# Mineral Analysis and Proximate Composition of Leaves of (*Brassica oleracea* var. *acephala*) in Response to Boron Application in Pot Experiments

by

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# DECLARATION

I, **Fatheya Mohamed**, declare that the thesis "**Mineral Analysis and Proximate Composition of Leaves of (*Brassica oleracea* var. *acephala*) in Response to Boron Application in Pot Experiments**" hereby submitted to the **Cape Peninsula University of Technology** for the degree of **Master of Technology (Agriculture)** represents my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

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# DEDICATION

***I dedicate this work to God Almighty the source of my strength.***

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***I wish to thank God, for divine strength, courage and grace that sustained me throughout my research work.***

- I am grateful to my husband (Yousif Eisa) for his constant support. And my lovely children
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# ABSTRACT

Choumollier (narrow-stem kale) (*Brassica oleracea*, L.) has been progressively used in recent years as a supplementary forage harvest in many countries with a temperate climate. Boron (B) and calcium (Ca) are the two most important elements for supporting plant structure and function of plasma membranes. Boron nutrition is vital for obtaining high quality yields in vegetables. The main objective of this study was to evaluate the extent to which boric acid concentration can affect growth parameters (plant height, leaf numbers, chlorophyll levels, and leaf size) of *Brassica olereacea* var. *acephala* at different stages of growth and development.

Treatment comprised of four concentrations of boron (0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg and 0.6 mg/kg). Yield and physiological growth responses were measured during the course of the study to ascertain effectiveness and influence of boron treatments on the test crops. Leaves of *B. oleracea* were harvested at weekly intervals (W1, W2, W3, W4 and W5) after each treatment regimen for approximate basic mineral analysis and composition. Soil pH did not vary much among the various orchard blocks tested, regardless of soil depth. Exchangeable cations $Na^+$ and $K^+$ levels did not vary significantly, but $Ca^{2+}$ and $Mg^{2+}$ levels fluctuated considerably among orchards analyzed.

The Control Orchard exhibited a higher P content than the other orchards. Ca, Mg, Cu and B levels did not vary significantly among the orchards, but Na, Fe and Zn levels were markedly raised in the Orchard treated with 0.3 mg/kg boron) relative to the Control Orchard. Chlorophyll fluorescence was significantly dependent on the treatment dose of boron as compared to control. Chlorophyll fluorescence also increased significantly with the growth period, i.e., the duration following the initial treatment at all doses of boron. Boron at all did not significantly affect leaf count, leaf length and plant height. The work may add to the body of knowledge on the influence of boron on the physiological performance, mineral contents and proximate composition of leaves of the species. Furthermore, the findings may have important applications in achieving high quality yields in vegetable crops.

**Keywords:** *Brassica oleracea*, boron, secondary metabolites, nutrient analyses, treatment, vegetable, concentrations

# LIST OF ABBREVIATIONS

| | |
|---|---|
| **ANOVA** | Analysis of Variance |
| **B** | Boron |
| **ºC** | Degrees Celsius |
| **Ca** | Calcium |
| **Chl** | Chlorophyll |
| **ChlF** | Chlorophyll Fluorescence |
| **CPUT** | Cape Peninsula University of Technology |
| **DV(s)** | Dependent Variable(s) |
| **FAO** | The Food and Agriculture Organization |
| **IV(s)** | Independent Variable(s) |
| **K** | Potassium |
| **$KH_2PO_4$** | Potassium Phosphate Monobasic |
| **KCl** | Potassium Chloride |
| **MANOVA** | Multivariate Analysis of Variance |
| **Mg** | Magnesium |
| **MP-AES** | Microwave Plasma-Atomic Emission Spectrometry |
| **N** | Nitrogen |
| **NOAEL** | No-Observed-Adverse-Effect-Level |
| **P** | Phosphorus |
| **TT** | Toxicity Thresholds |
| **Zn** | Zinc |

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# CHAPTER 1

## INTRODUCTION

### 1.1 Background

Vegetables are a substantial part of our nourishment. They provide not only the main dietary fibre component of our food, but also a range of micronutrients, including several compounds such as minerals, vitamins, antioxidants, carotenoids and polyphenols (Singh et al., 2010). It is well-established mineral nutrients are important for the growth, survival and reproductive success of plants (Stangoulis et al., 2001), and boron (B) and calcium (Ca) are the two most essential elements to keep plants healthy as well as important elements for supporting plant structural integrity and function of plasma membranes (Funakawa and Miwa, 2015; Jochner e*t a*l., 2013). Boron (B) is an obligatory non-metal in growth and development.

Boron plays a significant role in carbohydrate metabolism, ribonucleic acid (RNA) metabolism, respiration, indoleacetic acid metabolism, phenol metabolism, and as part of the cell membranes (Ahmad, 2009). In soils, B concentrations ranges from 20 to 200 mg/kg (Mengel and Kirkby, 1987). Due to its characteristics, B is absorbed by roots as a boric acid [B(OH)3] or H3BO3 (Marschner, 2012; Mengel and Kirkby, 1987), which enables it to complex with diols and polyols, mainly with cis-diols inside the plant system (Loomis and Durst, 1992). Boron is slightly immobile in the earth; therefore, its existence is necessary for all steps of growth, particularly during the fruit/seed progress.

However, recent physiological studies have revealed the presence of channelmediated facilitated diffusion and energy-dependent active transport against the gradients constructing the boron transport systems (Brown *et al.*, 2002; Dannel *et al.*, 2002; Stangoulis *et al.*, 2001). Boron deficiency affects crop production in many countries (Camacho-Cristóbal *et al.*, 2008; Shorrocks, 1997; Sillanpää, 1982). Boron plays a major role in cell division and structure, fruit and seed development (Bariya *et al.*, 2014; Koshiba *et al.*, 2009; Osman, 2013). The element is a micronutrient required for all plant nutrition and its absorption by plant roots is affected by several soil and non-soil environmental factors. As regards soil factors, the most important parameter is the pH (Gupta *et al.*, 1985).

## 1.2 Statement of the Research Problem

Mineral nutrients are essential for the growth, survival and reproductive success of plants (Ahmad, 2009; Bariya *et al.*, 2014; Stangoulis *et al.*, 2001). Boron (B) and Calcium (Ca) are the two most important elements for supporting plant structural integrity and function of plasma membranes. Boron nutrition is vital for obtaining high quality yields in vegetables (Bariya *et al.*, 2014; Mekki, 2015). Though total yield may not be affected, adequate quality for processing or fresh marketing of vegetables may not be obtained if available soil is deficient in boron (Mack, 1959; Oneida, 1993). A balanced supply of essential nutrients is one of the most important factors in increasing crop yields. The original leafy vegetables are recognized as sources of several nutrients, vitamins, antioxidants, minerals and important proteins (Odhav *et al.*, 2007).

Usually, the most consumed exotic leaf vegetables in southern Africa comprise various types of kale (also known as Chou Moellier), for example, various *Cruciferae* (rape, chou mollier, turnips, kale, Swedes, and radish rape). No report on the nutrient requirements of the *Brassica olerecea* var. *acelapha* was found in the literature. Experimental work will be carried out in a bid to test the response of boron in *Brassica olereacea* var *acephala* chlorophyll content, leaf length, leaf nutrient composition, leaf count, and plant height.

## 1.3 Research Questions

- What is the effect of boron treatment and availability on leaf nutrient and chlorophyll content of *B. oleracea* var. *acephala*?
- What is the influence of boron treatment on the growth parameters (leaf length, leaf count, and plant height) of *B. oleracea* var. *acephala*?

## 1.4 Objectives of the Study

### 1.4.1 Main Objective

The main objective of this study was to:

- Evaluate the extent to which boric acid concentration can affect leaf nutrient and chlorophyll content as well as growth parameters (leaf length, leaf count, and plant height) of *Brassica olereacea* var. *acephala*.

### 1.4.2 Specific Objectives

The specific objectives of this study were to:

- Evaluate the influence of boron concentration on the growth parameters of *Brassica oleracea* var. *acephala,* and
- Assess the effect of boron concentration on the nutrient composition of *Brassica oleracea* var. *acephala*.

## 1.5 Outline of the Thesis

The thesis is subdivided into the following chapters:

| | |
|---|---|
| **Chapter 1** | The introduction provides the background of boron application and also *Brassica oleracea* var. *acephala*. It also presents the problem statement, hypothesis, research objectives and the significance of the study. |
| **Chapter 2** | The literature review provides information on the role of boron in plants, its interaction with other nutrients and the implications of boron deficiency and toxicity to the agricultural industry. |
| **Chapter 3** | The research methodology summarizes the materials and methods used in this study to. It involves growing *Brassica olereacea* var. *acephala* in a greenhouse at various boron treatment levels, and their effects on the growth parameters and nutrient content of the plant. |
| **Chapter 4** | Entails an analysis and discussion the results. |
| **Chapter 5** | Provides the overall conclusion and recommendations of the study. |

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Role and Availability of Boron

Boron is classified as an essential micronutrient, required by vascular plants for optimum growth and for completion of the lifecycle (Oneida, 1993). Boron is involved in nucleic acid metabolism, carbohydrate biosynthesis, synthesis of plant hormones, cell division and elongation, and membrane function and structure (Marschner, 2012). In recent studies, it has been shown that B deficiency leads to a decline in concentrations of other macronutrients, such as ribose nucleic acid (RNA), magnesium (Mg), calcium (Ca) and potassium (K) and root nitrate content (Bolaños *et al.*, 2004; Camacho-Cristóbal *et al.*, 2008).

Boron availability is affected by increase in soil pH due to increase in adsorption of the element on soil particles (Lehto, 1995). Boron absorption by soils is typically at a pH range of 3 to 9 (Barrow, 1989), it has been reported that its absorption reduce in the range of pH 10 to 11 (Goldberg and Glaubig, 1986). In general, plant B absorption or B obtainability by the root is decreased with an increase in soil pH (Hu and Brown, 1997; Hu *et al.*, 1997).

Boron concentrations in soil vary from 2 to 200 mg/kg, but generally less than 5-10% of this is available for utilization by plants (Diana and Beni, 2006). The availability and utilization of B is affected by several factors, including parent material, texture, and nature of clay minerals, pH, liming, organic matter content, sources of irrigation, and its relationship with other elements (Lehto, 1995). Environmental conditions like moderate to heavy rainfall, dry weather and light intensity are also reported to affect B utilization by crops (Moraghan and Mascagni, 1991).

## 2.2 Interactions of Boron with Other Nutrients

Some functions of B interrelate with those of nitrogen (N), phosphorus (P), potassium (K) and calcium (Ca) in plants (Hosmane, 2011). Its interaction (synergistic, antagonistic) with most other essential nutrients (N, P, K, Ca, Mg, Al, and Zn) may occasionally influence its availability to plants in the soil. Application of B may improve the utilization of applied N in cotton plants by increasing the translocation of N compounds into the boll (Miley *et al.*, 1969). It has been reported that B uptake by barley (*Hordeum vulgare* L.) was lower when Zn was applied compared to in its absence (Graham *et al.*, 1987).

Furthermore, these authors also showed that rate of B accumulation in plants is increased even at low levels of Zn and high levels of P. Therefore, Zn fertilization may reduce B accumulation, and lessen the risk of toxicity in plants (Ahmed *et al.*, 2008). A significant relationship has been found between K and B fertilizers regarding their assimilation/uptake by crop plants as well as crop produce (Hill and Morrill, 1975). At heavy applications of K and other intensive production practices B may need to be applied to prevent reduction in corn yield (Woodruf *et al.*, 1987).

