globally there are concerns regarding the impact on the environment of synthetic, inorganic fertilizers, and the sustainable use of industrial manufactured fertilizers in agriculture (Galloway *et al.*, 2008). The uptake of mineral elements by plant roots, and their distribution in the leaves, has a direct impact on growth, yield and quality (Marschner, 1995; Mengel *et al.*, 2001; Watanabe *et al.*, 2007; Karley and White, 2009; Miller *et al.*, 2009; Miwa *et al.*, 2009; Puig and Penarrubia, 2009; Baxter, 2009; Chen *et al.*, 2009; White and Broadley, 2009; Broadley *et al.*, 2010).

Seaweed concentrates are known to cause many beneficial effects on plants as they contain growth promoting hormones (IAA and IBA, Cytokinins) trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins and amino acids (Challen and Hemingway, 1965). Liquid extracts obtained from seaweeds are successfully used as foliar sprays for several crops (Bokil *et al*., 1974). Booth (1966) observed that the value of seaweeds as fertilizers was not only due to nitrogen, phosphorus and potash content, but also because of the presence of trace elements and metabolites, which allow the plant the capability to utilise, distribute and ultimately enhance leaf nutrient content.

In a previous study aqueous extract of *Sargassum wightii* when applied as a foliar spray on *Zizyphus mauritiana* showed an increase in leaf nutrient content, quality of fruit and increased yield (Rama Rao, 1991). An increased leaf nutrient content in sesame crops as a result of application of seaweed extracts (*Enteromorpha intestinalis*) was recorded in a study conducted by Gandhiyappan and Perumal (2001). Moreover it was observed in various research work that seaweed as a foliar application increased the leaf nutrient content of several crops and ultimately resulted in increased yields, for crops such as beans by 25%, tomatoes by 99%, and cucumbers by 17% (Csizinszky, 1984; Temple and Bomke, 1989; Crouch and Van Standen, 1992; Passam *et al*., 1995).

Previous research has indicated that depending on the source of the liquid seaweed products, they will differ in their efficacy. (Blunden, 1972). These commercial products included Maxicrop, Algifert, Goemar, Kelpak, Seaspray, Seasol, SM3, Cytex and Seacrop. Gollan and Wright (2006), reported that seaweed extracts from *Caulerpa taxifolia* have the ability to increase growth in plants, due to the auxin content in the seaweed extracts. This resulted in an increased nutrient content in the leaves, and had an effective role in leaf cell division and enlargement, which resulted in an increase in shoot growth, leaf area, photosynthesis and ultimately plant dry weight. Eris *et al.* (2008), showed that a seaweed extract of *Ascophyllum nodosum* when sprayed on pepper plants led to increased growth, yield and concentration of nutrient elements in the leaves.

The primary focus of the present study was to quantify the effects of various concentrations of the seaweed extract Afrikelp® LG-1on the leaf nutrient content of the potato plant. It is envisaged that enhanced nutrient content in the potato leaves, would induce improved tuber yield. This study compared the effects of the method of seaweed extract application (drench and foliar), on the leaf nutrient content.

The hypothesis was that potato plants grown using the seaweed extract Afrikelp® LG-1 would show a direct relationship between potato leaf nutrient content, seaweed extract treatment and method of application over two growing seasons.

3.2 Material and Methods

3.2.1 Seaweed concentrate

The seaweed extract used in the trials was a commercial seaweed product, marketed as Afrikelp® LG-1, and produced in South Africa for the local and international agriculture and horticulture sectors. This product was produced from marine algae, *Ecklonia maxima* (Osbeck) Papenfuss, and prepared via cool fragmentation technology.

3.2.2 Crop identification and growth of plants

Solanum tuberosum, certified 'BP1' cultivar tubers were selected for planting. Tubers were planted 20 cm apart and 10 cm deep. The width between each row was 75 cm.

3.2.3 Site location & growing conditions

The field experiment was conducted at the Agricultural Research Council (ARC) Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa during the 2010 and 2011, in late autumn of both years. The field experiment was conducted under irrigation. The experimental site lies in the winter rainfall region of South Africa at an elevation of 146 m above sea level with mean annual rainfall of 713.4 mm and a mean annual maximum temperature of 22.6°C and minimum of 11.6°C. The soil type of the site was sandy loam (Glenrosa, Hutton form) which according to the Soil Classification Working Group (SCWG) is equivalent to skeletic leptosol (SCWG, 1991). The soil was ploughed to a depth of 40 cm prior to planting.

3.2.4 Experimental plot design

The experimental design followed a randomized complete block in a factorial arrangement of seven treatments (0, 2, 3, 4, 5, 6, 7 l/ha) and two application methods replicated four times. Potatoes were planted on the 21st May for the 2010 and 2011 seasons respectively. The experimental plot was 1225 meters square area (35 m X 35 m). Each treatment contained 4 rows of 12 potato plants, with a total of 48 plants per treatment, and 336 plants per block. A total of 2688 potato plants were used in the field experiment for each year. The first application of liquid seaweed was applied on the 7th July for each season. Thereafter, five applications were given with a two week interval in between each application (7/07; 21/07; 4/08; 18/08; 2/09 for each crop season). Foliar application was done in the form of fine droplets sprayed directly onto the leaf surfaces, and avoiding contact with the soil. Drench application was done by the application of the liquid directed to fill a reservoir at the base of the plants stem and in the root zone of the plant. Application was done early morning, when weather conditions were windless with no rain. Water was applied as a control treatment.

Weed management: This was done manually by the ARC staff, every three weeks from the commencement date (21st May 2010 and 2011). Disease control: Potato crops were sprayed for early blight on three occasions (with a fungicide mixture of copper oxychloride and mancozeb. Spray dates: 20/07; 3/08; 17/08 for 2010 and 2011 crops.

3.2.5 Plant harvest and sample preparation

At 60 days after planting (DAP), *Solanum tuberosum* 'BP1' cultivar, leaves were sampled for nutrient analysis. Ten plants were sampled from the middle rows of each plot. The border plants within each row were excluded. The leaves were oven-dried at 60ºC for 48 hours, weighed and ground into a fine powder (0.85 mm sieve) for the analysis of leaf nutrient content.

3.2.6 Determination of mineral nutrients in leaves

Concentration of nutrients (N, P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn and B) were determined by dry ashing 1 g ground sample in a porcelain crucible at 500ºC overnight. This was followed by dissolving the ash in 5 mL of 6 M HCl and placing it in an oven at 50ºC for 30 min; 35 mL, of deionised water were added and extract was filtered through Whatman no. 1 filter paper. Nutrient concentrations in plant extracts were determined using an inductively-coupled plasma (ICP) emission spectrophotometer (IRIS/AP HR DUO Thermo Electron Corporation, Franklin, Massachusettes, USA), and results were given in mg/kg (Giron, 1973).

3.2.7 Statistical analysis

The experimental data collected were analysed using the STATISTICA Software (StatSoft, 2013) and Two-Factor analysis of variance (ANOVA) was performed. The Fisher's least significant difference (LSD) was used to compare treatment means at $P \leq 0.05$ level of significance (Steel and Torrie, 1980).

3.3 Results

The concentrations of the various elements in the leaves are given in Tables 3.1 and 3.2 for 2010 and 2011 respectively. The results illustrated that for both years the method of application (drench and foliar) had no significant effect on the concentration of elements in the leaves. For both 2010 and 2011, there were no significant interactions between the methods of application (drench and foliar), and the level of seaweed extract concentrations of the elements, except for copper (Cu) in 2011 season.

3.3.1 Nitrogen concentration in the potato leaves (mg/kg)

For 2010 and 2011 season the application rates of 5 l/ha and above resulted in a significant increase by ($P \leq 0.001$ and $P \leq 0.01$) in nitrogen in the leaves. There is an anomaly with the 6 l/ha in 2011 in that its results do not differ from the control nitrogen content. This anomaly persist across all elements. The higher concentration of 7 l/ha represented a total mean increase of 84% and 85% for nitrogen content over the control respectively (Tables 3.1 and 3.2).

3.3.2 Phosphorous concentration in the potato leaves (mg/kg)

In 2010 and 2011 application rates of 7 l/ha resulted in a significant increase of leaf phosphorous by ($P \leq 0.01$ and $P \leq 0.001$), which was 67% for 2010 and 50% for 2011 respectively (Tables 3.1 and 3.2).

3.3.3 Potassium concentration in the potato leaves (mg/kg)

For 2010 and 2011 seasons at seaweed concentration of 7 l/ha the results show that the seaweed had a significant ($P \leq 0.01$) effect on the leaf potassium content, which represented a total mean increase of leaf potassium of 72 % and 76% over the control (Tables 3.1 and 3.2).

3.3.4 Calcium concentration in the potato leaves (mg/kg)

For the year 2010 and 2011, at seaweed concentration 7 l/ha the leaf calcium content significantly increased by ($P \leq 0.01$ and $P \leq 0.05$), which represented a total mean increase of leaf calcium content of 64% and 65% respectively over the control (Tables 3.1 and 3.2).

3.3.5 Magnesium concentration in the potato leaves (mg/kg)

In 2010 and 2011 at seaweed concentrations of 6 l/hand 7l/ha respectively, leaf magnesium content was significantly increased by ($P \leq 0.05$ and $P \leq 0.01$), which represented a total mean increase of 64% and 65% respectively over the control for both years (Tables 3.1 and 3.2).

3.3.6 Sodium concentration in the potato leaves (mg/kg)

In both 2010 and 2011 the highest seaweed extract concentration levels of 7 l/ha had significantly ($P \leq 0.05$) increased leaf sodium content by 63% and 70% respectively over the control (Tables 3.1 and 3.2).

3.3.7 Manganese concentration in the potato leaves (mg/kg)

For both 2010 and 2011 the highest seaweed concentrations of 7 l/ha had significantly increased leaf manganese content by ($P \leq 0.05$ and $P \leq 0.01$) respectively, which was a total mean increase of 55% and 62% over the control (Tables 3.1 and 3.2).

3.3.8 Iron concentration in the potato leaves (mg/kg)

For 2010 and 2011 the highest concentration of 7 l/ha significantly increased leaf iron content by ($P \leq 0.05$), which was a total mean increase of leaf iron content of 61% and 62% respectively over the control (Tables 3.1 and 3.2).

3.3.9 Copper concentration in the potato leaves (mg/kg)

For 2010 the results showed that no seaweed concentration had significantly affected the leaf copper content when compared with the experimental control.

However for 2011 this was not the situation. The concentration of 4 l/ha and above significantly ($P \leq 0.05$) increased the leaf copper content by 61% over the control. For 2011, methods of application and the concentration levels of seaweed had significant

($P \leq 0.05$) interactions on leaf copper content in the potato leaves. A quantitative interaction between methods of treatment (drench or foliar) and seaweed concentration applied in combination, had an additive effect on leaf copper content (Tables 3.1 and 3.2).

3.3.10 Zinc concentration in the potato leaves (mg/kg)

For both 2010 and 2011 the highest concentration 7 l/ha had significant effects of ($P \leq 0.001$ and $P \leq 0.01$) respectively on leaf zinc content, which was a total mean increase of 72% and 80% over the control (Tables 3.1 and 3.2).

3.3.11 Boron concentration in the potato leaves (mg/kg)

For both 2010 and 2011, the highest concentration of 7 l/ha had significant effects ($P \leq 0.001$ and $P \leq 0.01$) on leaf boron content, which was a total mean increase of leaf boron content 64% and 69% over the control respectively (Tables 3.1 and 3.2).

3.4 Discussion

Seaweed contains a variety of vitamins, including vitamins C, B, (thiamine), B2 (riboflavin), B12, D, E, K, niacin, pantothenic, folic and folinic acids. Seaweed extracts contains major and minor mineral nutrients, amino acids, cytokinins, auxins and abscisic acid (Yamamoto *et al.,* 1979; Mooney and Van Staden, 1986).

Minerals for human nutritional requirements are mainly derived from plants (White and Broadley, 2009; White *et al.,* 2012). Humans require at least twenty-five mineral elements for their well-being on a daily basis (Graham *et al.*, 2007; Stein 2010). The minerals often lacking in human diets are Fe, Zn, I, Se, Ca, Mg, and Cu (White and Broadley, 2009; Stein 2010). In the current study, potatoes, *Solanum tuberosum* 'BP1' were grown using seaweed extracts, Afrikelp® LG-1. For both 2010 and 2011 seasons the seaweed extracts promoted improved leaf nutrient content of N, P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn and B over the control. Torres *et al.* (2004), highlighted the influence of higher nutrient contents in the plant and their ultimate impact on the quality of the potato tuber. They mentioned the importance of high levels of nitrogen and essential macronutrients in the tuber, and it being one of the most important factors, which influences plant productivity and tuber quality. In our study macronutrients in the potato leaf such as nitrogen (N) content increased by 84% for both 2010 and 2011 growing seasons. Leaf phosphorous (P) content increased by 67% and 50% respectively, and likewise, leaf potassium (K) content increased by 72% and 76%. Also, Jensen (2004), reported that seaweed extracts contain various micro elements (Cu, Zn, Mo, B, Co) and when sprayed on plants lead to increase root growth ability, leaf nutrient concentration, stem thickness and larger yields.

Crouch *et al.* (1990), explained the relationship between nutrient content and seaweed. It outlined the usefulness of seaweed and its effects on plant nutrient uptake, leaf nutrient concentrations and the positive impacts on total yield. The report elaborated on the importance that seaweed has an additive effect on soil organic supply in the root zone area, and this lead to enhancement of nutrient uptake and finally an accumulative effect on leaf nutrient content. The usefulness of liquid seaweed Kelpak 66 was evaluated and applied to lettuce plants. The results illustrated increased nutrient concentration in leaf nutrient content and increased lettuce yields.

Glass (1989), concluded that nutrient movement and concentration in plants is under hormonal control. He explained how hormones from seaweeds has a stimulatory effect on the plant, which resulted in increased leaf nutrient content and proliferated growth of plant organs. Both Blunden (1977), and Temple and Bomke (1989), identified plant hormones such as cytokinins and auxins in commercial seaweed products, and linked their involvement to optimum plant growth, enhanced leaf nutrient content and optimal yields. The prime physiological responses of cytokinins are nutrient mobilization in various plant organs (Miller, 1963; Pozsar *et al.,* 1967). The application of seaweed concentrate has shown to increase leaf chlorophyll content, root and leaf surface area of treated plants (Featonby-Smith and Van Staden, 1983).

The results of the current study are comparable with the results of previous studies, where various marine algae in the form of commercial seaweed extracts, all resulted in increased leaf nutrient content in the plant. The positive effects were ascribed to seaweeds containing various mineral elements and growth hormones (Booth, 1964; Yamamoto and Ishibashi 1972).

Brady and Weil (1996), note that very few soils can sustain satisfactory crop production without the addition of nitrogen (N), phosphorous (P) and potassium (K). The results of the present study showed that leaf nutrient contents were significantly increased by the highest seaweed concentration for both growth seasons. N, P and K, are known to have a role in several vital functions in the plant, such as chlorophyll production, root and shoot growth, and increase leaf uptake and facilitate for higher yields (Okada *et al.*, 2004). A low presence of important nutrients in the soil will have a retarding effect on the plant, and thereby reduce leaf nutrient content and reduce plant productivity, eventually limiting the yield (Brady and Weil, 1996). Reduction in these nutrients will slow root development, which will negatively impact on uptake and mobility of other essential plant nutrients in the plant, and consequently restrict the overall movement of nutrients in the entire plant, reducing total yield (Zafar *et al.*, 2011).

Bambara and Ndakidemi (2010), and Ndakidemi *et al.* (2011), reported that the mechanism by which minerals are made available for uptake by the plant is not well known, but previous studies have reported on the possible mechanism of certain minerals increasing availability of other minerals in the soil. In the present investigation a possible mechanism, in the form of increased phosphorous (67% and 50%, 2010 and 2011 respectively) in the soil and its subsequent increased content in the leaf, may have acted as a mechanism which facilitated for further availability and mobility of other mineral nutrients and thereby improving the ability of the leaf and the roots for additional nutrient uptake**.**

Nitrogen (N) has a profound effect on crop development and a major influence on chlorophyll content (Brady and Weil, 1996). In the current study the application of liquid seaweed Afrikelp® LG-1 has increased leaf N by 84% for both 2010 and 2011 over the control. For nitrogen there exists an anomaly with the 6 l/ha in 2011 in that its results do not differ from the control nitrogen content. This anomaly persist across all elements. Haider *et al.* (2012), conducted a field trial to investigate the effect of foliar application of seaweed extract "Primo" as an organic bio-stimulant on potato *Solanum tuberosum* cv. Sante. The results illustrated that an improvement in growth, yield and tuber quality of potato was observed where treatment was applied. The treatments improved tuber nitrogen content, total soluble solids and proteins. The results of the study concluded a positive response of potato plant growth and tuber quality to the foliar application of seaweed extract.

Brady and Weil (1996), concluded that potassium (K) is an essential nutrient for plant growth and forms part of many crop production systems. Large amounts of K are required to maintain plant health and vigour. Specific functions of K in the plant include osmoregulation, internal cation and anion balance, enzyme activation, controls leaf water loss, and an increase the ability of the roots to take up water from the soil. Potassium plays a vital role in photosynthetic translocation, especially in grains, tubers, and fruit (Mikkelsen, 2007). In the current study liquid seaweed

Afrikelp® LG-1 promoted leaf potassium content (72% and 76% for 2010 and 2011 respectively). In the plant protein synthesis and nutrient mobility to the leaves are facilitated by an adequate supply of potassium (Mikkelsen, 2007; Miwa and Fujiwara, 2010). The consequences of inadequate K for crop growth will result in the inefficient utilization of other nutrients such as N and P (Mikkelsen, 2007). In the current investigation the results indicated that leaf content of K levels were significantly influenced by the higher concentrations of seaweed extracts for both growing seasons. In this study a high concentration of leaf K therefore positively affected the plants utilization and subsequently the leaf concentration of essential nutrients.

Makoi *et al.* (2013), reported that the availability of soil nutrients for uptake can be positively influenced by the activities of microorganisms in the immediate vicinity of the roots. Microorganism presence and there functions in the soil are encouraged by organic soil additives. The present research, found increased leaf nutrient content. This suggests that the seaweed extracts increased the mineral elements availability in the soil rhizosphere.

Whangchai *et al.* (2001), illustrated the connection between higher nutrient contents and potato tuber quality with reference to potassium, nitrogen and phosphorous. They found that potassium had a positive correlation with storage life of potato tubers**.** Potassium contents were found to increase in the tubers where seaweed extracts were applied. Potassium had a stimulatory effect on flower formation and ultimately tuber formation in the soil. Furthermore an increase in nitrogen contents was observed in potato tubers treated with seaweed extract. Seaweed extracts increased phosphorous contents in the tuber, which had a stimulatory effect on root mass in the potato plant, thereby increasing nutrient uptake, developed consistent tuber formation and improved tuber yield.

Martin *et al.* (2001), and Conn and Gilliham (2010), examined mineral element distribution in plant tissues. They observed the accumulation of K, P, Ca, and Na in various plant tissue, and suggested that the presence of these minerals assists with the mobilization of nutrients to various plant tissues and organs. In the current study leaf calcium (Ca) content increased by 64% and 65% over the control. Similarly, leaf sodium (Na) content increased by 63% and 70% over the control. In our study this may have assisted in mobilization of nutrients (N, P, K, Mn, Fe, Cu, Zn and B) in the leaves and resulted in an accumulative presence in the potato plant tissues, such as the leaves and possibly the tubers.

3.5 Conclusion

The findings of this study indicated that liquid seaweed extract Afrikelp® LG-1, improved leaf nutrient (N, P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn, and B) concentrations of *Solanum tuberosum* 'BP1'. The results showed that treatments applied by means of drench and foliar both had high results and are therefore considered to be equally effective methods of application. It can be noted that the significant increase of leaf nutrient concentrations were not associated with any evidence of leaf damage or nutrient concentrations toxicity due to substantial increase in macro and micro-nutrients in the leaves. The organic make-up of this product and therefore its safe nature will therefore help maintain the required nutrient balance needed for the plant, and in so doing avoid any deleterious harmful effects on the plant and the environment, as may be the case with synthetic, inorganic, industrial fertilizers. Further investigations will reveal whether the increase in various leaf nutrient contents due to Afrikelp® LG-1were linked to an increase in leaf chlorophyll content, growth and total tuber yield.

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Table 3.1

The effect of seaweed extract on the leaf nutrient content of *Solanum tuberosum* ‘BP1’ for 2010 growth season.

Treatments	**2010 season**										
Method of application	**Nitrogen (N)**	**Phosphorous (P)**	**Potassium (K)**	**Calcium (Ca)**	**Magnesium (Mg)**	**Sodium (Na)**	**Manganese (Mn)**	**Iron (Fe)**	**Copper (Cu)**	**Zinc (Zn)**	**Boron (B)**
					Mg/Kg						
Drench	0.62±0.03a	0.03±0.002a	0.99±0.06a	0.74±0.04a	0.18±0.01a	53.36±3.38a	17.90±1.14a	70.53±4.40a	4.86±0.30a	4.59±0.28a	1.44±0.07a
Foliar	0.56±0.04a	0.03±0.002a	0.94±0.06a	0.71±0.05a	0.17±0.01a	52.79±4.04a	17.96±1.38a	76.51±7.39a	6.03±0.52a	4.72±0.34a	1.40±0.09a
Afrikelp concentration (Litres/hectare) Drench & foliar applications combined											
0	0.44±0.04d	0.03±0.03cd	0.75±0.08c	0.56±0.06c	0.13±0.01c	40.79±4.78bc	15.18±2.35bc	58.84±8.50b	4.72±1.15ab	3.64±0.42cd	1.09±0.11c
2	0.47±0.04cd	0.03±0.002d	0.74±0.08c	0.56±0.06c	0.13±0.02bc	37.44±4.53c	11.93±1.53c	68.51±6.99ab	3.65±0.48b	3.05±0.39d	1.07±0.12c
3	0.51±0.04cd	0.03±0.002bcd	0.89±0.10c	0.67±0.07bc	0.16±0.02bc	48.37±6.50abc	18.01±2.13abc	75.85±12.98ab	5.66±0.80ab	4.49±0.47bc	1.27±0.10bc
4	0.55±0.05cd	0.03±0.002bcd	0.93±0.08bc	0.77±0.07ab	0.18±0.02ab	56.88±5.21ab	20.68±2.45ab	66.02±10.22ab	5.68±0.74ab	5.39±0.54ab	1.52±0.12ab
5	0.62±0.06bc	0.04±0.004abc	0.99±0.09bc	0.74±0.05abc	0.19±0.02ab	56.25±3.99ab	18.60±2.14ab	67.26±8.07ab	5.98±0.56a	4.88±0.49abc	1.57±0.09ab
6	0.73±0.04ab	0.04±0.003ab	1.17±0.08ab	0.88±0.07a	0.22±0.02a	65.37±5.26a	17.53±1.19abc	83.47±11.91ab	5.82±0.61ab	4.86±0.31bc	1.66±0.12a
7	0.81±0.10a	0.05±0.005a	1.29±0.13a	0.92±0.10a	0.21±0.02a	66.41±10.40a	23.59±2.63a	94.67±16.74a	6.61±0.97a	6.26±0.64a	1.79±0.18a
2-Way ANOVA (F- statistics)											
Method of application	1.69NS	0.25NS	0.64NS	0.36NS	0.01NS	0.01NS	0.001NS	0.45NS	3.73NS	0.11NS	0.18NS
Concentration	5.95***	3.96**	4.66**	4.01**	3.99**	3.15*	2.98*	1.08*	1.46NS	4.74***	5.25***
Method of application X Concentration	1.09NS	0.59NS	0.62NS	0.74NS	1.03NS	0.57NS	0.77NS	0.39NS	0.28NS	0.76NS	0.98NS

Values presented are Means ± SE. SE = Standard Error, *, **, *** = significant at *P*≤0.05, *P*≤0.01, *P*≤0.001 respectively, NS = not significant. Means followed by similar letter (s) in a column are not significant different.

Table 3.2

The effect of seaweed extract on the leaf nutrient content of *Solanum tuberosum* 'BP1' for 2011 growth season.

Treatments	**2011 season**										
Method of application	**Nitrogen (N)**	**Phosphorous (P)**	**Potassium (K)**	**Calcium (Ca)**	**Magnesium (Mg)**	**Sodium (Na)**	**Manganese (Mn)**	**Iron (Fe)**	**Copper (Cu)**	**Zinc (Zn)**	**Boron (B)**
					Mg/Kg						
Drench	0.83±0.06a	0.05±0.003a	1.41±0.09a	0.93±0.06a	0.21±0.02a	95.5±7.07a	20.7±1.41a	147.7±11.35a	6.9±0.55a	7.0±0.47a	2.16±0.14a
Foliar	0.75±0.06a	0.04±0.003a	1.29±0.10a	0.88±0.07a	0.21±0.02a	94.4±7.63a	20.0±1.66a	162.4±17.01a	7.4±0.54a	6.8±0.56a	2.10±0.13a
Afrikelp concentration (Litres/hectare) Drench & foliar applications combined											
0	0.59±0.05c	0.04±0.004c	1.01±0.10c	0.69±0.08c	0.16±0.02cd	71.8±12.50c	15.9±1.61c	119.5±15.60a	4.9±0.58c	5.1±0.55d	1.6±0.20b
2	0.63±0.08c	0.04±0.0.005c	1.05±0.14bc	0.73±0.09bc	0.15±0.02d	74.3±6.57bc	14.4±1.91c	181.9±35.85a	5.6±0.74bc	4.8±0.71d	1.7±0.16b
3	0.65±0.05c	0.004±0.002c	1.11±0.08bc	0.74±0.07bc	0.18±0.02bcd	72.2±7.62c	18.8±1.56abc	150.7±26.19a	6.9±0.98abc	6.0±0.47cd	1.8±0.13b
4	0.82±0.05bc	0.05±0.004abc	1.47±0.12ab	1.10±0.09a	0.26±0.02ab	122.3±14.40a	25.5±1.93a	145.3±12.72a	7.9±0.57ab	8.6±0.54ab	2.5±0.18a
5	0.94±0.11ab	0.06±0.006ab	1.58±0.15a	1.03±0.12ab	0.24±0.03abc	108.8±9.85ab	24.5±3.03ab	155.9±30.14a	9.0±1.10a	8.1±0.86abc	2.5±0.20a
6	0.82±0.11abc	0.04±0.005bc	1.42±0.21abc	0.92±0.14abc	0.20±0.03abcd	97.5±15.20abc	17.9±2.67bc	138.1±15.47a	7.0±1.20abc	6.5±0.91bcd	2.1±0.26ab
7	1.09±0.16a	0.06±0.008a	1.78±0.24a	1.14±0.15a	0.27±0.04a	117.5±15.75a	25.7±4.00a	193.8±40.21a	8.7±1.17a	9.2±1.34a	2.7±0.33a
2 - way ANOVA (F- statistics)											
Method of application	1.7NS	1.8NS	1NS	0.3NS	0.04NS	0.01NS	0.11NS	0.5NS	0.62NS	0.13NS	0.1NS
Concentration	4.3**	4.3***	3.4**	3*	2.8*	3.2*	3.3**	0.9NS	3.01*	4.06**	4.1**
Method of application X Concentration	0.6NS	1.2NS	1.1NS	1.4NS	0.8NS	1NS	0.6NS	0.9NS	2.4*	0.53NS	1.54NS

Values presented are Means ± SE. SE = Standard Error, *, **, *** = significant at $P \leq 0.05$, $P \leq 0.01$, $P \leq 0.001$ respectively, NS = not significant. Means followed by similar letter (s) in a column are not significant different.