## 2.3 Sources, Rates, Methods and Timing of Boron Application

There are eight different sources of B: borax ($Na_2B_4O_7 \cdot 10H_2O$ with 11% B), solubor -$Na_2B_8O_{13} \cdot 4H_2O$ (20% B), sodium borate ($Na_2B_4O_7 \cdot 5H_2O$ with 20%

B), sodium tetraborate $Na_2B_4O_2 \cdot 5H_2O$ with 14% B), boric acid ($H_3BO_3$ with 17% B), colemanite ($Ca_2B_6O_{11} \cdot 5H_2O$ with 10% B), B frits containing 2-6% B, and boronated superphosphate being used to prevent/correct B deficiency in crops (Smith and McBroom, 1992; Woods, 1994). Borax, solubor, sodium borate and sodium tetraborate have been most commonly used for soil application.

Boric acid, colemanite and B frits are considered to be more promising on highly leached sandy soils as well as for long duration field crops including perennial forages and fruit plants owing to their low solubility and slow release of B. Boronated superphosphate has also been tried to correct B deficiency in crops (Patil *et al.*, 1987). Among these B fertilizer sources, borax is the most commonly used B fertilizer to prevent and/or correct B deficiencies in crops. Because of the narrow margin between B sufficiency and toxicity (Camacho-Cristóbal *et al.*, 2008), an excess dose can easily occur and harm plant growth (Cakmak *et al.*, 1995; Gupta, 1972; Gupta *et al.*, 1985).

## 2.4 Vegetable Production

Leafy vegetables play an important part in the tradition and food culture of African households and some are also used for medicinal purposes (Adedokun *et al.*, 2016; Mensah *et al.*, 2008; Mingochi and Luchen, 1997). Traditional vegetables offer diversity in family diets and help to ensure household food security (Mingochi *et al.*, 1995). Indigenous leafy vegetables are known as sources of many nutrients, vitamins, antioxidants, minerals and important proteins (Odhav *et al.*, 2007). The most commonly consumed exotic leaf vegetables in southern Africa include various types of kale (for example, rape and choumollier) and Swiss chard (*Beta vulgaris* var. *cicla*), which is often wrongly referred to as spinach (*Spinacia oleracea*), as well as various types of cabbage (*Brassica oleracea* var. *capitata*).

There is also one traditional vegetable, *Brassica oleracea* var. *acephala* (leaf cabbage) that is now widely grown in both commercial and household scales in the southern African sub-region (Figure 2.1). This kale has several local names, for example, ‘rugare’ (comfort) or ‘covo’ in Zimbabwe, ‘muRhodesia’ in the northern part of South Africa and ‘sukuma wiki’(push out the week) in eastern Africa.

The Vavhenda farmers of Limpopo province refer to *B. oleracea* var. *acephala* as ‘muRhodesia’ implying that the lines of the vegetable found in their area were introduced from Zimbabwe (previously Rhodesia). (*B. olereacea* var *Acephala* is also called ‘walking stick cabbage’ due to the tall woody stalk it produces as it grows upwards for many months.

The vegetatively propagated types of leaf cabbage commonly grown in Zimbabwe were previously described (Mariga *et al.*, 2012; Mvere and van der Werff, 2004) as rugare and viscose. Rugare is vegetatively propagated, rarely by seed, except only at high altitudes, the plants are 2 to 3 m tall, offers repeated leaf pickings, has a long life, and has pale blue-green curly leaves, although clones with different leaf colours exist.

Choumollier (narrow-stem kale) (*Brassica oleracea* L.) has been progressively used in recent years as a supplementary forage harvest in many countries with a temperate climate (U.K. Minist. Agric. 1963, (Emarald, 1963)). In New Zealand, the increase has been from 8,000 acres in 1933 to 130,000 acres in 1963, as shown in the New Zealand Department of Agriculture Farm Production Statistics for 1962-63.

**Figure 2.1:** *Brassica olerecea* var. *acephala*

## 2.5 Brassicas in Africa

A wide diversity of brassica crops are grown in Africa, although kales and cabbages are usually the most significant crops in terms of quantity of production. Precise data are not easy to come by, except where focused studies have been undertaken, for example in Kenya and Ghana. In Kenya the estimated annual production of brassicas is 550,000 tons, with 95% of the production in the highlands on 35,000 ha (The Food and Agriculture Organization/FAO, 2007; http://www.fao.org/ faostat/en/#data). In East Africa as a whole, about 90% of the brassica production is by smallholder growers on plots of 0.1–0.5 ha and this may also apply to other areas of sub-Saharan Africa, although in South Africa larger scale commercial production is important too (Grzywacz *et al.*, 2010; Kfir, 2004).

## 2.6 Boron – An Essential Micronutrient for Plants

### 2.6.1 Physical and Chemical Properties of Boron

Boron is a member of the metalloid group of elements and has intermediate properties between metals and non-metals. The boron atom has only three valence electrons and it usually becomes oxidized in chemical reactions. In nature, boron exists as two types of stable isotopes, of which 80% is B11 and 20% is B10. The B10 isotope is good at capturing thermal neutrons from radiation. Boron is a relatively rare element widely distributed in the earth's crust, but only representing about 0.001% of the total composition (Smith *et al.*, 1995). It occurs in a variety of natural ores related to borax (sodium tetra borate, $Na_2B_4O_7 \cdot 10H_2O$).

Another common boron compound is boric acid ($H_3BO_3$), which is a very weak Lewis acid (pKa 9.24) (Woods, 1996). In normal animal and plant cytoplasm (pH 7.5), more than 98% of B is found in the form of free $B(OH)_3$, and a small amount of borate anion $B(OH)_4$ (Brown *et al.*, 2002; Woods, 1996). The equilibrium between these two forms in aqueous solution is (Hu and Brown, 1997):

$$\mathbf{B(OH)_3 + 2H_2O = B(OH)_4 - + H_3O+ \ (pKa\ 9.25)}$$

### 2.6.2 Chemistry of Boron in Nature

In nature, boron is found in the form of boric acid (Figure 2.2) borate (i.e., a salt of boric acid), or as a borosilicate mineral (Holleman-Wiberg, 2001). Boric acid, $H_3BO_3$ (or $B(OH)_3$), behaves as a weak Lewis acid in aqueous solution (Power and Woods, 1997). It accepts hydroxide ion from water and releases a proton into solution according to the following equilibrium equation ($K_a$ = 5.8x10-10; $pK_a$ = 9.24 @ 25 °C) (Dean, 1987):

$$B(OH)_3 + H_2O \Leftrightarrow B(OH)_4^- + H^+$$

Boric acid dissociation as a function of pH; above pH 9.24 the anion, $B(OH)_4^-$, is predominant, while below pH 9.24 the uncharged species is predominant. Boric acid is soluble in water (5.5 g/100 g solution at 25 °C) and its solubility increases with temperature (Waggott, 1969). At concentrations below 0.02 M (216 mg/l as B) only the mononuclear species $B(OH)_3$ and $B(OH)_4$ - are present. Polynuclear ions or ringed structures can exist at higher concentrations but these will not be discussed here due to their perceived rarity in nature (Power and Woods, 1997).

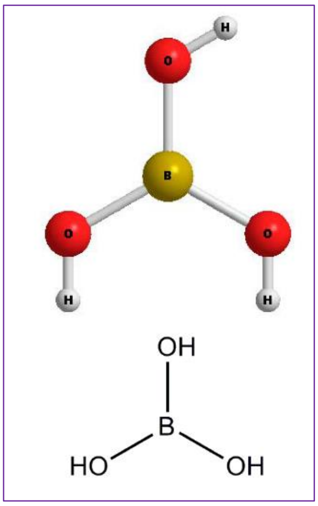


**Figure 2.2:** Molecular structure of boric acid

## 2.7 Industrial Uses of Boron

Boron has many uses worldwide. The principal industrial uses of boron compounds are in the production of fibreglass insulation, borosilicate glass, and detergents. Other uses include fertilizers, metallurgy, and nuclear shielding (Power and Woods, 1997). Boron is used in the fibreglass and glass industries because of its mechanical qualities. Boron oxide lowers the expansion coefficient in glass and therefore borosilicate glass has an increased shock resistance. Boron also gives glass increased mechanical strength and increased drawing quality that is especially useful in the manufacture of fibreglass (Woods, 1994).

Boron is used in the manufacture of detergents and bleaches as well. Boron in the form of sodium perborates is added to detergents as a bleaching agent. The hydrolysis of sodium per borate forms the hydro peroxide ion. This is only effective at temperatures above 60°C unless an activator is present. It also has been additional to diaper pails and to animal litter to decrease odour since it stops the development of ammonia by inhibiting the urease enzyme (Woods, 1994).

Boron has varied uses as a result of its nuclear possessions. Essential boron is used in alloys for nuclear reactor control rods because of its big neutron capture cross section (Waggott, 1969). This same property has led the medical establishment to use 10B in a procedure known as boron neutron capture therapy in the treatment of cancer patients (Hawthorne, 1993).

A recent innovation utilizing boron is the fuel cell as patented by Millennium Cell, Inc. Sodium borohydride is dissolved in water and passed over a catalyst producing liquid borax and hydrogen gas. The hydrogen can then be used in a fuel cell where it is converted into electricity and water. The borohydride fuel cell's advantage is that hydrogen is produced and there is no need to store it as is the case with other fuel cells. An obvious drawback is the need to recycle the borax back into its borohydride form (ABC News, 2001). Currently, Rohm and Haas® is the only producer of sodium borohydride in the United States. A fairly complex reaction sequence is utilized in borohydride production, including converting boric acid to trimethylborate, converting sodium to sodium hydride, then responding sodium hydride to make sodium borohydride (Mannsville Chemical Products Corp., 1999). Still, some designers trust this might be a feasible technology in the next decade (Parks and Edwards, 2005).

## 2.8 Boron Availability in Agricultural Lands

Although boron is a naturally occurring component in soil originating from boron-containing minerals in the earth's crust, its attentiveness in agricultural lands can vary significantly. The total boron level (soluble and insoluble) in soil can remain categorized as low-boron (less than 10 mg/kg soil) or high-boron (of the order of 100 mg/kg). The usual overall attentiveness of boron in all soil ranges from 10 to 20 mg/kg and in the oceans it is found at a concentration of about 4.5 mg/l (www.borax.com/borates1d.html) (Vengosh *et al.*, 1991).

Boron enters the agricultural environment mainly through natural processes from boron-containing rocks through weathering, seawater, boric acid vapor, volcanic action, and other geothermal releases (Green Facts, 2004; http://about.greenfacts.org/publications.htm). Turkey and California are the world's large commercial suppliers of boron. Turkey alone has nearly 63% of the world's boron potential and boron reserves (Kar *et al.*, 2006).

Soil B content has frequently been used to assess the B supplying capacity of soils for particular crops, but there are only a few regions in the world where B deficiency has been mapped (Shorrocks, 1997). Influences affecting B obtainability and extent of B adsorption in soils is: soil parent material, texture, pH, moisture, temperature, clay and climate. Soil parent material and soil texture are considered to be the dominant factors. Early examination of soils with low boron absorptions (0.5-0.6 mg/l) in mapped areas has been reported by the FAO/UNESCO (Sillanpää, 1982). Soil groups that were studied comprise: strongly weathered soils (Acrisols, Podzols, Ferralsols); coarse textured soils (Arenosols); shallow soils (Lithosols); thin soils covering calcareous material (Rendzinas); and volcanic ash soils (Andosols) (Sillanpää, 1982).