CHAPTER 4:

The effects of drench and foliar applications of a seaweed extract on the antioxidant levels and food proximate values of the tubers and leaves of *Solanum tuberosum* 'BP1'

CHAPTER 4

The effects of drench and foliar applications of a seaweed extract on the antioxidant levels and food proximate values of the tubers and leaves of *Solanum tuberosum* 'BP1'

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Abstract

A field trial was conducted to evaluate whether seaweed extract concentrations (Afrikelp® LG-1) have the capability to reduce plant oxidative stress of *Solanum tuberosum* 'BP1' tubers and leaves, and resulting in a reduction of antioxidants levels. Antioxidants levels of ascorbic acid, polyphenols, and ORAC (Oxygen Radical Absorbance Capacity) were measured over two growth seasons, namely 2010 and 2011. Simultaneously, food proximate values (crude fibre and protein) of the tubers were evaluated.

Field experiments were conducted at the Agricultural Research Council (ARC) Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa during the late autumn seasons. The experimental design followed a randomized complete block design in a factorial arrangement of seven treatments (0, 2, 3, 4, 5, 6, 7 l/ha) and two application methods (drench and foliar) replicated four times. Potato tubers and leaves were harvested 153 days after planting (DAP).

In 2010 the results showed that foliar method of seaweed extract application resulted in lower levels of ORAC in the tuber by 8.2% over drench. For polyphenols and ORAC in 2011 the higher seaweed concentrations of 6 l/ha and 7 l/ha, showed reduced levels of polyphenols (45%), and ORAC (40%) in the leaves over the controls, and thereby indicating lower plant oxidative stress. In the potato tuber food proximate values of crude fibre (50%) and protein (13%) in 2010 and 2011, respectively increased due to foliar application over drench.

Field trials showed that higher antioxidants values were present at lower seaweed treatments, an indication that the potato plant experienced higher oxidative stress.

Keywords: *Seaweed extracts, Antioxidants, Ascorbic acid, Polyphenols, ORAC, Potato tubers, Protein, Crude fibre, Solanum tuberosum 'BP1'*

4.1 Introduction

Potato is the fourth most important cultivated food crop in the world after maize, wheat and rice, with a production of 329 million tons. In terms of consumption, potato ranks third after rice and wheat (FAO, 2009). The importance of potato in the diet is higher in developed as compared to developing countries, accounting for 130 kcal per person per day for the developed world and for 41 kcal per person per day in the developing world (Burlingame *et al.*, 2009). In Europe, the per capita consumption reaches almost 90 kg per year, whereas in developing countries, its per capita consumption is smaller, reaching around 20 kg per year (André *et al.*, 2007, FAO, 2009).

Potatoes are mainly known to supply food proximate qualities such as dietary fibre, carbohydrates, high-quality proteins, vitamins and minerals. According to the Nutrient Data Laboratory, USDA (2001), contents would be 2.4 g for dietary crude fibre, 15.7 g for carbohydrates, 1.7 g for protein content, 19.7 g for vitamin C, each per 100 g of white, raw potato (flesh and skin) (Singh and Kaur, 2009). As for the minerals, iron and zinc contents are around 0.52 mg and 0.29 mg per 100 g (Singh and Kaur, 2009).

Cao *et al.* (1999), concluded that potatoes, when grown under stressful conditions produce antioxidant compounds, including polyphenols, ORAC (Oxygen Reactive Compounds), and ascorbic acid. In this study it was noted that the production of certain antioxidants, is considered to be a defence mechanism of the plant in response to challenging growing conditions. Antioxidants present in fruits and vegetables, have been positively linked to reduced levels of soil fertility, high day temperatures, and shortages of water. Various researchers supports the findings that an excess proliferation of antioxidants compromises the quality of fruit, and vegetables, reducing valuable minerals and vitamins (Hertog *et al.,* 1993; Knekt *et al.,* 1996; Cao *et al.,* 1998; Cao *et al.,* 1999; Wang *et al.,* 1999; Kruezer, 2001). However, in nature plants are exposed to severe environmental stresses, and adaptation is essential for their survival. Dixon and Paiva (1995), reported that plant phenols and antioxidants are inducible by environmental stress. As the potato is known to be a source of essential compounds (crude fibre and proteins), it is imperative to use optimum growing conditions to provide for a higher yield, marketable appearances, and higher minerals and vitamin content (Woolfe, 1987).

Ton *et al.* (2006), reports that plants possess many adaptive defence mechanisms to counteract pathogen, insect attack, and hostile growing factors. Following appropriate induction, plants are capable of mounting an enhanced defense capability, commonly referred to as induced resistance. Walters *et al.* (2005), describes that enhanced resistance to diseases, insect attacks, hostile environmental factors, locally and systemically, can also be achieved by treatment with different agents, such as virulent or avirulent pathogens, cell wall fragments, synthetic chemicals, bio-stimulants, and plant extracts, such as seaweed liquid fertilizers.

Scalbert *et al*. (2005), and Spencer *et al.* (2008), concluded that in plants, the production of free radicals and hence total antioxidants increases during biotic and abiotic stresses. Similar findings were found by Manach *et al*. (2004), numerous factors affect the total antioxidant accumulation in fruits and vegetables. These include degree of environmental stress, poor processing and storage quality.

The effect of the seaweed extract Afrikelp® LG-1 on antioxidants (ascorbic acid, polyphenols, and ORAC) and food proximate values (crude fibre and protein) of potato tuber and leaves was examined.

The hypothesis was that potato plants grown using seaweed extracts Afrikelp® LG-1 would show an inverse relationship with antioxidant levels (ascorbic acid, polyphenols, and ORAC), and a direct correlation between food proximate values (crude fiber and protein) and seaweed extract treatment.

4.2 Materials and methods

4.2.1 Seaweed concentrate

The seaweed extract used in the trials was a commercial seaweed product, marketed as Afrikelp® LG-1, and produced in South Africa for the agriculture and horticulture sectors. This product was produced from marine algae, *Ecklonia maxima* (Osbeck) Papenfuss, and prepared via cool fragmentation technology.

4.2.2 Crop choice and growth of plants

Solanum tuberosum 'BP1' cultivar tubers were selected for planting. Tubers were spaced at 25 cm in rows, 10 cm deep, with 75 cm between rows.

4.2.3 Site location & pre-planting soil analyses

The field experiment was conducted at the Agricultural Research Council Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosh, South Africa, in loam soil. The size of the experimental plot was 1225 m^2 (35m X 35m).

4.2.4 Experimental plot design

Potatoes were planted on the 21st May in 2010 and 2011. The experimental plot comprised the following layout: two crop feeding application methods viz, foliar and soil drench application. The trials consisted of one control and six treatment at application rates of 2, 3, 4, 5, 6, and 7 litres/ha, replicated four times. The first liquid seaweed application was on the 7th July for both the 2010 and 2011 crops. Thereafter, there were four more applications at two week intervals: 21/07; 4/08; 18/08; 2/09 for each year. Foliar application was done by means of a knapsack sprayer in the form of fine droplets applied directly onto the leaf surfaces, and avoiding contact with the soil. Drench application was done by filling a reservoir at the base of the plants stem. Water was applied to the control treatment in each block. Weed management: this was done manually by the ARC, every three weeks from the commencement date (21st May 2010 and 2011). Disease control: Potato crops were sprayed for early blight on three occasions (with a fungicide mixture of copper oxychloride and mancozeb. Spray dates: 20/07; 3/08; 17/08 for each year. This was applied as a fine droplet by means of a knapsack sprayer.

For each growth year potatoes were grown in randomized blocks, blocks A, B, C, and D. Each block contained one control and six treatments. Each treatment contained 4 rows, and each row was planted with 12 potato plants, with a total of 48 plants per treatment, and 336 plants per block, and a total of 2688 potato plants were used in the field experiment for each year, 2010 and 2011.

4.2.5 Sampling Preparation

Potato tubers and leaves were harvested 153 days after planting (DAP), for 2010 and 2011 crops. In each treatment, three plants were harvested from the second and third rows for anti-oxidant and food proximate analysis. These rows were selected since rows one and four had an increased risk of being exposed to unknown environmental factors.

Crude extracts of the potato tubers and leaves, were prepared by stirring the various dried, powdered plant materials (0.05 g of each) in 80% (v/v) ethanol (50 mL) (EtOH) (Saarchem, South Africa) thereafter the mixture was centrifuged at 3200 x g, for 5 min to remove cell debris. The supernatants were used for all analyses. The same sample preparation technique was followed for all assays and all analyses were performed in triplicate (Daniels *et al.,* 2011).

4.2.6 Determination of ORAC antioxidant-capacity and – content

According to Prior *et al*. (2005), it was recommended that three methods be considered for standardization of antioxidant capacity and total polyphenol determination in food and dietary supplements. Method 1: the oxygen radical absorbance capacity (ORAC) assay which represents a hydrogen atom transfer (HAT) reaction mechanism, which is most relevant to human biology. Method 2: the trolox equivalent antioxidant capacity (TEAC/ABTS) assays represents a single electron transfer (SET) based method which indicates reducing capacity. Method 3: the ferric reducing/antioxidant power (FRAP) assay which is also a SET-based assay and a direct test of the total antioxidant power of a biological sample. It was further suggested that experimental work using a series of assays gives a better understanding of the antioxidant capacity of a sample (Prior and Cao, 1999; Prior *et al.*, 2005).

4.2.7 Total polyphenol antioxidant-capacity and –content

The total polyphenol content of the various crude extracts was determined by the Folin Ciocalteu method (Swain and Hills, 1959; Singleton *et al.,* 1974). The method of Swain and Hills (1959) was adapted for use in a plate reader. Using a 96 well microplate, 25 µL of sample was mixed with 125 µL Folin-Ciocalteu reagent (Merck, South Africa), diluted 1:10 with distilled water. After 5 min, 100 µL (7.5%) aqueous sodium carbonate (Na_2CO_3) (Sigma-Aldrich, South Africa) was added to the well. The plates were incubated for 2 h at room temperature before the absorbance was read at 765 nm using a Multiskan plate reader (Thermo Electron Corporation, USA). The standard curve was prepared using 0, 20, 50, 100, 250 and 500 mg/L gallic acid in 10% EtOH and the results were expressed as mg gallic acid equivalents per g dry weight (mg GAE/g DW).

4.2.8 Total vitamin C assays

Total vitamin C (ascorbic acid + dehydroascorbic acid) was determined using the HPLC-UV method as described by Odriozola-Serrano *et al.* (2007), 2 g of cooked potato tubers were mixed with 20 mL MPA (4.5%, pH 3.5) and homogenized using a

Polytron homogenizer (S25N 18G blade, 9000 rpm) for 5 min. The samples were then centrifuged at 3200 x g, for 10 min and the resulting supernatant passed through a Millipore 45 μm membrane. In HPLC glass vials, 800 μL of each supernatant was separately mixed with 200 μL DTT (20 mg/mL). The vials were left for 30 min at room temperature, protected from light before the HPLC analysis. The vitamin C was analyzed on a Thermo Finnigan Spectra System HPLC with the UV wavelength detector set at 245 nm. Samples were introduced into the column (YMC-Pack Pro C18, 150 x 4.6 nm i.d., room temperature) with an automatic injector and a sample loop set at 20 μL. The flow rate of the mobile phase (0.01% solution of sulfuric acid, pH 2.6) was set at 0.9 mL/min. Total analysis time was 15 min and each sample was injected in triplicate. Quantification of the vitamin C in the samples was done by setting up a standard curve using L-ascorbic acid with concentrations varying from 5 to 50 μg/mL. The analytical variation was determined to be 4.9% and the recovery of L-ascorbic acid to be 95.3%.

4.2.9 Determination of protein by Kjeldahl Analysis

Approximately 1 g of dried potato tuber was accurately weighed and then transferred together with the paper to a digestion tube. Potassium sulphate/copper sulphate catalyst (7 g) and nitrogen-free H_2SO_4 (12 mL) were added, mixed by gentle swirling, followed by careful addition of H_2O_2 (5 mL) in a fume cupboard. The blank sample was also treated with the same chemicals. The digestion tubes were heated in a mantle to 420^0C and digested until clear green or colourless. Distilled water (75 mL) was added after cooling to room temperature. The digestion tube was then placed in the distillation unit. After sodium hydroxide was dispensed (50 mL), distillation was carried out and the distillate collected in the receiving flask containing 4% boric acid

(25 mL). The contents of the receiving flask were titrated with 0.1N hydrochloric acid (volume A). The blank gave volume B.

The percentage protein in the sample was calculated using the following equation:

$$\% \, Protein = \left(\frac{(\mathrm{A} - \mathrm{B})\ \mathrm{x\ N\ x}\ 14.007\ \mathrm{x\ f}}{\mathrm{mg}\ sample}\right) \mathrm{x}\ 100$$

Where, *A* = *ml acid for titrating the sample (titre).*

B = *ml acid for blank titration.*

N = *normality of acid used for titration.*

100 = *conversion to %*

F = *conversion factor which varies for different samples.*

(Conversion factor used for potato sample was 6.25) AOAC 920.53(1978)

4.2.10 Determination of crude fibre using the Fibretec System

Approximately 1 g of milled potato was accurately weighed into pre-weighed crucibles and loaded into the hot extraction unit (HEU) for digestion. A preheated 0.128 M H_2SO_4 (200 mL) was added into the column from the top using a funnel, followed by a few drops of iso-octanol. The system was then set to boil for 30 minutes, refluxing at a flow rate of 1-2 L/minute. After cooling to room temperature, filtration was done under vacuum, with washing three times with hot distilled water (30 mL). Preheated 0.233 M KOH (150 mL) was added and followed by repetition of refluxing, filtration, washing and drying crucibles with residues under vacuum. This was followed by three 25 ml portions of acetone were added and sucked dry each time. Crucibles were then dried overnight at 100^0C. The crucibles were cooled in a desiccator and the masses recorded. The percentage crude fibre in the sample was calculated using the following equation:

$$\% \text{ crude fibre} = \left(\frac{W2 - Wo}{W1 - Wo}\right) \text{x } 100$$

Where, W_o, is the weight of empty crucible

W_1, is the weight of the sample

W_2, is the weight of residue and crucible

AOAC 962.09 (1982).

4.2.11 Statistical analysis

The experimental data collected were analysed using the STATISTICA Software Programme (StatSoft, 2013) and Two-Factor analysis of variance (ANOVA) was performed. The Fisher's least significant difference (LSD) was used to compare treatment means at $P \leq 0.05$ level of significance (Steel and Torrie, 1980).

4.3 Results

4.3.1 Potato tuber antioxidants

Table 4. records the effects of various concentrations of Afrikelp® LG-1 seaweed extracts on ascorbic acid (mg/100g), polyphenols (mg GAE/100g), and ORAC (umol TE/g) in the potato tuber, *Solanum tuberosum* 'BP1' for 2010 and 2011.

4.3.1.1 Ascorbic acid, polyphenols and ORAC in the tuber

For both years 2010 and 2011, the methods of seaweed extract application, drench and foliar, had no significant effect on ascorbic acid and polyphenols in the potato tuber at any seaweed concentration (Table 4.1). For ORAC in 2010 (but not for 2011) the results indicated that the foliar method of seaweed extract application resulted in significantly ($P \leq 0.05$) lower levels of ORAC in the potato tuber by 8.2% over drench. The results indicated that in both 2010 and 2011 there were no significant interactive effects observed between the methods of application and the concentrations levels of seaweed extract on the antioxidants in the potato tubers (Table 4.1).

4.3.2 Potato plant leaves antioxidants

Table 4.2 records the effects of various concentrations of Afrikelp® LG-1seaweed extracts on polyphenols (mg GAE/100g) and ORAC (umol TE/g) in the leaves of the potato plant *Solanum tuberosum* 'BP1' for 2010 and 2011.

4.3.2.1 Polyphenols and ORAC in the potato leaves

The results in Table 4.2 illustrated that the method of seaweed extract application, drench and foliar, had no significant effect on polyphenols and ORAC in the leaves of the potato plant in both experimental years (2010 and 2011).

For 2010, all seaweed extract concentrations had no significant effects on the polyphenols and ORAC in the potato leaves compared with the experimental control. For 2011the higher seaweed concentrations reported significant ($P \leq 0.01$) reductions for both polyphenols and ORAC in the leaves. In particular 6 l/ha showed a mean total reduction of 45% on polyphenols over the control. For ORAC the higher seaweed extract concentration of 7 l/ha, had a mean total reduction of 40% over the control (Table 4.2).

For both 2010 and 2011 there were no significant interactions to report between the methods of application and the level of seaweed extract concentrations on polyphenols and ORAC levels (Table 4.2).

4.3.3 Potato tuber food proximate values

The results in Table 4.3 provide observations for the year 2010 and 2011. It records the effects of concentrations of Afrikelp® LG-1 seaweed extracts on crude fibre (%) and protein (%) in the potato tuber *Solanum tuberosum* 'BP 1'.

4.3.3.1 Crude fibre (%) and protein (%) of the tuber

For experimental seasons 2010 (crude fibre only) and 2011 (protein only), foliar application of seaweed had significantly ($P \leq 0.05$ and $P \leq 0.001$ respectively) affected crude fibre and protein over drench application. This represented a total mean increase of 50% for crude fibre and 13% for protein over drench. (Table 4.3). The results indicated that there were no significant effects of any seaweed extract concentration on the crude fibre and protein of the tuber. There was no significant interaction observed between the methods of application and the levels of seaweed extract concentrations on the crude fibre and protein of the tuber (Table 4.3)

4.4 Discussion

4.4.1 Antioxidants

Challen and Hemingway (1965), and Booth (1966), revealed by means of their investigations that seaweed concentrates have caused many beneficial effects on plants as they contain growth promoting hormones (IAA and IBA, Cytokinins), trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins, amino acids and metabolites. Bokil *et al*. (1974), and Metting *et al.* (1990) documented that commercial seaweed preparations has many beneficial effects on plants and are known to promote crops resistance to adverse growing conditions.

In the present study for 2010 season foliar method of application of seaweed extract Afrikelp® LG-1 reported a reduced level of ORAC (Oxygen Radical Absorbance Capacity) antioxidants by 8.2% over drench. In a separate study Rama Rao (1991) further emphasised the importance of foliar application of aqueous seaweed extracts *Sargassum wightii*, which was applied as a foliar spray on the tropical fruit tree species *Zizyphus mauritana*, also known as Chinese date or Indian plum. The plant responded positively and showed a general decrease in plant stress, which translated into an increase in vegetative tree characteristics and better fruit quality. In our study for growth season 2011, for potato leaves, but not the tubers, lower concentrations of seaweed extracts, and in particular the control, where there was an absence of Afrikelp® LG-1, showed higher values of polyphenols and ORAC. The reasons for higher values of polyphenols and ORAC at lower concentrations of seaweed extracts and at the control are attributed to the lack of essential plants nutrients, due to the absence of seaweed extracts. For all treatments in 2011 for polyphenols and ORAC the controls had the highest levels of antioxidants present.

Similar observations have been made in earlier studies, where it was found that oxidative stress, characterized by excess accumulation of reactive oxygen radicals, could result from various environmental stresses such as water stress and lack of essential nutrients (Gamble and Burke, 1984; Senaratna *et al.,* 1984; Smirnoff, 1988; Dhindsa, 1991; Seel *et al.,* 1992; Smirnoff, 1993). Stuhlfanth *et al.* (1990) and Bowler *et al.* (1992), concluded that plant are sensitive to stress and produce antioxidants as a protective measure. They further reported that the presence of antioxidants in the plant and their activity is directly related to the capacity of plant stress tolerance.

Polyphenols and ORAC are distributed in the cytosol as a water-soluble antioxidant. Dry conditions and lack of nutrients increased these antioxidants in turnip greens (Reder *et al.,* 1943), onions, and black currant (Shirochenkova *et al.,* 1985). Black currants grown in hot, dry condition and in soils with minimum nutrients were found to contain more than twice the antioxidants than those plants grown in wet conditions with adequate fertilizers (Stocker, 1960). In studies conducted by Challen and Hemingway (1965) on *Zea mays, Eleusine coracana,* and *Pennisetum glaucum* it was found reduced antioxidants were attributed to the presence of macro and micronutrients found in seaweed treatments. In a related study by Parr and Bolwell (2000) it was observed that the phenolic acid content decreases during maturing of fruits and vegetables. Many polyphenols, especially phenolic acids, are involved in the response of plants to different types of environmental stress. Polyphenols contribute to healing of damaged areas, possess antimicrobial properties, and their concentrations increase after infection, disease attack and nutrient malnutrition in the plant, especially in vegetables.

Alscher *et al.* (1997) highlighted the importance of ORAC (Oxygen Radical Absorbance Capacity) in plants. Their study identifies two main reasons for the synthesis and accumulation of ORAC in plants. The genetic make-up of plants imparts triggers the formation of ORAC to protect the plant from microbial pathogens, animal herbivores and hostile environmental conditions. Another reason for the synthesis of ORAC is the natural tendency of plants to respond to environmental stress conditions, including poor soil conditions, related to poor fertility (soils deficient in mineral contents).

According to Chinoy (1969), the plants natural defence to adverse conditions triggered an escalation in antioxidant activities. Mukherjee and Choudhuri (1983) noted that antioxidant activity increased significantly under water shortages and poor soil fertility. In a report by Rama Rao (1991), Zhang and Schmidt (2000) and, Zhang *et al.* (2003) on seaweed extracts, plant growth and yield, it provides evidence that due to the application of seaweed the plant developed a tolerance to environmental stress. Sexton and Woolhouse (1984), noted that seaweed extracts containing cytokinin or cytokinin-like growth regulators had the ability to inhibit the activity of free radical groups (antioxidants), such as hydrogen peroxide and superoxide, which are the major factors, which inherently reduce photosynthesis and result in chlorophyll degradation in plants. The results in our study provides evidence that antioxidants polyphenols and ORAC in 2011 in the potato leaves were significantly reduced by higher Afrikelp® LG-1 concentrations. Based on similar studies done by various researchers, this invariably have a positive impact on fundamental plant functions such as the process of photosynthesis and chlorophyll production. Ultimately this will improve potato tuber growth characteristics and yield.

4.4.2 Food proximate values

The results of the investigation revealed that food proximate values in 2010 and 2011 the foliar method of application of Afrikelp® LG-1, had a positive influence on the crude fibre and protein when compared with drench. Similar observations were made by Crouch *et al.* (1990), where it was recorded that seaweed depending on the method of application have the ability to promote protein synthesis in plants. Fletcher *et al.* (1988), have noted that a range of naturally occurring and synthetic cytokinins, present in liquid organic bio-stimulants, such as seaweed extracts can enhance protein formation such as polysomes and ribosomes in various ornamental and vegetable crops when applied onto the leaves. Anantharaj and Venkatesalu (2001 and 2002), conducted an investigation of seaweed extract on growth and protein of fenugreek (*Trigonella foenum-graecum* L.) using seaweed extracts of *Ulva fasciata, Sargassum ilicifolium* and *Gracilaria corticata*. The highest protein content in the plant was recorded at 50% concentration of seaweed liquid fertilizer soaked treatment in fenugreek. The increase in the protein content in the plant was evident at seedling stage. In trials done with liquid seaweed extracts (SLE), conducted by researchers Zodape, *et al.* (2010), on mung beans, Zodape, *et al.* (2008), and Arthur, *et al.* (2003), on peppers, it was evident that the application of seaweed extract significantly increased pepper yield, pod weight and improved protein, and carbohydrates of seeds.

4.5 Conclusion

The foliar method of application had a greater influence on ORAC reduction in the tubers of *Solanum tuberosum* 'BP1' when compared with drench. The results revealed that the leaves, which received the lower concentrations of Afrikelp® LG-1 and at the control, antioxidant values and therefore stress levels were higher. This indicates that the treatment crops, which received higher seaweed concentrations, had the ability prevent significant levels of ORAC and polyphenols from occurring. Seaweed extracts are not always able to supply all the essential nutrients in the quantities required by the plants (Schmidt *et al.*, 2003) but the results indicated that they will enhance plant growth and help prevent the formation of polyphenols and ORAC. In general, it was observed in the present study, that the higher seaweed extracts of Afrikelp® LG-1gave better results in all aspects of potato growth such as a reduction in antioxidants thereby lowering plants oxidative stress. Similar observations have been made in earlier studies, where it was found that oxidative stress, characterized by accumulation of antioxidants, could result from environmental stresses such as water and nutrient deficiency. The findings of this study suggest that Afrikelp® LG-1 has the capacity to reduce the need for an adaptive defence mechanism to counteract oxidative stress. The foliar method of seaweed application had an effect on the food proximate values, in particular protein and crude fibre values when compared with drench. The prospects for future research may require testing the chemical constituents of Afrikelp® LG-1, and how this product synergistically enhances the plants natural defence mechanism to resist extreme environmental conditions, and how this product promotes formation of other food proximate values. Further investigations will reveal whether these factors were adequate to sustain a stress free environment for the potato plant to enhance leaf chlorophyll content, increase growth parameters and tuber yield.

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Table 4.1

The effect of seaweed extract on the antioxidant values of the potato tubers of *Solanum tuberosum* 'BP1'

Treatments/season	2010			2011		
Method of application	Ascorbic acid (mg/100g)	Polyphenols (mgGAE/100g)	ORAC (Umol TE/g)	Ascorbic acid (mg/100g)	Polyphenols (mgGAE/100g)	ORAC (Umol TE/g)
Drench	9.4±0.98a	21.1±0.80a	7.6±0.20a	11.8±0.80a	30.5±1.39a	6.3±0.31a
Foliar	9.2±1.01a	19.6±0.80a	7.0±0.20b	12.9±0.64a	27.7±1.22a	5.8±0.34a
Afrikelp concentration (Litres/hectare) Drench & foliar applications combined						
0	8.4±1.28a	21.5±1.18a	7.4±0.53a	14.0±1.19a	30.0±2.20a	6.7±0.79a
2	11.1±2.76a	21.5±1.59a	7.4±0.37a	10.4±0.93a	31.9±1.96a	6.4±0.58a
3	7.9±1.99a	20.5±1.41a	7.4±0.47a	11.7±1.80a	31.9±2.10a	6.9±0.46a
4	7.2±1.07a	20.6±2.06a	7.2±0.34a	12.8±1.61a	28.5±2.70a	6.0±0.82a
5	11.4±1.67a	21.0±1.19a	7.0±0.23a	11.2±1.15a	26.7±2.80a	5.5±0.60a
6	9.8±2.04a	21.0±1.30a	7.5±0.42a	13.0±1.42a	29.2±2.69a	5.4±0.40a
7	9.3±1.80a	17.0±1.50a	7.1±0.39a	13.1±1.10a	25.3±2.70a	5.6±0.51a
2-Way ANOVA (F- statistics)						
Method of application	0.01NS	1.96NS	4.40*	1.47NS	2.23NS	1.00NS
Concentration	0.70NS	1.21NS	0.13NS	1.06NS	0.10NS	1.6NS
Method of application X Concentration	1.15NS	2.10NS	2.06NS	2.95	0.68NS	1.59NS

Values presented are Means ± SE. SE = Standard Error, * = significant at $P \leq 0.05$, NS = not significant. Means followed by similar letter (s) in a column are not significantly different from each other at $P=0.05$ according to Fisher least significance difference.