Soil texture is dependent on parent material and on the illuviation of clay from the top soil. Coarse-textured soils contain less available B so that B 9 deficiency occurs more frequently than on fine-textured soils. Boron deficiency is more likely to occur in plants growing on sandy soils because B, being mobile in the soil, is more likely to leach out of these soils (Fleming, 1980; http://agris.fao.org/agris-search/search.do?recordID=US201301302 283).

The amount of absorbed B is also dependent on soil texture, and increased with increasing clay content (Elrashidi and O'Connor, 1982; Goldberg, 1997; Nicholaichuk *et al.*, 1988). Boron availability generally decreases in dry soils because of the need for plants to absorb boron in a soluble form (Fleming, 1980; http://agris.fao.org/agris-search/search.do?recordID=US 201301302283). Climate is the most important factor affecting soil moisture and temperature both in the short and long term. Heavy rainfall can exacerbate B deficiency, because not only is soluble B easily leached out of the soil, but residual effects of B application are also minimized. In contrast, B deficiency symptoms on many crops also happen in hot and dry weather due to topsoil drying (Shorrocks, 1997). In the USA, three areas have traditionally been B deficient, namely the states along the Atlantic coast and the Gulf of Mexico, the Pacific Northwest, and the Great Lakes region.

Strongly weathered soils (Podzols) are found in the Great Lakes area (Shorrocks, 1997).

## 2.9 Boron Deficiency and Toxicity in Plants

Boron is essential for plants, but the range between deficient and toxic concentration is smaller than for any other nutrient element (Goldberg, 1997; Reid, 2013; Reid *et al.*, 2004). Of all micronutrient deficiencies in plants, boron deficiency is the most widespread and has been reported in the arena for a minimum 132 crops in 80 countries (Shorrocks, 1997). Boron deficiency in crops is a problem in horticultural settings due to low B concentration and alkalinity in the water sources (Krug *et al.*, 2009). However, there is a great variety of effects of low B on reproductive development among species.

The *Gramineae* appear to have the lowermost B requirement among the monocotyledons, although *Crucifers* and *Chenopodiaceous* are distinguished with respect to sensitivity to B deficiency among the cotyledons (Shorrocks, 1997). There are two main physiological responses in advanced plants to B deficiency, that affecting vegetative development and those affecting reproductive growth. In vegetative tissues, B deficiency inhibits root elongation through limiting cell enlargement and cell division in the root tips (Dell and Huang, 1997). In trees, B has been proposed to play a possible part with auxin in the biosynthesis of lignin and differentiation of xylem (Lewis, 1980), boron deficiency causes irregular growth of xylem and phloem cells. B-deficiency symptoms in the shoot appear at the shoot apex and the rising leaves, resulting in small leaves and that are dark-green colour (Dell and Huang, 1997). Sunflower is reported to be one of the most delicate crops to low B supply, and the deficiency symptoms first appear on the earlier leaves, which develop a bronze colour and become hardened, malformed, and necrotic (Asad *et al.*, 2003; Blamey *et al.*, 1987).

In contrast, growth of reproductive tissue appears to be more subtle to low B than vegetative growth in crops. Symptoms of B deficiency in reproductive growth may comprise: abortion of flower buds or flowers (Bell *et al.*, 1990); sterility of male flowers by damage of microgenesis and pollen tube growth (Dell and Huang, 1997; Rerkasem and Loneragan, 1994). In the case of wheat, floret sterility promoted by deficiency is mainly caused by male sterility (Huang *et al.*, 2000). Furthermore, boron deficiency reduces seed set or seed size (Dear and Lipsett, 1987), and malformed fruits (Dell and Huang, 1997).

Like boron deficiency, boron toxicity is also an important disorder that can limit plant growth worldwide (Ahmed *et al.*, 2008; Camacho-Cristóbal *et al.*, 2008; Gupta *et al.*, 1985; Reid, 2013; Reid *et al.*, 2004). The most common boron toxicity symptoms are leaf burn – chlorate and/or necrotic patches, often at the margins and tips of older leaves (Nable *et al.*, 1997).

However, in other species in which B is phloem mobile (e.g., *Prunus, Malus, Pyrus*), the evident symptoms of toxicity are fruit disorders, bark necrosis caused by the death of the cambial tissues, and stem die-back (Brown and Hu, 1996). Visible indications of B toxicity do not appear to grow in roots, because B concentrations in the roots remain relatively low compared to that in leaves (Nable, 1988).

## 2.10 Function of Boron in Plants

Several functions have been delineated for the biochemical and physiological roles of B in plants (Tariq and Mott, 2007). A brief mention of the evidence for each of these known and proposed functions is provided here. The biochemical roles of boron in plant cell wall structure include:

- Boron has an important cross-linking role in plant cell wall structure and purposes; and

- As a stabilizer of the cell-wall pectin network (Matoh, 1997). Over the years, researchers have observed that up to 90% of the cellular boron is localized in the cell wall fraction (Loomis and Durst, 1992).

## 2.11 Role and Availability of Boron

Boron (B) is classified as an essential micronutrient, required by vascular plants for optimum growth and for completion of the lifecycle. Although the exact role of boron in plant nutrition is not well understood, it is essential in many physiological processes (Pilbeam and Kirkby, 1983; Tariq and Mott, 2007). Boron is involved in nucleic acid metabolism, carbohydrate biosynthesis, synthesis of plant hormones, cell division and elongation, and membrane function and structure (Gupta, 1980; Marschner, 2012; Mengel and Kirkby, 1987; Pilbeam and Kirkby, 1983).

Pollen production, viability, germination and pollen tube growth are also affected by boron supply (Marschner, 2012; Mengel and Kirkby, 1987). In determining salt and boron tolerance of many crops and ornamental species, Maas (Maas, 1986) devised a boron tolerance rating system based on soil water boron threshold levels that did not reduce yields. Maas' ratings and threshold levels are: very sensitive (<0.5 mg/kg), sensitive (0.5-1.0 mg/kg), moderately sensitive (1.0-2.0 mg/kg), moderately tolerant (2.0-4.0 mg/kg), tolerant (4.0-6.0mg/kg), and very tolerant (6.0-15.0 mg/kg). Many factors influence the relative boron deficiency or toxicity to a crop including soil type, irrigation practices, fertilizer application, solubility of boron fertilizers, climate and crop varieties (Page and Paden, 1950).

Root uptake of boron from the soil solution is primarily a passive process associated with mass flow of water and transpiration rate. Translocation of B through the xylem is predicated by transpiration rate (Mengel and Kirkby, 1987). Other workers observed that the uptake of B by roots of table beets was directly related to the transpiration rate, but that xylem transport of B was independent of transpiration rate, but dependent on dry weight accumulation (Halbrooks *et al.*, 1986).

Boron is considered to be phloem immobile, but translocation of B from older leaves to developing sinks may occur under conditions of B starvation. In studies on rutabaga, radish and cauliflower (Brown and Hu, 1996; Brown and Shelp, 1997; Shelp *et al.*, 1987), the authors found that redistribution of B from older leaves occurred under conditions of B deficiency. Other studies demonstrated B export from deciduous tree fruit leaves after foliar application of B (Hanson, 1991).

Boron is the most widely deficient micronutrient, with deficiencies reported in 43 states in the USA (Sparr, 1970). Boron is present in most soils at 20-200 mg/kg, most of which is unavailable to plants (Mengel and Kirkby, 1987; Tisdale and Nelson, 1975). According to another report, plant available boron, measured by hot water extraction, ranged from 0.38-4.67 4 mg/kg

(Gupta, 1980). In agricultural soils ($pH<8$) soil solution boron exists as boric acid ($H_3B0_3$). Boric acid is easily leached from the soil (Marschner, 2012; Mengel and Kirkby, 1987; Tisdale and Nelson, 1975). Boron is present in many minerals, of which tourmaline (3-4% B) is the most important (Berger, 1949; Mengel and Kirkby, 1987).

Tourmaline is resistant to weathering, so replacement of soil B from this mineral might not supply adequate B to cropping systems with heavy boron demand (Berger, 1949; Tisdale and Nelson, 1975). Other factors that affect boron availability are soil organic matter, soil texture, soil pH, and soil moisture. Most of the obtainable boron in acid soils is held by the organic matter (Berger, 1949; Tisdale and Nelson, 1975) and is released by microbial activity (Gupta, 1980).

It was found that in three related sandy Newberg soils, the soil with the higher organic matter content (4%) retained more of the applied boron than the soils with 0.9% and 1.1% organic matter (Parker and Gardner, 1982). Fine textured soils adsorb more boron than course textured soils. The amount and the type of clay mineral affect boron adsorption (Keren *et al.*, 1985). Soil pH and moisture influence boron availability. Boron uptake by plants exhibitions a negative relationship above pH 6.5 (Keren *et al.*, 1985).

As the pH increases, the anion $B(OH)_4$ is formed and is adsorbed onto clay 5 minerals. Repeated wetting and drying cycles increase the amount of boron fixed to clay particles. The degree of obsession is often greater in limed than in unlimed soils (Berger, 1949; Keren *et al.*, 1985). Soil moisture alters the accessibility of boron. In very wet, acidic, highly leached conditions, boron deficiencies may happen. Below drought conditions, boron fixation may increase, and organic matter decomposition may slow, thereby reducing plant available boron in the surface soil. This drought decrease the available B by a split root system on tomatoes (Hobbs and Bertramson, 1950). Half of the root system was placed in dry topsoil that contained adequate boron, the other half in a moist subsoil with actual low levels of boron. As long as the highest soil remained dry, the plants did not find adequate boron from the subsoil. In dry conditions, reduced boron obtainability and delivery, and low boron levels in the moist subsurface horizons can create plant of boron deficiency in soils that contain adequate levels of extractable boron.

## 2.12 Boron in Vegetable Production

Boron nutrition is energetic for obtaining high quality yields in vegetables. Though total yield may not be affected, adequate quality for processing or fresh marketing of vegetables may not be obtained if available soil boron is inadequate (Mack, 1959). Heart rot of sugar beet, black heart in turnips, cracked stem in celery, brown rot in cauliflower and canker in red beets have all been characterized as diseases or disorders that are now known to result from boron deficiency (Atwater, 1941). Boron has a narrow concentration range between deficiency and toxicity.

Boron deficiency is first expressed as abnormal or retarded growth in the apical tissues. If the deficiency persists, the terminal growing point dies, growth is reduced and the plant will take on a bushy, resettled appearance. Young leaves are misshapen and internodes are shortened. Boron toxicity is expressed in leaves as marginal and tip chlorosis with subsequent necrosis (Gupta, 1980; Marschner, 2012; Maynard, 1979; Mengel and Kirkby, 1987). Boron toxicity is generally due to high concentrations of boron in the water supply, use of municipal compost high in boron, or misapplication of boron-containing fertilizers (Gupta, 1980; Maynard, 1979). Vegetable species and even strains differ widely in their requirements for, and their tolerance to boron. Eaton grew 50 species of plants in sand culture and rated them as boron sensitive, semi-tolerant, and tolerant (Eaton, 1944). His ratings were based on estimates of leaf injury as few crops were grown to maturity.

Recent studies indicated that Eaton's rankings may not be indicative of boron tolerance of a species when grown to horticultural maturity (Francois, 1984; 1986; 1988; 1991). Eaton reported the lowest boron attentiveness in soil solution for injury on tomato, radish, celery, lettuce, and onion to be 5, 10, 25, 1 and 1 mg/kg, respectively. All of these vegetables were rated as boron semi-tolerant.