Table 4.2

The effect of seaweed extract on the antioxidant values of the leaves of *Solanum tuberosum* 'BP1'

Treatments/season	2010		2011	
Method of application	**Polyphenols** (mg GAE/100g)	**ORAC** (Umol TE/g)	**Polyphenols** (mg GAE/100g)	**ORAC** (Umol TE/g)
Drench	700±51.20a	180±8.67a	600±30.81a	177±10.72a
Foliar	690±41.0a	171±7.90a	590±40.40a	158±9.01a
Afrikelp concentration (Litres/hectare) **Drench & foliar applications combined**				
0	770±a76.53a	192±15.60a	820±65.10a	220±24.37a
2	820±96.60a	190±14.63a	660±86.70ab	187±17.60ab
3	710±103.04a	170±17.40a	610±34.23bc	170±6.64bc
4	740±37.62a	190±7.90a	610±64.72bc	180±18.30abc
5	540±71.00a	160±14.14a	510±27.70bc	150±10.06bc
6	600±106.60a	150±19.49a	450±55.43bc	140±20.73bc
7	700±80.27a	170±15.50a	470±31.50c	131±11.73c
2-Way ANOVA (F- statistics)				
Method of application	0.02NS	0.30NS	0.06NS	2.33NS
Concentration	1.17NS	1.0NS	4.41**	3.31**
Method of application X Concentration	0.29NS	0.32NS	0.60NS	0.80NS

Values presented are Means ± SE. SE = Standard Error, ** significant at $P \leq 0.01$, NS = not significant. Means followed by similar letter (s) in a column are not significant different.

Table 4.3

The effect of seaweed extracts on the food proximate values of the potato tubers of *Solanum tuberosum* ‘BP1’

Treatments/season	**2010**	**2011**
Method of application	**Crude fibre** (%)	**Protein** (%)
Drench	0.0±0.00b	1.4±0.02b
Foliar	0.3±0.11a	1.6±0.04a
Afrikelp concentration (Litres/hectare) Drench & foliar applications combined		
0	0.1±0.05a	1.5±0.06a
2	0.2±0.17a	1.4±0.08a
3	0.2±0.22a	1.5±0.09a
4	0.1±0.10a	1.4±0.04a
5	0.2±0.22a	1.4±0.07a
6	0.1±0.07a	1.5±0.06a
7	0.2±0.22a	1.5±0.07a
2-Way ANOVA (F- statistics)		
Method of application	5.78*	13.21***
Concentration	0.20NS	0.74NS
Method of application X Concentration	0.20NS	0.38NS

Values presented are Means ± SE. SE = Standard Error, *, *** = significant at $P \leq 0.05$, $P \leq 0.001$ respectively, NS = not significant. Means followed by similar letter (s) in a column are not significant different.

CHAPTER 5

The effects of soil drench and foliar application of a commercial seaweed extract on chlorophyll, growth and yield of *Solanum tuberosum* 'BP1'

CHAPTER 5

The effects of soil drench and foliar application of a commercial seaweed extract on chlorophyll, growth and yield of *Solanum tuberosum* 'BP1'

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Abstract

A field trial was conducted to investigate the effects of a locally produced seaweed extract (Afrikelp® LG-1) on leaf chlorophyll content, growth and yield of potato tuber, *Solanum tuberosum* 'BP1' under foliar and drench applications over two growing seasons, namely 2010 and 2011. The field experiment was conducted under irrigation at the Agricultural Research Council's (ARC) Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa. The experimental plot followed a randomized factorial block design with 4 replications per treatment. Each block contained one control and six seaweed extract treatments (0, 2, 3, 4, 5, 6, and 7 litres/ha) for both drench and foliar applications.

For chlorophyll sampling a total of 168 leaf samples were collected and analyzed per growth season. For growth parameters and treatment of samples potato plants were harvested 153 days after planting.

Chlorophyll *a*, *b* and total leaf chlorophyll increased with increasing seaweed extract concentrations with 7 l/ha resulting in the most significant effect over the control. For growth parameters: foliage fresh and dry mass, plant height, and tuber diameter (except in 2011 for tuber diameter) increased significantly with increasing seaweed extract concentrations. The highest concentration of 7 l/ha had the most significant effect on total tuber yield in 2010 (125%) and 2011 (109%) respectively over the control.

Field trials showed that a locally produced seaweed extract enhanced growth and total yield of *Solanum tuberosum* 'BP1' and can therefore be used as an agent to facilitate growth of potato plants.

Keywords: *Seaweed extract; Potato tuber; Total yield; Growth parameters; Chlorophyll; Solanum tuberosum 'BP1'; Drench and foliar.*

5.1 Introduction

5.1.1 Chlorophyll

Sims and Gamon (2002), outlined the importance leaf pigment content, and how this characteristic can provide valuable insight into the physiological performance of leaves, impact on growth, and total yield of crop. Chlorophyll tends to decline more rapidly than carotenoids when plants are under stress or during leaf senescence (Gitelson and Merzlyak, 1994a, 1994b, Merzlyak *et al*., 1999) and impact negatively on plant growth. Metting *et al.* (1990) investigated the application of commercial seaweed preparations and concluded that they have many beneficial effects on plants. One of our objectives in this study was to measure the effects of seaweed extracts

Afrikelp® LG-1applied by means of foliar and soil drench methods on the chlorophyll values (Chl*a*, Chl*b* and total chlorophyll) of *Solanum tuberosum* 'BP1' leaves grown over two seasons.

5.1.2 Growth parameters

Challen and Hemingway (1965), reported that the application of seaweed liquid extracts are beneficial for various plant types. It was noted in their study that seaweed extracts concentrates contained growth promoting hormones (IAA and IBA, Cytokinins), trace elements (Fe, Cu, Zn, Co, Mo, Mn, and Ni), vitamins and amino acids. Marine algae are used in agricultural and horticultural crop production, and beneficial effects, in terms of enhancement of yield and quality have been reported (Blunden and Wildgoose, 1977; Crouch and Van Staden, 1994). Liquid extracts obtained from seaweeds have gained importance as foliar sprays for many crops including various grasses, cereals, flowers and vegetable species (Crouch and Van Staden, 1994). In work done by Blunden (1972a) and Stephenson (1974), improvement in tomato yield was recorded after the application of seaweed to the plants as either a soil drench or foliar spray.

Pise and Sabale (2010), identified some of the more common commercial seaweed liquid fertiliers products available in market, which included Maxicrop, Algifert, Goemar, Kelpak, Seaspray, Seasol, SM3, Cytex and Seacrop. Seaweed fertilizers are claimed to be better than other fertilizers and very economical. Many different beneficial effects have been recorded for crops treated with seaweed extracts. These benefits include increase crop yields (Senn *et al.*,1961, and Blunden 1972a), improved seed germination, increased uptake of inorganic constituents from the soil, increased resistance of plants to frost (Senn *et al.*, 1961), increase resistance to insect attack (Stephenson, 1966), and improve storage quality of fruit (Skelton and Senn, 1969).

Blunden (1972b), recorded that potato plants treated with seaweed extracts gave increased yields of tubers, and the potatoes from the treated plants were more even in size. The findings of previous researchers were mainly based on commercial observations and not always on properly conducted field trials.

This experiment was to determine the most effective application method (drench or foliar) and the optimum concentration of Afrikelp® LG-1in order to promote plant growth (foliage fresh and dry mass, plant height, and tuber diameter) and total yield of *Solanum tuberosum* 'BP1' cultivar. The field experiment was conducted over two growth seasons, years 2010 and 2011.

This study revealed correlations between concentrations of the seaweed extract (Afrikelp® LG-1), leaf chlorophyll content, plant growth features, and total yield. The effects of foliar and drench application methods were compared on chlorophyll concentration, growth variables and ultimately total yield in the potato plant over two growth seasons.

The hypothesis was that potato plants grown using seaweed extracts Afrikelp® LG-1 would show a direct relationship between the potato leaf chlorophyll content, growth, total yield, and seaweed extract treatment.

5.2 Material and Methods

5.2.1 Seaweed concentrate

The seaweed extract used in the trials was a commercial seaweed product, marketed as Afrikelp® LG-1, locally produced in South Africa for the local and international agriculture and horticulture sectors. This product was produced from the marine algae, *Ecklonia maxima* (Osbeck) Papenfuss, and prepared via cool fragmentation technology.

5.2.2 Crop identification and growth of plants

Solanum tuberosum 'BP1' cultivar tubers were selected for planting. Tubers were planted in rows, in each row the tubers were planted 20 cm apart and 10 cm deep. The width between rows was 75 cm. Standard techniques were used to ensure uniformity of planting depth and spacing.

5.2.3 Site location & growing conditions

The field experiment was conducted under irrigation at the Agricultural Research Council's (ARC) Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa during the 2010 and 2011 growth seasons. The experimental site lies in the winter rainfall region at an elevation of 146 m above sea level with mean annual rainfall of 713.4 mm and a mean annual maximum of 22.6°C and minimum 11.6°C. The soil type of the experimental site was sandy loam (Glenrosa, Hutton form) which according to the Soil Classification Working Group (SCWG) is equivalent to skeletic leptosol (SCWG, 1991). The soil was ploughed to a depth of 40 cm prior to planting.

5.2.4 Experimental plot design

A randomized factorial block design with 4 replications per treatment was used. Planting took place on the 21st May for both years. The experimental plot was

1225 m^2 (35 m X 35 m). Each block contained one control and six treatments for both drench and foliar applications. Each treatment measuring 3 m X 4 m, contained 4 rows, and each row was planted with 12 potato plants, with a total of 48 plants per treatment, and 336 plants per block. A total of 2688 potato plants were used in the field experiment each year.

Application rates of seaweed extracts were 2, 3, 4, 5, 6, and 7 litres/ha. The first application was on the 7th July for each season. Thereafter, five applications were made with a two week interval in between each application, on the following dates: 7/07; 21/07; 4/08; 18/08; 2/09. A knapsack sprayer, calibrated according to the required dosage applied the seaweed extract in the form of fine droplets sprayed directly onto the leaf surfaces, avoiding contact with the soil. Around the base of each plant and in the drip-line area plastic covers were placed to avoid contact of foliar spray with the soil. Drench application was done by the application of the liquid directed to fill a reservoir at the base of the plants stem and in the root zone of the plant. An applicator with a knapsack was assigned to each treatment. Application was done early morning, when weather conditions were windless with no rain. Water was applied to the control treatment in each block.

Weed management was done manually by the ARC staff, every three weeks from the commencement date (21st May 2010 and 2011). For disease control the crops were sprayed for early blight on three occasions (with a fungicide mixture of copper oxychloride and mancozeb), 20/07; 3/08; 17/08 in both years.

5.2.5 Chlorophyll

5.2.5.1 Sample preparation

A total of 168 leaf samples were collected and analyzed per growth season. Three leaves were collected from three plants in the each treatment. A total of 21 leaves per experimental row, and 42 leaves per experimental block were collected. These leaves were selected to represent as large a range of the potato plants. All leaves collected in the field were placed in plastic bags and kept cool until they were brought back to the laboratory. Three replicate measurements were made on each leaf, a total of 1008 across both seasons, and the results averaged.

5.2.5.2 Quantification of chlorophylls

For chlorophyll analysis, 0.64 cm^2 leaf samples were ground in 2 ml cold acetone/tris buffer solution (80:20 vol; vol, pH=7.8), centrifuged to remove particles, and this supernatant was diluted to a final volume of 6 ml with additional acetone/tris buffer.

The absorbance of the extract solutions was measured with the Unispec spectrometer reconfigured as a spectrophotometer using an external cuvette holder (model 664.000, Hellma Plainview, NY). This allowed better comparisons between the leaf reflectance and pigment absorbance measurements since both were made with the same type of spectral detector. An initial test showed that the spectrophotometer response was linear with pigment concentration up to an absorbance of one. When the peak absorbance of the extract solutions exceeded one, the solutions were diluted further and remeasured.

The maximum error that would be introduced into the chlorophyll calculations would be less than 3%, according to Lichtenthaler's (1987). The following equations were used:

$Chl\,a = 0.01373A_{663} – 0.000897A_{537} - 0.0030406A_{647}$

$Chl\,b = 0.024005A_{647} – 0.004305A_{537} – 0.005507A_{663}$

A_x is the absorbance of the extract solution in a 1-cm path length cuvette at wavelength x.

5.2.6 Collection of growth data parameters and treatment of samples

Potato plants were harvested 153 days after planting, for 2010 and 2011 crops. In each treatment for foliar and drench application, 24 plants were sampled respectively from the middle rows in each plot, i.e. 2nd and 3rd rows only. The border rows were excluded i.e. rows 1 and 4 since there was an increased risk of exposer to unknown environmental factors. A total of 168 plants per experimental row, and a total of 336 plants per block were measured. A combine total of 1344 plants were measured for the four blocks. Plants were carefully dug out with their entire root system, and potatoes were harvested, washed and labeled.