Francois found that tomato fruit weights were not significantly affected by up to 10 mg/kg boron in the soil solution. Radish leaf necrosis was not observed with boron solution concentrations of 16 mg/kg. In celery, Francois reported a yield decrease when boron concentration exceeded 10 mg/kg, but at no time did the plant leaves exhibit boron toxicity symptoms. Southport White Globe onions showed no significant difference in yield components at B concentrations up to 5 mg/kg in soil

Gupta reported boron toxicity symptoms of reduced growth and marginal leaf burn that occurred at 2 and 4 mg/kg soil-added boron and tissue boron levels of 125 and 232 mg/kg, respectively (Gupta *et al.*, 1985). Boron 2013 deficiency symptoms such as yellowing of the tops slow flowering and pod formation, occurred under conditions of soil added boron and boron tissue levels less than 12 mg/kg. Deficiency symptoms reported in beans grown in solution culture include root and top dwarfing, a darkening of the leaves and spot necrosis of the oldest leaves (Teare, 1974). The bean roots exhibited stunting within three days of transplanting to the boron deficient nutrient solution. In other work, beans grown under boron deficiency develop thickened and brittle roots with necrotic tips (Odhnoff, 1961). The extensive use of boron fertilizers has led to concerns over residual soil boron and the effect on subsequent cropping of boron sensitive species.

In other studies, bean yields and plant dry weights were not adversely affected until available soil boron concentration exceeded 2.30 mg/kg from a fall application of 17.9 kg/ha (Mack, 1959). Spring applications of boron above 2.2 kg/ha decreased yields and reduced plant dry weights.

These results agree with work on applied and residual boron on beans (Gupta and Cutcliffe, 1984). Application rates of up to 8.8 kg B/ha the year prior to planting beans did not have a damaging effect on yield. Cucumbers (*Cucumissati vus*) are considered moderately sensitive.

## 2.13 Boron in Food

Food is the main source of boron that is ingested by humans. Studies demonstrate that the regular adult in the United States consumes about 1 mg of boron per day in their diet (Meacham and Hunt, 1998). The richest bases of boron comprise fruits and nuts. Wine is also a major contributor. Typical values of boron content in foods have been documented for various countries (Rainey and Nyquist, 1998).

Importantly, boron consumption is determined by the individual's nutritional habits in addition to the boron content in drinking water and can therefore vary extensively.

## 2.14 Health Effects of Boron

This part describes the effects boron has on living organisms in the environment. This comprises plants, microorganisms, animals, and humans.

### 2.14.1 Plants

There is a small variety amongst boron deficiency and boron toxicity in plants. Boron has been shown to play a part in carbohydrate metabolism, sugar translocation, pollen growth, hormone action, normal growth and functioning of the apical meristem, nucleic acid synthesis, membrane structure and function (Howe, 1998).

Indications of boron deficiency comprise discontinuance of root and leaf growth, bark splitting, retardation of enzyme reactions, reduced pollen germination, and even death (Tombuloglu *et al.*, 2015). The first stages of boron toxicity in plants contain yellowing of leaf tips progressing into the leaf blade. Death of chlorotic tissue is followed by leaf damage, eventually resulting in a loss of photosynthetic capacity and a decline in plant production (Lovatt and Dugger, 1984).

Boron deficiency can occur in heavy-textured soils with high pH as under these situations boron is readily adsorbed (Howe, 1998). Boron toxicity might also happen in boron-rich soils or in soils exposed to boron-contaminated irrigation waters or excessive application of boron-rich fertilizers, sewage sludge, and fly ashes (Nable *et al.*, 1997). Some plants are extra sensitive to boron. Sensitive plants can tolerate irrigation waters with only 0.3 mg/l boron while very tolerant plants may be able to survive where 4 mg/l boron irrigation water is used (Keren *et al.*, 1985).

### 2.14.2 Microorganisms

Toxicity thresholds (TTs) were established for various microorganisms in a previous study (Bringmann and Kühn, 1980). The authors found that the bacteria, *Pseudomonas putida*, had a TT of 290 mg/l boron. Toxicity threshold was defined as the concentration at which the inhibitory action of a chemical leads to a >3% difference in the quantity of organisms versus a control group. The green alga, *Scendesmus quadricauda*, had a TT of 0.16 mg/l, while the protozoan, *Entosiphon sulcatum*, had a TT of 0.28 mg/l. Activated sewage treatment was affected by a boron concentration of 20 mg/l (Howe, 1998). On the other hand, another study observed an important decrease in chemical oxygen demand (COD) elimination at concentrations greater than 10 mg/l on aerobic activated sludge biological treatment (Banerji *et al.*, 1968).

### 2.14.3 Animals

Boron is nutritionally significant to animals. Boron has been originate to enhance the maturing of the development plate in the extended bones in chicks (Hunt, 1994). Boron has effects on brain activity in mature rats (Penland and Eberhardt, 1993). In pests, a lack of B also reduced the absorption of calcium, magnesium, and phosphorus (Hegsted *et al.*, 1991). Another study substantiated this by discovering that B supplementation to boron-deficient chicks augmented femur calcium, phosphorus, and magnesium concentrations (Hunt, 1994).

A study on rabbits indicated boron is not able to penetrate intact skin, but is readily absorbed through broken skin (Draize and Kelley, 1959). Several studies have been completed on rats, mice, rabbits, ducks, and dogs (Fail *et al.*, 1998; Fail *et al.*, 1991; Ku *et al.*, 1991; Price *et al.*, 1996a; Price *et al.*, 1996b; Seal and Weeth, 1980; Smith and Anders, 1989) and have been summarized extensively (Moore, 1997). Moore concluded that a no-observed-adverse-effect-level (NOAEL) of 9.6 mg B/kg body wt/day was appropriate based on developmental toxicity in rats, the most sensitive organism in the studies reviewed. Data indicated NOAELs for female and male generative toxicity were 24 and 17 mg/kg body wt./day, respectively (Moore, 1997).

### 2.14.4 Humans

Boron may be an essential element for higher animals, including humans. Boric acid and borax are entirely absorbed by the oral route of exposure. Absorption through intact skin is deemed insignificant, although absorption can occur through damaged skin. Boron levels in the body are rapidly eliminated upon cessation of exposure. Humans may be exposed to B through three primary sources: 1) consumption of private, municipal, or commercial (bottled) sources of drinking water; 2) dietary consumption of crops and other foodstuffs (including dietary supplements for body building); and 3) inhalation of boron compounds during their mining, manufacturing, and other industrial processing. While boron has been detected in 81.8% of the municipal water systems, it is a minor source of boron in most parts of the U.S. (Moore, 1997).

WHO established an acceptable safe range of population mean intakes for boron of 1-13 mg/day (IPCS, 1998; WHO, 1996). However, B has not been accepted to be an essential component in the human diet since a specific biochemical role for it has not been established (Nielsen, 1997). However, there is strong incidental evidence that this can be the case (Nielsen, 1996; Parks and Edwards, 2005). Boron is significant in the metabolism and utilization of calcium in humans (Nielsen, 1997). Other benefits of boron include development of brain function, psychomotor response, and the response to oestrogen ingestion in postmenopausal women (Nielsen, 1994). There is evidence that boron plays a role in healthy bones and joints (Newnham, 1994).

Boron has recently been studied for its potential as a synergistic agent with several anticancer drugs to treat various malignancies, including endometrial cancer (Tuluce *et al.*, 2017), head and neck cancer (Gonzalez *et al.*, 2017), prostate cancer (Li *et al.*, 2017), colon cancer (Trivillin *et al.*, 2017), hepatic cancer (Yanagie *et al.*, 2017), non-small cell lung cancer (Yu *et al.*, 2017), clinical trials on radiation-induced dermatitis in breast cancer (Aysan *et al.*, 2017), and as nanocarriers for antitumour drugs (Emanet *et al.*, 2017). Current advances on the effects of B on various aspects of human health have been amply documented and will not be detailed here.

# CHAPTER 3

# METHODOLOGY

## 3.1 Plant Material

Seeds for this experiment were collected from the department of Horticulture, Cape Peninsula University of Technology (CPUT). Seedlings were raised to maturity (minimum of 4 leaves and average length of 7 cm), acclimatized and transplanted to pots after 2 weeks in the glasshouse.

Top soil samples were collected from existing commercial farmer's, filed, sieved and potted in 25-cm pots. Thirty pots were used for the experiment. The pH was maintained at an average of 6.5. Each 25-cm pot was subjected to different treatments, with six replicates per treatment. The chemical (boric acid) was obtained from Unilab®.

## 3.2 Greenhouse Experiment

The experiments were conducted in the greenhouse of the Department of Horticultural Sciences, Cape Peninsula University of Technology, Cape Town Campus. Inside, the greenhouse was equipped with a 40% Alunet shade cloth suspended 2 m above ground. Steel tables (2.5 x 1 m) were used as flat surfaces for plastic gutters (2 x 0.15 m), and 30 brown plastic gutters (2 x 0.15 m) were placed on two steel tables (Figure 3.1).

## 3.3 Nutrient Preparation

Analytical grade chemicals were purchased from Merck (Pty) Ltd (South Africa); boric acid [$H_3BO_3$] instead of boron.

## 3.4 Treatments

Treatments comprised of five concentrations of boron and the control. Each experimental unit was a pot of one plant replicated 6 times making a total of 30 experimental units, including the control.

**Figure 3.1:** Preparation of potting soil

Prior to treatment of the soil with boric acid, soil was analyzed to show a baseline of boron level which then was adjusted according to the level of each treatment with boric acid. Various ranges of toxicity of boron levels (T5=Control; T4=0.3 mg/kg, T3=0.4 mg/kg, T2=0.5 mg/kg and T1=0.6 mg/kg) were used. In the control treatment (T5), distilled water was used to irrigate the plants. The following were determined: soil and leaf mineral composition, leaf chlorophyll content, leaf count, leaf length and plant height.

### 3.5 Plant and Soil Analysis

Prior to conducting the experiment, soil samples were taken for laboratory analysis for the following nutrients: boron (B), nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), zinc (Zn), pH, soil texture as well as base saturation. For the analysis, we harvested the whole plant using distilled water to wash the plant and used the oven to dry the plant overnight on filter paper at 60°C and analyzed. Dried plant materials were cut into smaller pieces and ground using a Jakel and Kunkel Model a 10 mill into fine powder. Powdered foliage material (5 g) was sent to BembLab for analysis of micronutrients.

### 3.6 Measurement of Chlorophyll Fluorescence

Chlorophyll (Chl) is found within living plants cells and is a key biochemical component in the molecular apparatus that is responsible for photosynthesis, the critical process in which the energy from sunlight is transduced to produce life-sustaining oxygen. In this study, Chl was determined by using a portable fluorimeter to measure discrete molecular chlorophyll fluorescence (Figure 3.2). The total leaf Chl content was monitored after 1, 2, 3, 4 and 5 weeks following the initial boron treatment regimens (T5=Control; 0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg and 0.6 mg/kg). Chlorophyll was extracted from leaf tissue in 95% alcohol. Leaf slices weighing about 0.2 g were placed in 25 ml of alcohol in 50 ml tubes protected from light overnight prior to fluorimetry. Four replicates per line per treatment were sampled for this test.