For each treatment the average measurements for 24 plants combined per treatment were taken. For the variable total yield, potato tubers were harvested, washed and weighed (kg/ha). The variable foliage fresh weight, plants were weighed (kg/ha) on site. The total fresh weight of the potato plants was calculated and the average was recorded. For the variable foliage dry weights, foliage was oven dried at a temperature of 65 degrees celcius for a total 120 hours and weighed. This was then expressed as kg/ha. The total dry mass for all potato plant foliage were calculated and the average was recorded. The variable plant height, each of the plants were measured (cm) for its tallest stem and the average was calculated. The potato tuber diameter: tubers were individually measured (cm) and recorded.

5.2.7 Statistical analysis

The experimental data collected were analyzed using The STATISTICA Software (StatSoft 2013) and Two-Factor analysis of variance (ANOVA) was performed. The Fisher's least significant difference (LSD) was used to compare treatment means at $P \leq 0.05$ level of significance (Steel and Torrie, 1980).

5.3 Results

5.3.1 Chlorophyll

The results in Table 5.1 clearly demonstrate the effects of the various concentrations of Afrikelp® LG-1 seaweed extract on the levels of Chl*a*, Chl*b* and total chlorophyll in the leaves of *Solanum tuberosum* 'BP1' cultivar, for the years 2010 and 2011. For both years the method of application (drench and foliar), had no significant effect on the Chl*a*, Chl*b* and total chlorophyll values in the leaves of the potato plant. The results indicate that both the methods of application and the concentration levels of seaweed extract there were no significant interactions for Chl*a*, Chl*b* and total chlorophyll values for both 2010 and 2011 (Table 5.1).

5.3.1.1 Chl*a* (mg/g)

The results in Table 5.1 clearly indicate that increasing levels of seaweed concentrations had a significant increasing effect on the Chl*a* for both years. For the year 2010 and 2011, the concentration level that had the most significant ($P \leq 0.001$) effect on the increase of potato leaf Chl*a* was 7 l/ha, a total mean increase of 100% and 50% respectively over the control.

5.3.1.2 Chl*b* (mg/g)

The results in Table 5.1 clearly indicate that increasing levels of seaweed concentrations had a significant increasing effect on the Chl*b* for both years. For 2010 and 2011 years the concentration level that had the most significant ($P \leq 0.001$) effect on the increase of Chl*b* levels was 7 l/ha, which had a total mean increase of 500% (2010) and 400% (2011) respectively over the control.

5.3.1.3 Total chlorophyll (mg/gram)

Table 5.1., demonstrate that with increasing seaweed concentrations the total leaf chlorophyll levels significantly ($P \leq 0.001$) increased for both years. The highest concentration 7 l/ha had a total mean increase of total leaf chlorophyll levels of 700% (2010) and 600% (2011) over the control.

5.3.2 Growth parameters and total yield

The results in Table 5.2 clearly demonstrate the effects of various concentrations of Afrikelp® LG-1seaweed extract on the growth parameters and total yield of *Solanum tuberosum*, BP 1 cultivar for the years 2010 and 2011. For both years the method of application (drench and foliar) had no significant effect on the foliage fresh mass, foliage dry mass, tuber diameter and total yield, except for plant height in 2010, which was significantly higher with foliar treatment. There were no significant differences to report between the interactions of the method of application and the concentration levels of seaweed extract for all the variables tested for both years (Table 5.2).

5.3.2.1 Foliage fresh mass (kg/ha)

The results in Table 5.2 clearly demonstrate that for the year 2010, from 3 l/ha on there was an increase in foliage fresh mass compared with the control. The highest treatment 7 l/ha showed a significant ($P \leq 0.001$) increase of foliage fresh mass of 97% over control. For 2011 the treatment 6 l/ha had the most significant effect, and showed a total mean increase of 45% over the control for foliage fresh mass.

5.3.2.2 Foliage dry mass (kg/ha)

The results in Table 5.2 clearly demonstrate that for the years 2010 and 2011 from 5 l/ha and 4 l/ha respectively, the dry mass showed a significant increase over the control. For the highest treatments 7 l/ha, there was a significant ($P \leq 0.001$) increase of 66% in 2010 and a 69% in 2011 over the control.

5.3.2.3 Plant height (cm)

For the year 2010 only the plant height was significantly ($P \leq 0.01$) greater (12%) with foliar application than with drench. Overall there was a steady increase in plant height with increasing applications. The highest seaweed concentration 7 l/ha gave a mean total increase in plant height of 42% over the control. For the year 2011, plant height was not affected by methods of application. When comparing with the experimental control, 0 l/ha, from 3 l/ha on there was an increase over the control. The concentration level that had the most significant ($P \leq 0.001$) effect on plant height was 7 l/ha, which had a mean plant height of 49 % over the control.

5.3.2.4 Tuber diameter (cm)

The results in Table 5.2 clearly demonstrate that all seaweed concentrations in 2010 and 2011 had similar effects on tuber diameter with no significant observations to report.

5.3.2.5 Total yield (kg/ha)

The results in Table 5.2 clearly demonstrate that for both years 2010 and 2011, increasing seaweed concentrations had increasing results on total yield. The highest concentration of 7 l/ha had the most significant ($P \leq 0.001$) effect on total yield in 2010 (125%) and 2011 (109%) respectively over the control.

5.4 Discussion

5.4.1 Chlorophyll

Dhargalkar and Untawale (1983) provides evidence that seaweed extracts as liquid fertilizers (SLF) have the potential to positively impact on chlorophyll levels in the plant. This is due to the nitrogen, phosphorus, potash, trace elements and metabolites content of seaweed. The growth promoting hormones contained in seaweed include auxin, gibberellin and cytokinins, vitamins, amino acids and micronutrients, have a functional role in plant metabolism, in particular the utilization of nitrogen in the plant leaves.

The findings of the present study as in Table 5.1 substantiate that all chlorophyll levels were highest at higher seaweed concentrations of Afrikelp® LG-1 when supplied as drench or foliar applications, in particular at 7 l/ha for both seasons. The results from the current study therefore imply a correlation exists between chlorophyll levels and nitrogen concentration in the soil and leaves, which were significantly increased as a result of seaweed application. Nitrogen is a prominent component of chlorophyll, and is a basic mineral in plants, especially in the leaves. It is evident from the results (Table 5.1) that seaweed extract and therefore nitrogen had a positive effect on the levels of Chl*a*, Chl*b* and total chlorophyll values. It implies that high seaweed extract concentrations and nitrogen levels produce high chlorophyll values, which helps the plants photosynthetic ability to function optimally and produce an increased yield. In the lower seaweed concentrations, especially the control, all chlorophyll levels were at its lowest in the leaves. Here the plant were under stress, due to lower mineral levels, which resulted in lower levels of chlorophyll.

The results of the present study are supported by previous studies where leaf pigment content of chlorophyll tends to decline more rapidly than carotenoids when plants are under stress or during leaf senescence and lack of soil fertility (Gitelson and Merzlyak, 1994a, 1994b, and Merzlyak *et al.,* 1999). Chlorophyll can provide valuable insight into the physiological performance of leaves and the plant. Metting *et al.* (1990), suggests that the application of commercial seaweed preparations increases the nitrogen supply and consequently chlorophyll. Blunden *et al.* (1996), reported that the seaweed extract applied as foliar spray enhanced the leaf chlorophyll level in potatoes. The findings of the current research (Table 5.1) compares with the results of a study conducted by Thirumaran *et al*. (2007), where seaweed liquid fertilizer increased total chlorophyll content of *Cyamopsis tetrogonolaba* (L) Taub and radish (*Raphanus sativus)* by 20% over the control crop. Our findings coincide with earlier findings by Whapham *et al.* (1993), where it was observed that the application of seaweed liquid fertilizer of *Ascophyllum nodosum* increased the chlorophyll of cucumber cotyledons and tomato plants.

5.4.2 Growth parameters and yield

Hartman *et al.* (1990), reaffirms that in horticulture and agriculture the success of various vegetative growth parameters of plants may be influenced by several growth factors, including the method of application of nutrients and the type of plant nutrients. In our present field experiment growth variables including foliage fresh and dry mass, plant height, and total yield were significantly improved due to seaweed extract Afrikelp® LG-1 application, during the 2010 and 2011 growth seasons (Table 5.2).

In earlier studies conducted by Crouch and Van Staden (1994), similar results were obtained where the application of liquid extracts gave significant results when applied as foliar sprays for crops including grasses, cereals, flowers and vegetable species. Blunden and Wildgoose (1977), Crouch and Van Staden (1994), utilised marine algae for agricultural and horticultural crops, and beneficial effects, in terms of enhancement of yield and crop quality have been reported. Crouch and Van Staden (1992) confirmed the importance of commercial seaweed concentrate, where a seaweed extract prepared from *Eklonia maxima* (Osbek) Papenfuss, applied as a soil drench or foliar spray, significantly improved the growth and yield of tomato plants. Chapman and Chapman (1980) noted that seaweed extracts contain macronutrients, trace elements, organic substances like amino acids, plant growth regulators such as auxin, cytokinins and gibberellins. Nelson and Van Staden (1984) concluded that it is due to seaweed extracts nutrient and hormonal composition that they have the ability to promote, the growth and yield of crop plants.

The results (Table 5.2) indicates that for plant height in 2010, the drench application had a lesser effect when compared with foliar. This implies that once the seaweed extracts made full contact with the soil, its ability to be utilized by the plant may have been compromised. This could be due to seaweed extract being leached out of the soil, or components being bound in the soil. There may have been inconsistencies of seaweed utilization when applied by drench.

Seaweed utilisation by means of drench application may be affected by the degree of soil porosity, which affects the infiltration rates of liquid fertilizer into the soil. In Table 5.2 it shows that foliar application of nutrient uptake for plant height in 2010 generally favoured the overall growth of the potato plant, and in 2010 and 2011. Drench and foliar applications had similar effects on the plant height and all research variables.

At higher seaweed concentration levels, for the year 2010 and 2011 growth seasons, growth variables plant height (cm), foliage fresh (kg/ha) and dry weight (kg/ha) and total yield (kg/ha), were significantly promoted. This pattern of results therefore illustrate that incremental, (small) increases in seaweed concentrations have a similar or minimal influence on the potato yield (kg/ha), but when comparing the experimental control of 0 litres per hectare with the highest concentration of 7 l/ha the kg/ha result was very significant. The higher seaweed concentrations are recommended for plant growth to increase significantly.

Blunden (1972b), recorded that potato plants treated with seaweed extracts gave increased tuber yields and that the potatoes from the treated plants were more even in size. Similar observations was made in a study conducted by Crouch *et al.*, 1990. It recorded that seaweed enhanced nutrient uptake, regulated plant growth substances, increased chlorophyll content, protein synthesis, promoted root and shoot growth (Button and Noyes, 1964; Beckett and Van Staden, 1990; Yan, 1993). Results presented in the current study regarding potato yield validate the findings of Abetz and Young (1983) who had concluded that seaweed extract can improve total yield. The obtained results are in line with findings of Fornes *et al.* (2002) who reported that yield of mango was increased by seaweed extract foliar application. The findings of the study also coincide with those of earlier studies carried out on *Zizyphus mauritiana* Lamk (Rao, 1991). In the same way, Selvaraj *et al.* (2004) reported that foliar application of seaweed extract was more effective in increasing vegetative growth and yield components of *Lycopersicon esculentum*. Similarly, a substantial increase in yield was achieved in peppers when treated with a liquid seaweed extract (Arthur *et al.*, 2003).

The supply of Afrikelp® LG-1 over both growth seasons increased chlorophyll content. Due to an enhanced photosynthetic ability, this had a positive correlation with plant fresh mass, and culminated in substantial tuber formation.

5.5 Conclusion

Our findings confirms that drench and foliar application of seaweed extract Afrikelp® LG-1will have a measurable influence on the chlorophyll content, vegetative growth parameters and yield of the plant. It appears that the increased chlorophyll pigment, foliage mass and shoot size resulted in the highly significant increases in tuber yield, more than doubling the yield in each of the years of study.

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Table 5.1

Values of chlorophyll *a*, *b* and total chlorophyll of *Solanum tuberosum* 'BP1' supplied with seaweed extract.

Treatments/season	**2010**			**2011**		
Method of application	**Chl*a*** (mg/g)	**Chl*b*** (mg/g)	**Chl Total** (mg/g)	**Chl*a*** (mg/g)	**Chl*b*** (mg/g)	**Chl Total** (mg/g)
Drench	0.1±0.01a	0.4±0.04a	0.5±0.05a	0.1±0.01a	0.3±0.03a	0.3±0.04a
Foliar	0.1±0.01a	0.4±0.04a	0.5±0.06a	0.1± 0.01a	0.2±0.03a	0.3±0.04a
Afrikelp concentration (Litres/hectare)						
Drench & foliar applications combined						
0	0.1±0.01c	0.1±0.02d	0.1±0.02c	0.1±0.00d	0.1±0.03d	0.1±0.01f
2	0.0±0.01c	0.1±0.03d	0.2±0.03c	0.0±0.01cd	0.1±0.02cd	0.1±0.03ef
3	0.1±0.01b	0.3±0.04c	0.4±0.05b	0.1±0.01c	0.2±0.03c	0.2d±0.03e
4	0.1±0.01b	0.4±0.05c	0.5±0.06b	0.1±0.01c	0.3±0.03b	0.3±0.05cd
5	0.2±0.01a	0.5±0.03b	0.7±0.04a	0.1±0.01b	0.3±0.03b	0.4±0.2bc
6	0.2±0.01a	0.6±0.02a	0.8±0.04a	0.09±0.01b	0.4±0.04b	0.5±0.05b
7	0.2±0.01a	0.6±0.04a	0.8±0.05a	0.15±01a	0.5±0.04a	0.7±0.05a
2-Way ANOVA (F- statistics)						
Method of application	0.03NS	2.29NS	1.8NS	0.09NS	1.38NS	0.75NS
Concentration	27.68***	42.42***	40.92***	26.36***	21.53***	24.70***
Method of application X Concentration	0.16NS	0.76NS	0.57NS	0.18NS	0.31NS	0.25NS

Values presented are Means ± SE. SE = Standard Error, *** = significant at $P \leq 0.001$, NS = not significant. Means followed by similar letter (s) in a column are not significantly different from each other at $P=0.05$ according to Fisher least significance difference.