### 3.7 Microwave Plasma-Atomic Emission Spectrometry (MP-AES)

A Microwave Plasma-Atomic Emission Spectrometer (Figure 3.3) was used for sample analysis to determine the concentration of metals in the samples. The

MP-AES spectrometer was chosen for this study for its multi-element capabilities and it is less subjective to chemical and matrix interferences. The MP-AES, which was used for our analysis, has several advantages: Runs on air, eliminates need for source/hollow cathode lamps, and simple installation – no chiller. It has a higher resolution, it is more flexible in the number and the selection of lines is infinitely variable, automatic background correction and it is safer as no flammable gases are required.

**Figure 3.2:** Measurement of chlorophyll fluorescence

**Figure 3.3:** Milestone-MLS 1200 Mega microwave oven

## 3.8 Statistical Analysis

Growth parameter (leaf number, plant height, and leaf chlorophyll content) recordings were taken at weekly intervals (W1-W5) in the morning. Data were analysed using repeated measures ANOVA (Wilks, 1932) and Tukey's post-hoc test to compare the means at a level of significance, p<0.05. These analyses were performed using Statistica software (StatSoft, version 2015). Chlorophyll was measured using chlorophyll fluorescence. For measurement of the plant height, a normal ruler was used. Leaf counts were made weekly for a period of 5 weeks.

# CHAPTER 4

## RESULTS AND DISCUSSION

### 4.1 Introduction

In the early 1920s, B has been categorized as an essential element for normal plant growth and development (Warington, 1923) A steady supply of essential nutrients is one of the most critical considerations in increasing crop yields and sustaining human life (Osman, 2013). A steady supply of essential nutrients is one of the most critical considerations in increasing crop yields and sustaining human life (Osman, 2013). This chapter presents the data on the effects of treatment of the soil with increasing concentrations of boron (mg boric acid/kg soil) on *Brassica oleracea* var. *acephala* chlorophyll fluorescence, leaf count, leaf length, and plant height [values are means ± SEM (n=4)]. MANOVA (multivariate analysis of variance), applying the Wilks' Lambda test, and Tukey's multiple comparisons test were used to test the hypothesis that increasing concentrations of boron (independent variables; IVs) have an effect on chlorophyll fluorescence, leaf count, leaf length, and plant height (dependent variables; DVs) at various weekly intervals (W1 to W5). In addition, laboratory reports of mineral compositional analyses of *Brassica oleracea* var. *acephala* leaves and soil samples in which the plants were grown are tabulated and described.

### 4.2 *Brassica oleracea* var. *acephala* Soil and Base Analysis

Table 4.1 shows the laboratory analysis of the pottery soil used to cultivate *Brassica oleracea* var. *acephala*. Soil pH did not vary much among the various orchard blocks tested, regardless of soil depth. Soil resistance (resistivity) was high in the middle orchard blocks (2380 and 2310 Ω) and highest in the bottom orchard block (3130 Ω). Differences in soil type and seasonal changes in the soil's electrolyte content may account for these variations, but this aspect was not investigated.

The available phosphorus, using Bray II solution as extractant, was highest in the top block orchard (33 mg/kg), but the bottom block orchard exhibited the highest K levels (96 mg/kg) which correlated with a low phosphorus content (14 mg/kg) as compared to values for the other orchard blocks. In terms of exchangeable cations (cmol/kg), Na+ and K+ levels did not vary significantly, but $Ca^{++}$ and $Mg^{++}$ levels fluctuated considerably among orchards analyzed (Tables 4.1 and 4.2).

## 4.3 *Brassica oleracea* var. *acephala* Leaf Composition Analysis

Table 4.3 shows the compositional analysis of minerals in samples of the plant, for each treatment group (i.e., 0.6, 0.5, 0.4, 0.3 mg/kg boron, control). The nutritional profiles of 5 orchards labelled A, B, C D and F were analyzed. Comparison of the nitrogen (N) content indicates that the 0.6 and 0.5 mg/kg boron treatments (Orchards A and B) resulted in a decreased N content (2.43% and 2.02%, respectively) compared to the other treatments (Orchards C, D and F= control). Orchard F (Control) had a higher phosphorous (P) content (0.37%) than the other orchards. Ca, Mg, Cu and B levels did not vary significantly among the orchards, but Na (3047 mg/kg), Fe (878 mg/kg) and Zn (45 mg/kg) levels were markedly raised in Orchard D (Treatment with 0.3 mg/kg boron) relative to the Control Orchard. These results were confirmed in a separate similar laboratory analysis of the mineral contents of leaves samples from the respective orchards (Table 4.4).

## 4.4 Effects of Boron on *Brassica oleracea* var. *acephala* Chlorophyll Fluorescence

Figure 4.1 ad Table 4.5 show the chlorophyll fluorescence (ChlF) induced in *Brassica oleracea* var. *acephala* pottery samples in response to increasing doses of boron (0.3 to 0.6 mg/kg) and measured at various weekly time intervals (W1, W2, W3, W4, and W5) after initial soil treatment.

**Table 4.1:** *Brassica oleracea* var. *acephala* soil analysis

**Soil Analyses Report No. GR2488_a (*Supplement to Test Report No. GR002483.doc*)**

**Date received: 04/02/2014**

**Date tested: 06/02/2014**

| **Orchard** | **Lab No** | **Depth (cm)** | **Soil** | **pH (KCl)** | **Resist. (Ohm)** | **$H^+$ (cmol/kg)** | **Vol (%)** | **Stone (mg/kg)** | **P Bray II K** | **Exchangeable cations (cmol(+)/kg)** | | | | **C (%)** |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | **Na** | **K** | **Ca** | **Mg** | |
| **Top Block** | 2483 | 30 | Sand | 6.1 | 1910 | | 2 | 33 | 59 | 0.07 | 0.15 | 4.06 | 0.86 | 1.04 |
| **Top Block** | 2484 | 60 | Sand | 5.9 | 1810 | 0.35 | 1 | 8 | 68 | 0.06 | 0.18 | 2.73 | 0.70 | 0.61 |
| **Middle Block** | 2485 | 30 | Sand | 6.0 | 2380 | 0.30 | 1 | 20 | 62 | 0.05 | 0.16 | 3.88 | 0.92 | 1.08 |
| **Middle Block** | 2486 | 60 | Sand | 5.9 | 2310 | 0.40 | 1 | 4 | 54 | 0.06 | 0.14 | 2.79 | 0.79 | 0.63 |
| **Bottom Block** | 2487 | 30 | Sand | 5.9 | 3130 | 0.30 | 1 | 23 | 41 | 0.07 | 0.10 | 3.82 | 0.86 | 0.87 |
| **Bottom Block** | 2488 | 60 | Sand | 6.2 | 1860 | | 1 | 14 | 96 | 0.07 | 0.24 | 4.03 | 1.12 | 1.00 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Methods**[#] | 3108 | 3106 | 3109 | 3117 | 3113 | 3113 | 3113 | 3113 | 3107 |

If the pH>7.0, the Olsen method (3118) is used to determine P; # Refers to BemLab work instructions.

Cerificate of Analyses issued by BemLab (Report No. GR2483, www.bemlab.co.za)

**Table 4.2**: *Brassica oleracea* var. *acephala* soil base analysis

| **Base Saturation** | | | | | | |
|---|---|---|---|---|---|---|
| **Orchard No.** | **Lab No.** | **Na (%)** | **K (%)** | **Ca (%)** | **Mg (%)** | **T-Value (cmol/kg)** |
| **Top Block** | 2483 | 1.46 | 2.95 | 78.85 | 16.75 | 5.14 |
| **Top Block** | 2484 | 1.53 | 4.35 | 67.99 | 17.42 | 4.02 |
| **Middle Block** | 2485 | 1.02 | 3.00 | 73.1 | 17.27 | 5.31 |
| **Middle Block** | 2486 | 1.40 | 3.29 | 66.85 | 18.88 | 4.17 |
| **Bottom Block** | 2487 | 1.35 | 2.04 | 74.07 | 16.72 | 5.15 |
| **Bottom Block** | 2488 | 1.30 | 4.48 | 73.66 | 20.57 | 5.47 |
| Cerificate of Analyses issued by BemLab (Report No. GR2483, www.bemlab.co.za) | | | | | | |

According to the Multivariate Tests (Figure 4.1), the one-way MANOVA was statistically significant, i.e., a significance value of 0.000164 (which means $p=0.000164$) indicates that ChlF was significantly dependent on the treatment dose of boron as compared to control. Boron at a concentration of 0.6 mg/kg produced a significant increase in *Brassica oleracea* var. *acephala* ChlF for all the weeks (W1 to W5) analyzed. A similar trend in ChlF was observed for boron concentrations of 0.5 and 0.4 mg/kg, whereas in W2 through W5, ChlF reached a plateau at a boron concentration of 0.3 mg/kg. However, after W4, at all concentrations of boron, ChlF did not change significantly from control, while at W5 notable differences in ChlF occurred.

### 4.5 Effects of Boron on *Brassica oleracea* var. *acephala* Leaf Count

Figure 4.2 and Table 4.6 show the leaf count on samples of *Brassica oleracea* var. *acephala* in response to increasing doses of boron (0.3 to 0.6 mg/kg) and measured at various weekly time intervals (W1, W2, W3, W4, and W5) after initial soil treatment. None of the boron treatments produced any significant (p=0.978724) changes in leaf count for all the weeks analyzed.

**Table 4.3**: *Brassica oleracea* var. *acephala* leaf composition analysis – part 1

**Leaf Analyses Report**

**Date received: 30/06/2014**

**Date tested: 01/07/2014**

| Orchard | Lab No | % | | | | | mg/kg | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **N** | **P** | **K** | **Ca** | **Mg** | **Na** | **Mn** | **Fe** | **Cu** | **Zn** | **B** |
| **A (5 g)** | 20564 | 0.07 | 0.15 | 4.06 | 0.86 | 1.04 | 1892 | 19 | 376 | 6 | 39 | 23 |
| **B (5 g)** | 20565 | 0.06 | 0.18 | 2.73 | 0.70 | 0.61 | 1259 | 15 | 341 | 5 | 29 | 34 |
| **C (5 g)** | 20566 | 0.05 | 0.16 | 3.88 | 0.92 | 1.08 | 1366 | 14 | 403 | 6 | 28 | 34 |
| **D (5 g)** | 20567 | 0.06 | 0.14 | 2.79 | 0.79 | 0.63 | 3047 | 22 | 878 | 7 | 45 | 31 |
| **F (5 g)** | 205688 | 0.07 | 0.10 | 3.82 | 0.86 | 0.87 | 1558 | 13 | 188 | 5 | 22 | 30 |
| **Methods**[#] | | 3127 | 3105 | 3105 | 3105 | 3105 | 3105 | 3105 | 3105 | 3105 | 3105 | 3105 |

D=Deficient; L=Low; H=High; V=Very High; T=Toxic

Certificate of Analyses issued by BemLab (Report No.: BL20564, www.bemlab.co.za); **A:** Represents the 0.6 mg/kg boron treatment, **B:** 0.5 mg/kg, **C:** 0.4 mg/kg, **D:** 0.3 mg/kg, **F:** Control; 5 g indicate that the 6 replicate samples had been pooled to provide sufficient material for laboratory analysis.

**Table 4.4**: *Brassica oleracea* var. *acephala* leaf composition analysis – part 2

**Leaf Analyses Report**

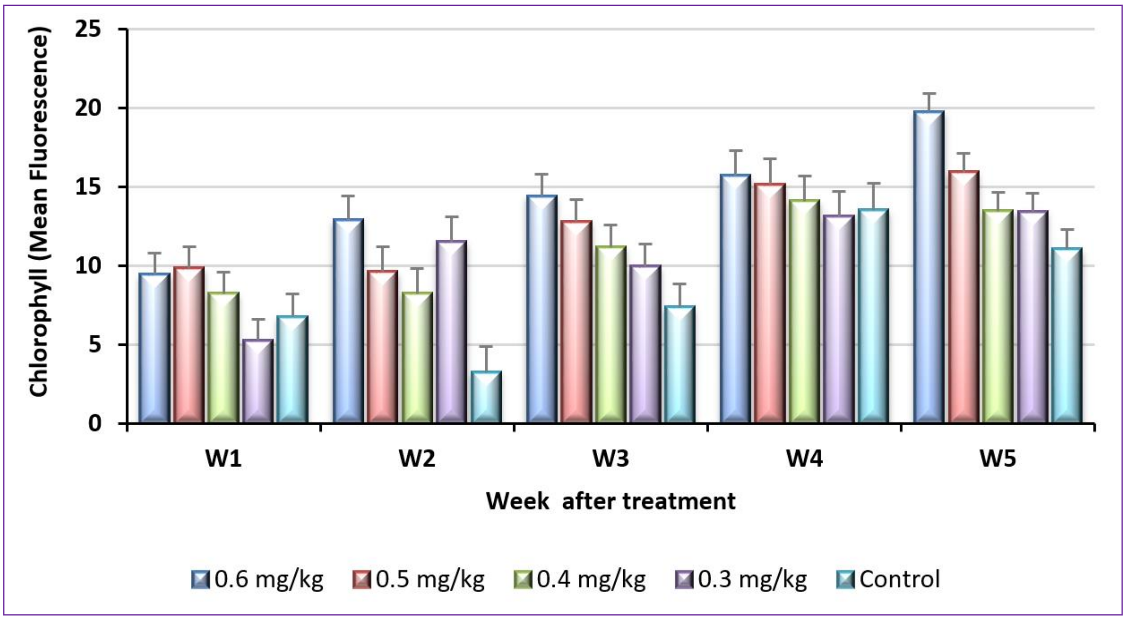
**Date received: 02/02/2015**

**Date tested: 11/02/2015**

| **Orchard** | **Lab** | **%** | | | | | **mg/kg** | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | **No** | **N** | **P** | **K** | **Ca** | **Mg** | **Na** | **Mn** | **Fe** | **Cu** | **Zn** | **B** |
| **A1** | 7282 | 1.86 | 0.39 | 2.55 | 1.92 | 0.41 | 2152 | 17 | 574 | 5 | 30 | 34 |
| **A2** | 7283 | 1.70 | 0.32 | 2.24 | 1.48 | 0.30 | 1960 | 16 | 1005 | 5 | 28 | 26 |
| **B1** | 7284 | 1.71 | 0.44 | 2.49 | 1.94 | 0.37 | 1375 | 17 | 244 | 4 | 33 | 36 |
| **B2** | 7285 | 1.24 | 0.37 | 2.21 | 1.44 | 0.37 | 1532 | 12 | 235 | 3 | 30 | 27 |
| **C1** | 7286 | 1.68 | 0.35 | 2.61 | 1.76 | 0.37 | 2046 | 17 | 799 | 5 | 44 | 31 |
| **C2** | 7287 | 1.54 | 0.32 | 1.89 | 2.09 | 0.41 | 1970 | 16 | 446 | 4 | 44 | 31 |
| **D1** | 7288 | 1.51 | 0.35 | 2.23 | 1.58 | 0.35 | 2277 | 15 | 1103 | 4 | 29 | 28 |
| **D2** | 7289 | 1.62 | 0.32 | 2.07 | 1.91 | 0.43 | 2074 | 17 | 473 | 4 | 37 | 32 |
| **F1** | 7290 | 1.39 | 0.36 | 1.98 | 2.00 | 0.41 | 1670 | 21 | 376 | 4 | 53 | 32 |
| **F2** | 7291 | 1.51 | 0.37 | 2.31 | 1.75 | 0.37 | 1562 | 23 | 1618 | 5 | 54 | 31 |

D=Deficient; L=Low; H=High; V=Very High; T=Toxic

Certificate of Analyses issued by BemLab (Report No: BL7282, www.bemlab.co.za); A total of 60 samples were analyzed. **A1 and A2:** represent the 0.6 mg/kg boron treatment; . **B1 and B2:** represent the 0.5 mg/kg boron treatment; **C1 and C2:** represent the 0.4 mg/kg boron treatment; **D1 and D2:** represent the 0.3 mg/kg boron treatment; **F1 and F2:** represent the control.



Values are means ± SEM (n=4)

**Multivariate Tests of Significance (Final data set)**
**Sigma-restricted parameterization**
**Type I decomposition**

| | **Test** | **Value** | **F** | **Effect** | **Error** | **Significance (p)** |
|---|---|---|---|---|---|---|
| **Intercept** | Wilks' Lambda | 0.069604 | 211.1976 | 5 | 79.0000 | 0.000000 |
| **Treatment** | Wilks' Lambda | 0.537212 | 2.7091 | 20 | 262.9632 | 0.000164 |

**Figure 4.1:** Effects of boron on *Brassica oleracea* var. *acephala* chlorophyll fluorescence

None of the boron treatments produced any significant (p=0.978724) changes in leaf count for all the weeks analyzed.

## 4.6 Effects of Boron on *Brassica oleracea* var. *acephala* Leaf Length

Figure 4.3 and Table 4.7 illustrate the leaf length on samples of *Brassica oleracea* var. *acephala* in response to increasing doses of boron (0.3 to 0.6 mg/kg) and measured at various weekly time intervals (W1, W2, W3, W4, and

W5) after initial soil treatment. None of the boron treatments produced any significant (p=0.070530) changes in leaf length for all the weeks analyzed.

## 4.7 Effects of Boron on *Brassica oleracea* var. *acephala* Plant Height

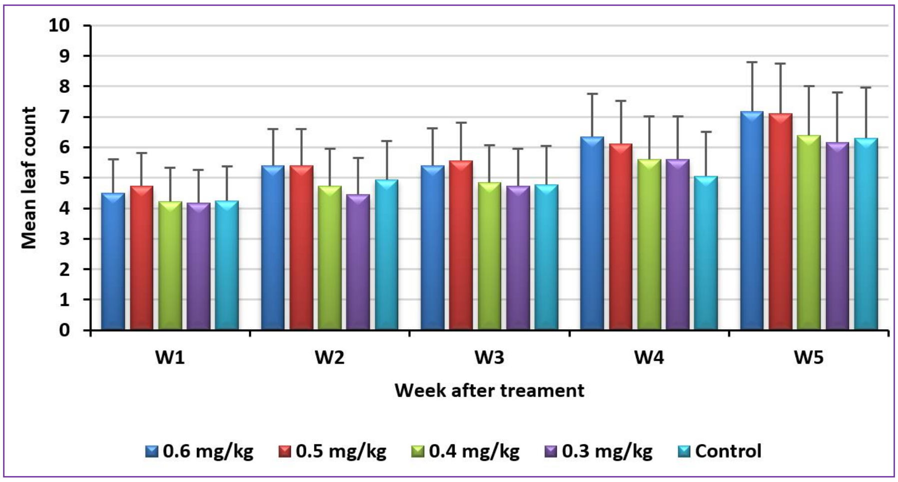
Figure 4.4A and Table 4.8 display the plant height of *Brassica oleracea* var. *acephala* in response to increasing doses of boron (0.3 to 0.6 mg/kg) and measured at various weekly time intervals (W1, W2, W3, W4, and W5) after initial soil treatment. None of the boron treatments produced any significant (p=0.070530) changes in plant height for all the weeks analyzed. This was further confirmed by Tukey’s multiple comparisons test (Figure 4.4B).

**Table 4.5:** Effects of boron on *Brassica oleracea* var. *acephala* chlorophyll fluorescence

| **Treatment** | | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **N** |
|---|---|---|---|---|---|---|---|---|---|---|
| **No.** | mg/kg | Week_1 | Week_1 | Week_1 | Week_1 | Week_2 | Week_2 | Week_2 | Week_2 | |
| **1** | 0.06 | 9.416667 | 1.358848 | 6.713974 | 12.11936 | 12.86111 | 1.576066 | 9.726380 | 15.99584 | 18 |
| **2** | 0.05 | 9.811111 | 1.358848 | 7.108418 | 12.51380 | 9.60000 | 1.576066 | 6.465269 | 12.73473 | 18 |
| **3** | 0.04 | 8.227778 | 1.358848 | 5.525085 | 10.93047 | 8.21111 | 1.576066 | 5.076380 | 11.34584 | 18 |
| **4** | 0.03 | 5.261111 | 1.358848 | 2.558418 | 7.96380 | 11.50000 | 1.576066 | 8.365269 | 14.63473 | 18 |
| **5** | Control | 6.743750 | 1.441275 | 3.877111 | 9.61039 | 3.20625 | 1.671670 | -0.118634 | 6.53113 | 16 |

| **Treatment** | | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **N** |
|---|---|---|---|---|---|---|---|---|---|---|
| **No.** | mg/kg | Week_3 | Week_3 | Week_3 | Week_3 | Week_4 | Week_4 | Week_4 | Week_4 | |
| **1** | 0.06 | 14.38889 | 1.421911 | 11.56076 | 17.21701 | 15.68333 | 1.613433 | 12.47428 | 18.89239 | 18 |
| **2** | 0.05 | 12.77222 | 1.421911 | 9.94410 | 15.60035 | 15.13889 | 1.613433 | 11.92984 | 18.34794 | 18 |
| **3** | 0.04 | 11.13333 | 1.421911 | 8.30521 | 13.96146 | 14.08333 | 1.613433 | 10.87428 | 17.29239 | 18 |
| **4** | 0.03 | 9.93889 | 1.421911 | 7.11076 | 12.76701 | 13.11111 | 1.613433 | 9.90206 | 16.32016 | 18 |
| **5** | Control | 7.35625 | 1.508165 | 4.35657 | 10.35593 | 13.48750 | 1.711304 | 10.08379 | 16.89121 | 16 |

| **Treatment** | | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **N** |
|---|---|---|---|---|---|---|
| **No.** | mg/kg | Week_5 | Week_5 | Week_5 | Week_5 | |
| **1** | 0.06 | 19.73333 | 1.191435 | 17.36362 | 22.10305 | 18 |
| **2** | 0.05 | 15.90000 | 1.191435 | 13.53028 | 18.26972 | 18 |
| **3** | 0.04 | 13.45556 | 1.191435 | 11.08584 | 15.82527 | 18 |
| **4** | 0.03 | 13.41111 | 1.191435 | 11.04139 | 15.78083 | 18 |
| **5** | Control | 11.01250 | 1.263708 | 8.49904 | 13.52596 | 16 |