Table 5.2

The effect of soil drench and foliar applications of seaweed extracts on the growth and yield of *Solanum tuberosum* 'BP1'

Treatments/ Season	2010					2011				
Method of application	**Foliage fresh** (kg/ha)	**Foliage dry** (kg/ha)	**Plant height** (cm)	**Tuber diameter** (cm)	**Total yield** (kg/ha)	**Foliage fresh** (kg/ha)	**Foliage dry** (kg/ha)	**Plant height** (cm)	**Tuber diameter** (cm)	**Total yield** (kg/ha)
Drench	235.8±13.6a	35.2±1.5a	12.0±0.30b	3.8±0.8a	7881.4±721.8a	216.9±13.8a	50.1±3.1a	13.0±0.40a	3.0±0.11a	7133.3±421.9a
Foliar	216.9±12.8a	34.7±2.03a	13.4±0.31a	3.0±0.1a	7807.1±538.9a	218.2±12.1a	49.7±3.0a	13.0±0.34a	2.8±0.10a	7261.9±398.2a
Afrikelp concentration (Litres/hectare) Drench & foliar applications combined.										
0	150.4±16.3c	27.1±2.2d	11.1±0.21d	2.9±0.10a	5025.0±563.5d	178.5±26.4a	38.4±3.6d	10.5±0.31e	2.9±0.11a	4341.7±525.4c
2	184.5±20.3bc	27.6±2.4d	12.0±0.23c	3.2±0.12a	5984.2±933.5cd	176.0±26.7a	42.6±3. 4cd	10.9±0.33e	2.7±0.23a	5291.7±479.5c
3	210.0±14.1b	32.1±2.2cd	12.2±0.18c	3.1±0.10a	5999.2±705.8cd	214.5±20.5a	42.1±3.4cd	12.2±0.35d	2.8±0.18a	7000.0±536.4b
4	219.7±17.4b	34.2±2.1cd	13.2±0.15b	5.7±2.70a	8364.2±774.0bc	207.3±20.5a	54.8±3.4abc	13.0±0.27cd	2.9±0.12a	7729.2±456.4ab
5	230.0±14.7b	36.2±2.9bc	13.7±0.35b	2.8±0.07a	8770.0±927.6abc	235.9±23.2a	56.9±5.5ab	13.6±0.31bc	3.2±0.24a	8020.8±607.9ab
6	294.1±19.8a	42.1±2.6ab	13.9±0.41b	3.1±0.12a	9434.5±494.8ab	259.3±18.7a	49.5±6.3bcd	14.4±0.27b	3.0±0.26a	8895.8±334.7a
7	295.7±23.4a	45.1±3.2a	15.8±0.35a	3.1±0.15a	11333.3±1785.2a	251.7±21.3a	64.9±7.4a	15.6±0.36a	3.0±0.17a	9104.2±553.6a
2-Way ANOVA (F- statistics)										
Method of application	1.72NS	0.05NS	10.07**	1.0NS	0.01NS	0.01NS	0.01NS	1.0NS	1.9NS	0.11NS
Concentration	7.99***	7.10***	34.5***	1.0NS	4.89***	1.89NS	3.73**	31.0***	0.6NS	12.17***
Method of application X Concentration	0.28NS	1.13NS	0.74NS	0.9NS	0.36NS	0.17NS	1.28NS	0.5NS	0.7NS	0.88NS

Values presented are Means ± SE. SE = Standard Error, ** = significant at $P \leq 0.01$ respectively, NS = not significant. Means followed by similar letter (s) in a column are not significantly different from each other at *P=0.05* according to Fisher least significance difference

CHAPTER 6:

The effects of a commercial seaweed extract on the growth and yield of *Vitis vinifera* var. sultana grapes, under soil drench and foliar applications

CHAPTER 6

The effects of a commercial seaweed extract on the growth and yield of *Vitis vinifera* var. sultana grapes, under soil drench and foliar applications.

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Abstract

A field trial was conducted to evaluate the effect of seaweed extracts (Afrikelp® LG-1) on the growth parameters and total yield of *Vitis vinifera* var. sultana grapes. The field experiment was conducted at the Agricultural Research Council Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa, during the 2011 and 2012 growing seasons. In total 168 *Vitis vinifera* var. sultana grape vines were used. A randomized block experiment consisting of two application methods (foliar and drench), seven application levels (0, 2, 3, 4, 5, 6 and 7 l/ha), replicated four times. After 169 days samples were collected and analyzed for the number of bunches per vine, bunch length (cm) and diameter (cm), and final yield (kg/ha).

The results showed that for both years the highest seaweed extract concentrations, 7 l/ha produced the highest results for all vine growth parameters over the control. It was noted that

foliar application significantly increased the length of grape bunches over drench for both years. Drench application resulted in a significantly higher total yield in both years over foliar. For total yield, at the highest concentration, 7 l/ha this was a commercially important increase over the control of 355% in 2011, and 210% in 2012.

The field trials showed that a seaweed extract had the capability to promote growth and total yield of *Vitis vinifera* var. sultana.

Keywords: *Afrikelp® LG-1, Growth parameters, Number of bunches, Length of bunches, Diameter of bunches, Total yield, Vitis vinifera var. sultana, Growth seasons*

6.1 Introduction

Seaweeds extracts are marketed as liquid fertilizers. They contain many growth regulators and nutrients such as gibberellins (Wildgoose *et al.*, 1978), betaines (Blunden *et al.*, 1991; Wu *et al.*, 1997), cytokinins (Durand *et al.*, 2003; Stirk *et al.*, 2003), auxins (Stirk *et al.*, 2004), macronutrients (Ca, K, P) and micronutrients (Fe, Cu, Zn, B, Mn, Co, and Mo). Khan *et al.* (2009), confirms the importance of seaweed extracts for plant development due to their nutrient components, and reinforces that these are necessary for the development and growth of fruits and vegetables.

Blunden and Gordon (1986), Ugarte *et al.* (2006) and Hong *et al.* (2007), noted various liquid seaweed products are commonly used in agriculture and horticulture as bio-fertilizers. These products are mainly sourced from three main groups of seaweeds based on their pigmentation (for example, *Phaeophyta*, *Rhodophyta*, and *Chlorophyta*, also known as the brown, red, and green algae, respectively.

Studies conducted by Beckett and Van Staden (1989), Van Staden *et al*. (1994), Washington *et al.* (1999), and Norrie and Keathley (2006), revealed a wide range of beneficial effects of seaweed extract applications on various fruits including grapes.

These benefits include early seed germination and establishment, improved crop performance, yield, and enhanced postharvest shelf-life. Other physiological plant features enhanced by seaweed extracts includes, increased foliage, deep root development, improved lateral root formation (Atzmon and Van Staden 1994) and improved appearances and size of the fruit (Allen *et al.*, 2001; Cluzet *et al.*, 2004 and Mancuso *et al.*, 2006).

Norrie *et al*. (2002), explored the benefit of seaweed extract used for grapes. The results showed an increase in grape berry size, length of bunches, and total yield. Avenant and Avenant (2006), utilized seaweed extracts for grapes for the improvement of colour and yield. Red globe grapes were treated with a 1.5% *Ecklonia maxima* extract solution at 12 mm and 16 mm berry size, and applied by means of drench and foliar application. These grapes produced a higher percentage of bunches with a darker colour. Kok *et al.* (2010), conducted a similar study, which reported on the use of seaweed extracts on Trakya Ilkeren grapes. Seaweed extract *Ascophyllum nodosum* were applied by means of foliar application at 15 cm to 20 cm shoot/vine length, pre-bloom and at pea berry size. The results showed improved brightness, redness, an increase in bunches, diameter and yield. Norrie *et al*. (2002), further documented the importance of seaweeds utilized for grapes. Spray applications of seaweed extract improved berry firmness, number of bunches, diameter of bunches, and yield of Sultanina. This is substantiated by related research conducted by Norrie and Keathley (2006), which demonstrated that supplementary foliar sprays with seaweed products can also improve berry size, yield and grape quality.

Abetz (1980) and Metting *et al.* (1990), highlighted the importance of industrial manufactured products and the effects on the environment. They provide a discussion related to the increasing costs of energy and raw materials, and the elevated awareness of detrimental environmental effects related to excessive use of conventional fertilizers, herbicides, and pesticides. They emphasised that it's important to improve fertilizer and chemical efficiency, and find less harmful alternative methods to improve crop quality and yields.

For the purpose of the current research, viticulture is an agricultural activity extensively practiced in the Western Cape, South Africa, which is regarded as a coastal region. The sultana table grape industry is one of the most important fruit growing sectors in this region and provides employment to a large sector of the Western Cape population.

The South African table grape export industry is situated in mild Mediterranean and arid subtropical climates. More than 80% of table grape production in South Africa occurs in the Western Cape region. Other production areas include the Northern Cape, Eastern Cape, Limpopo, Free State and Mpumalanga. According to the Dept. of Agriculture, Forestry and Fisheries (2012), internationally, table grapes are among the most traded fruit types in the world. In the table grape industry, berry quality and certain physical growth characteristics play an important role for marketing table grapes. In the local table grape industry the crops quality is measured in terms of seediness, appearance, eating quality characteristics, which includes sweetness, firmness, texture and flavour. Clingeleffer (1985), provides the physical characteristics important for marketing table grapes, which includes bunch formation, size, diameter and length of bunches.

The objective of this research was therefore to evaluate the effect of different concentrations of a local seaweed extract, Afrikelp® LG-1, produced from marine algae, *Ecklonia maxima* (Osbeck) Papenfuss, on the growth (number, length and diameter of bunches) and yield of *Vitis vinifera* var. sultana under foliar and soil drench application. The Afrikelp® LG-1was evaluated under local agricultural conditions, which are experienced by local grape farmers. The results will thus be applicable under local farming practices.

The hypothesis was that for sultana grape parameters and total yield there would be a direct relationship to seaweed extract treatment.

6.2 Materials and methods

6.2.1 Seaweed concentrate

A cool fragmentation seaweed extract derived from marine algae, *Ecklonia maxima* (Osbeck) Papenfuss, were used in the trials. It is a commercial product, marketed as Afrikelp® LG-1, and produced in South Africa for the local agriculture and horticulture sectors.

6.2.2 The vineyard

In total 168 *Vitis vinifera* var. sultana grape vines were used. The vines were planted in December 1995 on loam soil and were pruned in August each year.

6.2.3 Experimental site

The field experiment was conducted at the Agricultural Research Council Nietvoorbij site (33° 54' S, 18° 14' E) in Stellenbosch, South Africa, on a loam soil.

6.2.4 Experimental design

A randomized block experiment involving two application methods (foliar and drench), seven application levels (0, 2, 3, 4, 5, 6 and 7 l/ha) and four replicates was carried out. Established vines were used and treatments were started on the 1st September in both 2010 and 2011.

Thereafter, liquid seaweed extract diluted in water were applied in five applications with a two week interval in between each application (29/09, 6/10, 20/10, 3/11, 17/11). Applications was by means of a knapsack sprayer, calibrated according to the required dosage. Foliar application was done in the form of a fine droplet applied directly onto the leaf surfaces, and avoiding no contact with the soil. Drench application was done by the application of the fluid directed to fill the reservoir at the base of the vine stem. For drench, a reservoir was created, which act as a collecting device to ensure no run-off of applied fluids. An applicator with a knapsack was assigned to each treatment. Water was applied to the control treatment in each block.. Each treatment contained 3 vines. Each block contained two rows (i.e. foliar and drench). Each row had a total of 21 vines. Each block (i.e. replication) had a total of 42 vines. A total of 168 vines were used in the field experiment. Weed management and disease control was done manually by the ARC, every three weeks from the start date. No diseases or pest related incidents were found on the vines.

After 169 days, from 1/09/2010 to 16/02/2011 and in the second year, was from 1/09/2011 to 16/02/2012, samples were collected. The following variables: the number of bunches per vine, bunch length (cm) and diameter (cm), and final yield (kg/ha).

6.2.5 Statistical analysis

The experimental data collected were analysed using StatSoft (2013), and Two-Factor analysis of variance (ANOVA) was performed. The Fisher's least significant difference (LSD) was used to compare treatment means at $P \leq 0.05$, level of significance (Steel and Torrie, 1980).

6.3 Results

The results in Table 6.1 clearly demonstrate the effects of various concentrations of Afrikelp® LG-1 seaweed extract on the physical growth parameters of *Vitis vinifera* var. sultana grapes, over a period of two years, 2011 and 2012. The results indicated that there were no significant interactions between the methods of application (drench and foliar) and the concentration levels on the number of bunches, length of bunches, diameter of bunches and yield of bunches, for both experimental years.

6.3.1 Number of grape bunches (per vine)

The results presented in Table 6.1 indicated that for both years (2011 and 2012) there were no significant differences due to the method of application (drench and foliar) on the number of bunches per vine.

However the results in Table 6.1 clearly demonstrated that for both years (2011 and 2012) that concentrations (5, 6, and 7 l/ha) significantly ($P \leq 0.01$) increased the number of bunches per vine. This represented a total mean increase for 7 l/ha of 186% and 111% over the control for 2011 and 2012 respectively, which were the highest increases (Table 6.1).