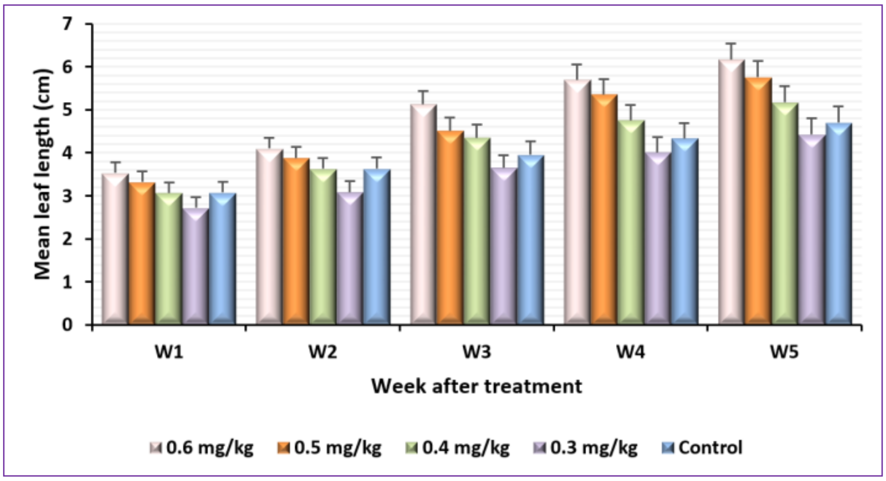


Values are means ± SEM (n=4)

**Multivariate Tests of Significance (Final data set)**
**Sigma-restricted parameterization**
**Type I decomposition**

| | Test | Value | F | Effect | Error | Significance (p) |
|---|---|---|---|---|---|---|
| **Treatment** | Wilks' Lambda | 0.894816 | 0.459351 | 20 | 269.5965 | 0.978724 |

**Figure 4.2:** Effects of boron on *Brassica oleracea* var. *acephala* leaf count



Values are means ± SEM (n=4)

**Multivariate Tests of Significance (Final data set)**
**Sigma-restricted parameterization**
**Type I decomposition**

| | Test | Value | F | Effect | Error | Significance (p) |
|---|---|---|---|---|---|---|
| **Intercept** | Wilks' Lambda | 0.064289 | 232.8769 | 5 | 80.0000 | 0.000000 |
| **Treatment** | Wilks' Lambda | 0.696888 | 1.5316 | 20 | 266.2799 | 0.070530 |

**Figure 4.3**: Effects of boron on *Brassica oleracea* var. *acephala* leaf length

**Table 4.6:** Effects of boron on *Brassica oleracea* var. *acephala* leaf count

| **Treatment** | | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **N** |
|---|---|---|---|---|---|---|---|---|---|---|
| **No.** | mg/kg | Week_1 | Week_1 | Week_1 | Week_1 | Week_2 | Week_2 | Week_2 | Week_2 | |
| **1** | 0.06 | 3.533333 | 0.24277 | 3.05056 | 4.016107 | 4.1 | 0.252711 | 3.597457 | 4.602543 | 18 |
| **2** | 0.05 | 3.322222 | 0.24277 | 2.839448 | 3.804996 | 3.877778 | 0.252711 | 3.375235 | 4.38032 | 18 |
| **3** | 0.04 | 3.066667 | 0.24277 | 2.583893 | 3.54944 | 3.627778 | 0.252711 | 3.125235 | 4.13032 | 18 |
| **4** | 0.03 | 2.722222 | 0.24277 | 2.239448 | 3.204996 | 3.088889 | 0.252711 | 2.586346 | 3.591432 | 18 |
| **5** | Control | 3.076471 | 0.249808 | 2.5797 | 3.573241 | 3.629412 | 0.260037 | 3.1123 | 4.146524 | 16 |

| **Treatment** | | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **N** |
|---|---|---|---|---|---|---|---|---|---|---|
| **No.** | mg/kg | Week_3 | Week_3 | Week_3 | Week_3 | Week_4 | Week_4 | Week_4 | Week_4 | |
| **1** | 0.06 | 3.533333 | 0.24277 | 3.05056 | 4.016107 | 5.7 | 0.348072 | 5.00782 | 6.39218 | 18 |
| **2** | 0.05 | 3.322222 | 0.24277 | 2.839448 | 3.804996 | 5.355556 | 0.348072 | 4.663375 | 6.047736 | 18 |
| **3** | 0.04 | 3.066667 | 0.24277 | 2.583893 | 3.54944 | 4.755556 | 0.348072 | 4.063375 | 5.447736 | 18 |
| **4** | 0.03 | 2.722222 | 0.24277 | 2.239448 | 3.204996 | 4.011111 | 0.348072 | 3.318931 | 4.703291 | 18 |
| **5** | Control | 3.076471 | 0.249808 | 2.5797 | 3.573241 | 4.335294 | 0.358164 | 3.623047 | 5.047542 | 16 |

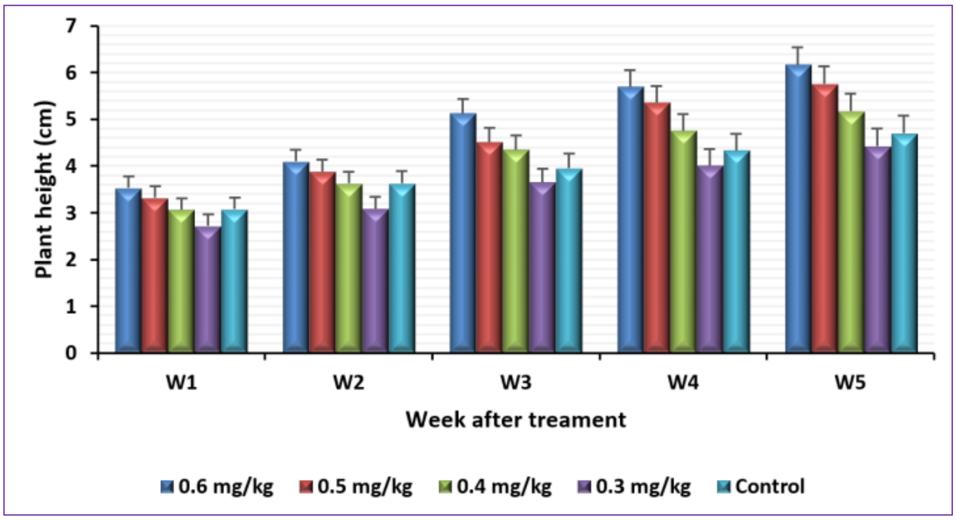
| **Treatment** | | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **N** |
|---|---|---|---|---|---|---|
| **No.** | mg/kg | Week_5 | Week_5 | Week_5 | Week_5 | |
| **1** | 0.06 | 6.166667 | 0.371484 | 5.42793 | 6.905404 | 18 |
| **2** | 0.05 | 5.755556 | 0.371484 | 5.016819 | 6.494292 | 18 |
| **3** | 0.04 | 5.172222 | 0.371484 | 4.433485 | 5.910959 | 18 |
| **4** | 0.03 | 4.427778 | 0.371484 | 3.689041 | 5.166515 | 18 |
| **5** | Control | 4.7 | 0.382254 | 3.939846 | 5.460154 | 16 |

**Table 4.7:** Effects of boron on *Brassica oleracea* var. *acephala* leaf length

| **Treatment** | | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **N** |
|---|---|---|---|---|---|---|---|---|---|---|
| **No.** | mg/kg | Week_1 | Week_1 | Week_1 | Week_1 | Week_2 | Week_2 | Week_2 | Week_2 | |
| **1** | 0.06 | 3.533333 | 0.24277 | 3.05056 | 4.016107 | 4.1 | 0.252711 | 3.597457 | 4.602543 | 18 |
| **2** | 0.05 | 3.322222 | 0.24277 | 2.839448 | 3.804996 | 3.877778 | 0.252711 | 3.375235 | 4.38032 | 18 |
| **3** | 0.04 | 3.066667 | 0.24277 | 2.583893 | 3.54944 | 3.627778 | 0.252711 | 3.125235 | 4.13032 | 18 |
| **4** | 0.03 | 2.722222 | 0.24277 | 2.239448 | 3.204996 | 3.088889 | 0.252711 | 2.586346 | 3.591432 | 18 |
| **5** | Control | 3.076471 | 0.249808 | 2.5797 | 3.573241 | 3.629412 | 0.260037 | 3.1123 | 4.146524 | 16 |

| **Treatment** | | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **N** |
|---|---|---|---|---|---|---|---|---|---|---|
| **No.** | mg/kg | Week_3 | Week_3 | Week_3 | Week_3 | Week_4 | Week_4 | Week_4 | Week_4 | |
| **1** | 0.06 | 5.133333 | 0.299445 | 4.537853 | 5.728813 | 5.7 | 0.348072 | 5.00782 | 6.39218 | 18 |
| **2** | 0.05 | 4.516667 | 0.299445 | 3.921187 | 5.112147 | 5.355556 | 0.348072 | 4.663375 | 6.047736 | 18 |
| **3** | 0.04 | 4.35 | 0.299445 | 3.75452 | 4.94548 | 4.755556 | 0.348072 | 4.063375 | 5.447736 | 18 |
| **4** | 0.03 | 3.65 | 0.299445 | 3.05452 | 4.24548 | 4.011111 | 0.348072 | 3.318931 | 4.703291 | 18 |
| **5** | Control | 3.952941 | 0.308127 | 3.340197 | 4.565685 | 4.335294 | 0.358164 | 3.623047 | 5.047542 | 16 |

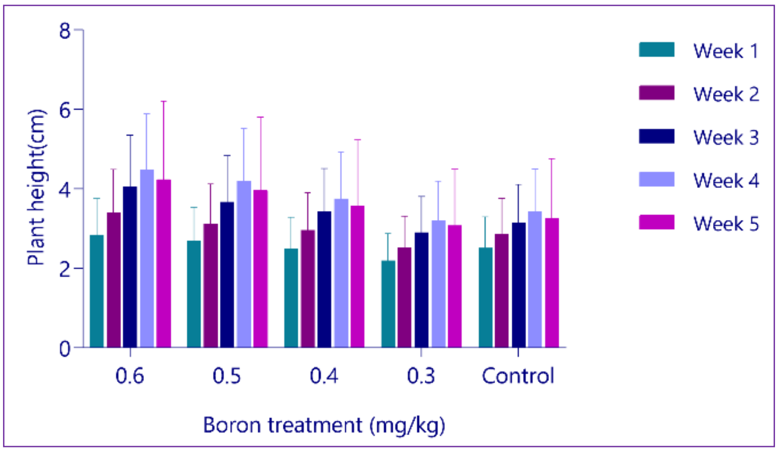
| **Treatment** | | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **N** |
|---|---|---|---|---|---|---|
| **No.** | mg/kg | Week_5 | Week_5 | Week_5 | Week_5 | |
| **1** | 0.06 | 6.166667 | 0.371484 | 5.42793 | 6.905404 | 18 |
| **2** | 0.05 | 5.755556 | 0.371484 | 5.016819 | 6.494292 | 18 |
| **3** | 0.04 | 5.172222 | 0.371484 | 4.433485 | 5.910959 | 18 |
| **4** | 0.03 | 4.427778 | 0.371484 | 3.689041 | 5.166515 | 18 |
| **5** | Control | 4.7 | 0.382254 | 3.939846 | 5.460154 | 16 |



Values are means ± SEM (n=4)

| Multivariate Tests of Significance (Final data set)<br>Sigma-restricted parameterization<br>Type I decomposition | | | | | | |
|---|---|---|---|---|---|---|
| | **Test** | **Value** | **F** | **Effect** | **Error** | **Significance (p)** |
| **Intercept** | Wilks' Lambda | 0.064289 | 232.8769 | 5 | 80.0000 | 0.000000 |
| **Treatment** | Wilks' Lambda | 0.696888 | 1.5316 | 20 | 266.2799 | 0.070530 |

**Figure 4.4A:** Effects of boron on *Brassica oleracea* var. *acephala* plant height – Wilks' test



Values are means ± SEM (n=4)

Tukey's multiple comparisons test showed no significant diffirences between means.

**Figure 4.4B:** Effects of boron on *Brassica oleracea* var. *acephala* plant height – Tukey's test

**Table 4.8:** Effects of boron on *Brassica oleracea* var. *acephala* plant height

| **Treatment** | | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **N** |
|---|---|---|---|---|---|---|---|---|---|---|
| **No.** | mg/kg | Week_1 | Week_1 | Week_1 | Week_1 | Week_2 | Week_2 | Week_2 | Week_2 | |
| **1** | 0.06 | 3.533333 | 0.24277 | 3.05056 | 4.016107 | 4.1 | 0.252711 | 3.597457 | 4.602543 | 18 |
| **2** | 0.05 | 3.322222 | 0.24277 | 2.839448 | 3.804996 | 3.877778 | 0.252711 | 3.375235 | 4.38032 | 18 |
| **3** | 0.04 | 3.066667 | 0.24277 | 2.583893 | 3.54944 | 3.627778 | 0.252711 | 3.125235 | 4.13032 | 18 |
| **4** | 0.03 | 2.722222 | 0.24277 | 2.239448 | 3.204996 | 3.088889 | 0.252711 | 2.586346 | 3.591432 | 18 |
| **5** | Control | 3.076471 | 0.249808 | 2.5797 | 3.573241 | 3.629412 | 0.260037 | 3.1123 | 4.146524 | 16 |

| **Treatment** | | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **N** |
|---|---|---|---|---|---|---|---|---|---|---|
| **No.** | mg/kg | Week_3 | Week_3 | Week_3 | Week_3 | Week_4 | Week_4 | Week_4 | Week_4 | |
| **1** | 0.06 | 5.133333 | 0.299445 | 4.537853 | 5.728813 | 5.7 | 0.348072 | 5.00782 | 6.39218 | 18 |
| **2** | 0.05 | 4.516667 | 0.299445 | 3.921187 | 5.112147 | 5.355556 | 0.348072 | 4.663375 | 6.047736 | 18 |
| **3** | 0.04 | 4.35 | 0.299445 | 3.75452 | 4.94548 | 4.755556 | 0.348072 | 4.063375 | 5.447736 | 18 |
| **4** | 0.03 | 3.65 | 0.299445 | 3.05452 | 4.24548 | 4.011111 | 0.348072 | 3.318931 | 4.703291 | 18 |
| **5** | Control | 3.952941 | 0.308127 | 3.340197 | 4.565685 | 4.335294 | 0.358164 | 3.623047 | 5.047542 | 16 |

| **Treatment** | | **Mean** | **STD error** | **Lower 95%CI** | **Upper 95%CI** | **N** |
|---|---|---|---|---|---|---|
| **No.** | mg/kg | Week_5 | Week_5 | Week_5 | Week_5 | |
| **1** | 0.06 | 6.166667 | 0.371484 | 5.42793 | 6.905404 | 18 |
| **2** | 0.05 | 5.755556 | 0.371484 | 5.016819 | 6.494292 | 18 |
| **3** | 0.04 | 5.172222 | 0.371484 | 4.433485 | 5.910959 | 18 |
| **4** | 0.03 | 4.427778 | 0.371484 | 3.689041 | 5.166515 | 18 |
| **5** | Control | 4.7 | 0.382254 | 3.939846 | 5.460154 | 16 |

# CHAPTER 5

## CONCLUSION AND FUTURE PERSPECTIVES

### 5.1 Introduction

Boron (B) is an important metalloid element, i.e., it possesses characteristics of both metals and non-metals(Bariya *et al.*, 2014). A deficiency or toxic accumulation of B in plants impedes various biochemical and physiological processes, including an increase in oxidative metabolism, DNA strand breaks, impairment of DNA repair systems and membrane functions, or the inhibition of protein folding, and protein function (Camacho-Cristóbal *et al.*, 2008;Uluisik *et al.*, 2018; Uluisik *et al.*, 2011). High doses of B are usually exploited to control bacterial and fungal infections. In fungi and possibly also in higher (vascular) plants, the toxicity of boron is exerted at the level of the mitochondria (Ali *et al.*, 2014; Uluisik *et al.*, 2011).

Boron is ubiquitously distributed at wide-ranging concentrations in all layers of soil, rocks, oceans and rivers (Kot, 2009; Power and Woods, 1997). Boron is used generally in agriculture given its roles in plant growth and development. Boron deficiency and toxicity often result in growth impairment in many crop plants, and hence may adversely impact the agricultural and economic sectors of many countries worldwide. Boron uptake into the plant occurs through the xylem and it is generally not absorbed by other tissues, but is deposited at the end of leaf veins where necrosis (damage to leaf tissue) progresses. Boron toxicity is a poorly understood phenomenon, but likely implicates disruption of genetic processes, such as transcription and translation. A wide variation in tolerance to boron toxicity has been reported for different crop species but also for different cultivars of the same species. Therefore, an increased understanding the biochemical mechanisms of boron action may improve crop yield (Berger, 1949; Gupta, 1980; Mack, 1959; Maynard, 1979; Oneida, 1993; Reid, 2013; Shelp *et al.*, 1987). 5.2 The Scope and Objectives of this Study

Boron is essential for attaining high quality yields in vegetables (Bariya *et al.*, 2014; Mack, 1959; Mekki, 2015; Oneida, 1993). Leafy vegetables are important sources of several nutrients, vitamins, antioxidants, minerals and proteins (Adedokun *et al.*, 2016; Mensah *et al.*, 2008; Mingochi and Luchen, 1997; Odhav *et al.*, 2007). Arguably, the most consumed leafy vegetables in southern Africa include various types of kale (also known as Chou Moellier), for example, various *Cruciferae* (rape, Chou Mollier, turnips, kale, Swedes, and radish rape).

Cabbage, e.g., *Brassica oleracea* is one of the most important vegetables grown worldwide, including in sub-Saharan Africa (Ongeng *et al.*, 2011). Cabbage contains substantial amounts of bioactive compounds such as glucosinolates, vitamin C, carotenoids, polyphenols and antioxidants which protect the human body against free radicals, thus reducing the risks of many chronic diseases (Hallmann *et al.*, 2017) and cancer (Choi and Park, 1999; Hallmann *et al.*, 2017; Licznerska *et al.*, 2013; Podsędek, 2007). There are very limited literature data on the responses of *Brassica olereacea* var *acephala* to boron treatment. The objectives of the present study were to examine the effects of boron treatment and availability on leaf nutrient and chlorophyll content as well as the growth parameters (leaf length, leaf count, and plant height) of *B. oleracea*. var. *acephala.*

## 5.3 Summary of the Study Findings

The main objective of this study was to evaluate the extent to which boric acid concentration can affect growth parameters (plant height, leaf numbers, leaf chlorophyll content, and leaf size) of *Brassica olereacea* var. *acephala* at different stages of growth and development. Treatment comprised of four concentrations of boron (0.3 mg/kg, 0.4 mg/kg, 0.5 mg/kg and 0.6 mg/kg and distilled water as control). Soil pH did not fluctuate much among the various orchard blocks tested, irrespective of soil depth or layer.

Exchangeable cations $Na^+$ and $K^+$ levels did not differ, but $Ca^{2+}$ and $Mg^{2+}$ levels varied significantly among orchards analyzed. The Control Orchard displayed a higher P content than the other orchards. Ca, Mg, Cu and B levels did not change significantly among the orchards in terms of boron treatment applied, but Na, Fe and Zn levels were markedly raised in the Orchard treated with 0.3 mg/kg boron) compared to the Control Orchard.

Chlorophyll fluorescence (a measure of leaf chlorophyll content) increased significantly with the growth period, i.e., the duration following the initial treatment at all doses of boron. Boron at all did not significantly affect leaf count, leaf length and plant height. These findings may have important applications in achieving high quality yields in vegetable crops, especially *Brassica oleracea*. However, more refined research is needed to explain the observed effects reported in this study.

## 5.4 Research Context

Boron (B) is an interesting metalloid micronutrient required for normal growth and development of plants (Ahmad, 2009; Warington, 1923). Boron is also a promising nutrient for increasing growth and yield of plants and crops (Bariya *et* *al.*, 2014). The last two decades have witnessed remarkable advances in our knowledge of the molecular basis of B deficiency and toxicity responses in plants (Bariya *et al.*, 2014; Brown *et al.*, 2002; Camacho-Cristóbal *et al.*, 2008; Gupta *et al.*, 1985; Krug *et al.*, 2009; Reid, 2013). A small concentration range exists between boron deficiency and toxicity in soil-plant systems (Tariq and Mott, 2007). Boron is almost certainly the trace element which most commonly limits crop yield and, therefore, is most widely used in agriculture, horticulture and in forestry.

Boron deficiency has significant adverse effects on leguminous crops such as luceme, red clover and alfalfa and cruciferous crops such as cabbage, cauliflower, rutabagas, turnips and radish (Tariq and Mott, 2007). A recent study of the effects of B foliar application on the yield and quality of some sunflower (*Helianthus annuus* L.) cultivars concluded that it plays a vital role for increasing the productivity and quality of sunflower plants, particularly when grown in B-deficient soil (Mekki, 2015). Against the background of *Brassica oleracea* var. *acephala* (kale), it was selected for the present study since cabbage is a staple food globally, including in various regions of Africa and B may increase the quality and yield of such crops (Kfir, 2004; Mvere and van der Werff, 2004).

## 5.5 Recommendations for Future Studies

Given the importance of B in normal plant growth and development, it is critical to establish the cut-off point between B deficiency and toxicity in soil-plant systems, especially since the optimal B concentration required for crop health, yield and nutritional value will be key considerations in developing sub-Saharan countries and many other countries globally where *Brassica oleracea* var. *acephala* (kale) is a staple food source (Bariya *et al.*, 2014; Mekki, 2015; Mvere and van der Werff, 2004; Odhav *et al.*, 2007; Reid, 2013). In the present study, B treatments generally enhanced *Brassica oleracea* var. *acephala* leaf chlorophyll content over several weeks after the initial exposure to the regimens selected.

However, over the same concentration range of B, no significant effects were observed with regard to leaf mineral content, leaf count and plant height. Since these parameters are important criteria for proof of concept analysis of the effects of B on such crops, the experimental design needs to be refined as has been achieved in other related recent studies (Bariya *et al.*, 2014; Mekki, 2015; Oneida, 1993; Osman, 2013; Saadati *et al.*, 2013; Sah and Brown, 1997; Tariq and Mott, 2007).

In addition, factors affecting B bioavailability in soils such as parent material, texture, nature of clay minerals, pH, liming, organic matter content, sources of irrigation, interrelationship with other elements, and environmental conditions like moderate to heavy rainfall, and dry weather all need to be incorporated into future experimental designs that warrant a proper assessment of B toxicity and deficiency in plant crops (Ahmad *et al.*, 2012).

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# APPENDIX

## CONFIRMATION BY LANGUAGE EDITOR

Professor D.C. Hiss

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16 January 2018

To Whom it May Concern

This serves to confirm that I have edited the language, spelling, grammar and style of the Master of Technology (Agriculture) by **Fatheya Mohamed**, titled: “**Mineral Analysis and Proximate Composition of Leaves of (*Brassica oleracea* var. *acephala*) in Response to Boron Application in Pot Experiments**” The manuscript was also professionally typeset by me.

Sincerely Yours

Dip. Freelance Journalism, Dip. Creative Writing, MSc (Medicine), PhD