6.3.2 Length of the grape bunches (cm)

For the year 2011 and 2012, the results in Table 6.1 indicated that there was a significant ($P \leq 0.01$) increase in length due to foliar application when compared with drench. In 2011 this represented a total mean increase for foliar of 10% over drench, and in 2012 this represented a total mean increase for foliar of 16% over drench application.

For 2011 seaweed concentration 7 l/ha had a significant ($P \leq 0.01$) effect on the length of grape bunches, which represented a total mean increase of 38% over the control. For the year 2012, the concentration levels of seaweed had no effect in the length of the grape bunches (Table 6.1).

6.3.3 Diameter of grape bunches (cm)

The results in Table 6.1 indicated that for the two experimental years (2011 and 2012), there were no significant differences resulting from the method of application.

For 2011 the results showed that seaweed treatments (4, 5, 6, and 7 l/ha) had significantly ($P \leq 0.01$) improved the diameter of grape bunches. The highest concentration of 7 l/ha represented a total mean increase of 47% over the control.

For the year 2012, the concentration levels of seaweed extracts had no significant effects on the diameter of grape bunches (Table 6.1).

6.3.4 Total yield of vines (kg/ha)

The results from this study indicated that drench application resulted in a significantly ($P \leq 0.05$) higher yield than foliar application in both years (Table 6.1). For the year 2011 and 2012 the drench application gave a total mean increase of 28% and 35% over the foliar application for yield.

Seaweed concentrates of 4 l/ha and above in 2011 and 2012 resulted in significant ($P \leq 0.01$) yield increases. At the highest concentration of 7 l/ha, this was a commercially important increase over the control of 355% in 2011 and 210% in 2012. (Table 6.1).

6.4 Discussion

This study determined that for 2011 and 2012 the number of bunches per vine increased significantly by 186% and 111% over the control when Afrikelp® LG-1 seaweed concentrations of 7 l/ha were applied. The results in our study also showed that length of bunches in 2011 and 2012 increased 10% and 16% due to foliar application when compared with drench. Winkler *et al.* (1974), discussed the importance of spraying different growth regulators and bio-stimulants as a management technique to achieve quality attributes and berry mass, when growing commercial grapes. Our study provides conclusive evidence that seaweed extracts enhanced bunch formation. Kok *et al.* (2010), reported on the use of seaweed extracts on grapes, where the seaweed extract of *Ascophyllum nodosum*, was applied by means of foliar application and increased shoot/vine length, berry size, number of bunches, diameter and yield of cv. Trakya Ilkeren grapes (*Vitis vinifera* L.).

Many studies show enhancement and improved functioning of other crops by seaweed extracts applications (Abetz and Young, 1983; Featonby-Smith and Van Staden 1987; Crouch and Van Staden, 1992 and 1994; Van Staden *et al.,* 1994; Arthur *et al.,* 2003; Leclerc *et al.,* 2006; Khan *et al.,* 2009; Craige, 2010).

Although foliar application had a more profound effect on length of grape bunches, in the present study the results illustrates that Afrikelp® LG-1 applied as a drench application increased total yield of the sultana grapes for both years, more so than foliar application.

Norrie and Keathley (2006), reported that *A. nodosum* extracts showed positive effects on the yield of 'Thompson seedless' grape (*Vitis vinifera* L.). They observed that the *A. nodosum*-treated plants outperformed the controls in terms of berries per bunch, berry size, berry weight, rachis length, and the number of bunches per plant.

6.5 Conclusions

Our findings were that the liquid seaweed extract Afrikelp® LG-1 enhanced certain physical growth parameters of *Vitis vinifera* var. sultana grapes and outperformed the controls consistently over a two year period. The considerable increase in total yield, could have far-reaching benefits for the grape industry economically. The results serves as an endorsement that seaweed extracts are a reliable alternative form of bio-fertilizer as they are eco-friendly, cheaper and can overcome the ill-effect of chemical fertilizers. It will therefore be beneficial to the grape industry to test seaweed extracts like Afrikelp® LG-1 on other popular grape cultivars in the Western Cape region. For future research prospects, the mechanism of action of seaweed extracts-elicited growth responses is largely unknown.

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Table 6.1

Growth parameter values of *Vitis vinifera* var. sultana grapes supplied with seaweed extract.

Treatments/season	2011				2012			
Method of application	**Number of bunches** (per vine)	**Length of bunches** (cm)	**Diameter of bunches** (cm)	**Total Yield** (kg/ha)	**Number of bunches** (per vine)	**Length of bunches** (cm)	**Diameter of bunches** (cm)	**Total Yield** (kg/ha)
Drench	56.9±4.55a	22.5±0.55b	12.2±0.26a	34031.3±3567.9a	51.8±3.00a	22.8±0.80b	16.0±0.9a	35911.2±2511.4a
Foliar	63.1±6.29a	24.8±0.75a	13.2±0.56a	26573.7±3166.6b	49.7±4.80a	26.4±0.80a	14.4±0.80a	26600.0±2630.4b
Afrikelp concentration (Litres/hectare) Drench & foliar applications combined								
0	32.5±3.54d	20.5±1.61d	10.5±0.38c	10992.2±1762.45d	32.6±3.0c	24.7±1.75a	14.0±1.40a	15406.3±1463.8f
2	37.0±2.77d	21.3±1.07cd	11.8±0.67bc	19554.7±3006.6cd	39.8±3.0c	24.0±1.18a	16.4±2.04a	19221.9±2397.4ef
3	50.8±2.78cd	21.7±0.83cd	12.1±0.40bc	20929.7±2794.0cd	35.8±3.0c	23.7±1.0a	14.0±1.10a	26384.4±2732.01de
4	61.8±4.45bc	23.5±0.90bc	12.6±0.40b	32296.9±5302.9bc	47.6±4.0bc	23.0±1.71a	14.8±1.34a	32339.1±3587.0cd
5	66.9±7.36bc	25.0±0.74b	13.4±0.40b	35695.3±5326.3b	61.9±6.0ab	24.6±2.0a	15.3±1.70a	36112.5±3486.9bc
6	77.9±4.87ab	25.2±0.70b	13.6±0.40ab	42625.0±7461.4ab	69.1±8.1a	26.7±1.4a	15.4±2.20a	41451.6±3905.7ab
7	93±16.09a	28.3±1.25a	15.4±1.42a	50023.4±4971.0a	69.0±9.5a	25.7±2.15a	13.4±1.14a	47873.4±5175.9a
2-Way ANOVA (F- statistics)								
Type	1.04NS	10.10**	3.21NS	4.24*	0.22NS	9.90**	0.29NS	15.66**
Concentration	7.26**	8.91**	5.33**	8.35**	6.70**	0.69NS	0.42NS	14.19**
Method of application X Concentration	0.35NS	0.32NS	0.64NS	0.27NS	0.16NS	0.76NS	0.35NS	0.31NS

Values presented are Means ± SE. SE = Standard Error, *and ** = significant at $P \leq 0.05$ and $P \leq 0.01$ respectively, NS = not significant. Means followed by similar letter (s) in a column are not significantly different from each other at $P=0.05$ according to Fisher least significance difference.

CHAPTER 7: CAN SEAWEED EXTRACTS PREPARATIONS ENHANCE CROP YIELD?

CHAPTER 7

Of major importance is the fact that all experimental work in this study involved statistically planned field trials.

7.1 Potatoes

7.1.1 Leaf nutrient content

Potato plants grown using seaweed extracts (Afrikelp® LG-1) would show a direct relationship between potato leaf nutrient content, and the seaweed extract treatment.

It was found that after the application of a commercial seaweed product, leaf nutrients (N, P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn and B) in *Solanum tuberosum* 'BP1' for growing seasons, 2010 and 2011, increased. The leaf macronutrient nitrogen increased by 84% for both growing seasons, phosphorous increased by 67% and 50%, respectively, and likewise, potassium increased by 72% and 76%. Stephenson (1974), Senn and Kingman (1978), noted that the N, P, K, in seaweeds have been positively linked to increasing nutrient concentration in the plant.

7.1.2 Antioxidants and food proximate values

Potato plants grown using a seaweed extract would show an inverse relationship with antioxidant levels (ascorbic acid, polyphenols, and ORAC), and a direct correlation between food proximate values (crude fiber and protein), and seaweed extract treatment.

For 2011(but not for 2010) the higher seaweed concentrations reduced polyphenols and ORAC in the leaves of potato tuber *Solanum tuberosum* 'BP 1' by 45% and 40%. The results show that potatoes in the untreated soils had the highest levels of antioxidants. Bowler (1992) and Stuhlfauth *et al.* (1990) concluded that plants are sensitive to stress and produce antioxidants as a protective measure. Clearly the extracts reduced stress. For food proximate values for 2010 (crude fibre only) and 2011 (protein only), the foliar application of seaweed increased crude fibre (50%) and protein (13%) over drench.

7.1.3 Chlorophyll, growth and yield

Potato plants grown using a seaweed extract would show a direct relationship between the potato leaf chlorophyll content, growth, total yield, and seaweed extract treatment. Commercial seaweed product treatment resulted in increased chlorophyll values in the leaves of *Solanum tuberosum* 'BP1' for growing seasons, 2010 and 2011. Leaf Chl*a* increased by 20% and 15% respectively, Chl*b* increased by 500%.

Gitelson and Merzlyak (1994a and 1994b) concluded that chlorophyll tends to decline more rapidly when plants are under stress. Merzlyak *et al*. (1999) reported that a decrease in chlorophyll pigments due to stressful conditions, generally have negative growth implications for the crop. Attememe (2009), identified that seaweed extracts had significant macronutrients, increasing the nitrogen, potassium and phosphorous content in the leaves, which had an enhancing effect on chlorophyll pigmentation and plant photosynthesis.

The supply of a seaweed extract for *Solanum tuberosum* 'BP1' growth, increased all growth variables. Foliage fresh mass increased by 97% and 45%. Likewise, foliage dry mass increased by 66% and 69%. In 2010 plant height was significantly increased by 42% (2010) and 49% (2011). Total tuber yield were substantially improved by 125% in 2010 and 109% in 2011.

7.1.4 Methods of treatment

For *Solanum tuberosum* 'BP1' antioxidants, ORAC in 2010 the results indicated that the foliar method of seaweed extract application of Afrikelp® LG-1 reduced levels of ORAC in the potato tuber by 8.2% over drench.

Food proximate values for crude fibre (50%) and protein (13%) in 2010 and 2011 respectively, foliar application and uptake was more effective over drench. Sivasankar and Oaks (1996), and Stitt (1999), evaluated the effects of seaweed liquid extract (SLE) on the protein activity and concluded that protein was increased by seaweed extracts of *Fucus spiralis* and *Ulva rigida.* For growth parameters in 2010 plant height was significantly affected by foliar application of seaweed extracts by 12% over drench.

In our study, although foliar treatment gave better results in certain aspects of potato growth, as compared to drench (soil feeding), drench has also proven to produce significant results. An ideal approach for potato cultivation, may well be a combination of both. Ease of application will probably be the deciding factor for farmers.

7.1.5 Integrating the information on potatoes

It can be observed that seaweed extracts enhanced many parameters over two growth seasons. The leaf nutrients increased, the antioxidants was reduced, indicating reduced plant stress. Torres *et al.* (2004), highlighted the influence of higher nutrient contents and how this is represented in the quality of the potato tuber.

For reduced antioxidants, plant height and food proximate values, foliar method of nutrient application was more effective than drench. This suggests that seaweed extract absorption through the leaves was more direct and effective than through the roots. Chlorophyll content improved in the leaves, as did foliage fresh and dry mass. All effects culminated in increased yield.

7.2 Sultana

Sultana grape parameters and total yield would be a directly proportional to the seaweed extract treatment.

The results demonstrated that for 2011 and 2012, seaweed extract concentrations of 7 l/ha increased the number of bunches per vine of *Vitis vinifera* var. sultana's by 186% and 111% respectively. For both years foliar application increased the length of bunches over drench by 10% and 16%. In 2011 seaweed concentration 7 l/ha increased the length of grape bunches by 38%. In the same year diameter of grape bunches increased by 47% over the control. For yield 2011 and 2012 drench application gave a total mean increase of 28% and 35% over the foliar application. Seaweed concentrations of 7 l/ha in 2011 and 2012 resulted in commercially important yield increases of 355% and 210%.

This study notes that both methods of applications, drench and foliar are beneficial ways of fertilizing vines, and increasing grape yields. Mancuso *et al.* (2006), reported that the presence of marine bioactive substances in seaweed extract improved nutrient content of macronutrients, in particular nitrogen, phosphorous and potassium in plants. Mundy (2007) concluded that after the vine has extracted the nitrogen and other macronutrients via the leaves or from the soil, the vines have an ability to store reserves and use them at key growth stages. Research has shown that nitrogen application at key times can influence vine physiology beneficially. Nitrogen in the vine is converted into biologically active amino acids, including arginine. Arginine is converted into a range of products within each plant cell of the vine, which potentially increases yield (Mundy *et al*, 2006). Although yield was significantly increased the food components of size, shape, colour and flavour of the grapes, and the effect on wine quality must still be determined.

7.3 Recommendations

These field trials were conducted over two growth seasons, with the same crop management practice and identical growing conditions being implemented. Under such circumstances, the results were verified, and final recommendations can therefore be made to local potato farmers and grape growers. The significance of the results, confirms that there is no need for speculation regarding the extraordinary plant growth elicited by seaweed extracts. It is recommended that this product or similar formulations become a common component of crop feeding regimes for agriculture, viticulture, and horticulture. In a South African context, the high yields elevates financial returns for growers, and makes seaweed extracts a viable economical product and a practical reality for the end user. This potentially contribute towards social economic improvement in economically marginalized rural settlements of the Western Cape.

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