THE EFFECT OF MORINGA LEAF EXTRACT (MLE) ON GROWTH AND DEVELOPMENT, MINERAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF RADISH (*RAPHANUS SATIVUS*) AND GREEN BEANS (*PHASEOLUS VULGARIS*)

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DECLARATION 1 - PLAGIARISM

The research work reported in this thesis was a result of experiments carried out in the School of Agricultural, Earth and Environmental Sciences, University of KwaZulu-Natal, Pietermaritzburg, from February 2018 to June 2019, under the supervision of Prof. Isa Bertling.

By submitting this thesis electronically, I, **Makungu Charmaine Mabaso**, hereby declare that the entire research was a result of my own investigations. It therefore represents my original work except where otherwise stated and due acknowledgments are recorded.

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I certify that the above statement is correct.

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GENERAL ABSTRACT

Besides enhancing food production, one of the major challenges of the agricultural sector is to provide essential minerals and nutrients to humans for the maintenance of a healthy body, not only from a caloric perspective, but also through the provision of antioxidant compounds. It is believed that two-thirds of the world's plants have medicinal properties and many of these plants have high antioxidant potential. Natural antioxidants, such as flavonoids, vitamin C, tocopherols and other phenolic compounds are known to be present in many plants. *Moringa oleifera* is one of such plants that has been identified to contain natural antioxidants; particularly the leaves of moringa are a good source of natural antioxidants due to the presence of phenolics, carotenoids, ascorbic acid and flavonoids. While the effect of such plant material on human health has become common subject of investigation, little is known on the effect of moringa leaf extracts applied to plants to enhance their resistance and antioxidant potential. The aim of the experiment was to evaluate the effect of moringa leaf extract (MLE) on the growth and development, mineral composition and antioxidant properties of radish (*Raphanus sativus*) and green beans (*Phaseolus vulgaris*). The experiment was laid out in a completely randomized design with five replications and comprised of three treatments, viz. (Control, only inorganic fertilizer Calmag+B (5 g/plant) (T1), common fertilizer plus MLE 100% (T2) (20 g/L dried moringa powder (obtained from Run KZN, Pietermaritzburg, South Africa)) and MLE 50% (T3) (T2 diluted to 50% with 100% methanol). Applications of MLE was carried out during the flowering stage, pod formation stage and prior to harvest. The obtained results demonstrate that MLE applications increased growth and development of both crops (leaf size, pod size, number of flowers, number of matured leaves and, at final harvest, above and below ground fresh and dry mass). Among the various MLE treatments, MLE 50% resulted in higher growth development and yield parameters on both radish and green bean plants compared with the MLE 100% and control plants. The mineral composition of radish leaves, storage roots and

green bean pods was carried out by an independent laboratory. Applications of the treatments had significant influence ($p < 0.05$) on plants, with MLE-treated plants obtaining higher mineral concentrations compared with the control plants. Treatment with MLE also significantly ($p < 0.05$) increased antioxidant properties, particularly total antioxidants, anthocyanin, ascorbic acid and total chlorophyll concentrations, with MLE 50% producing plants of the highest overall antioxidant properties. This treatment could, therefore, be possibly employed as a method to obtain healthier, organic vegetables.

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CHAPTER 1

GENERAL INTRODUCTION AND RESEARCH AIMS

1 Introduction

Moringa oleifera, belonging to the Moringaceae family, is one of 13 species of the genus *Moringa*, known as the "drumstick tree" or "horseradish tree" (Gopalakrishnan et al., 2016), but sometimes also referred to as the "miracle tree", indicating its pharmacological properties. Moringa leaves are a good source of highly digestible protein, calcium, iron and vitamins (Yasmeen, 2011). The tree can grow in semi-arid, tropical and subtropical areas (Thanaa et al., 2017); it is deciduous, fast-growing and drought-resistant and can reach a height of up to 10 m (Fuglie, 2000). It is considered one of the world's most diversely used trees, as almost every plant part can be used for food, industrial purposes and medication (Khalafalla et al., 2010). Plant parts that can be consumed include roots, fruit, leaves and flowers. These plant organs are used as vegetables in Africa, Arabia, India, South Asia, America and Pakistan (Yasmeen, 2011). The young leaves are commonly cooked and eaten like Swiss chard or used to make soups and salads (Fuglie, 2005); they are a good source of vitamin A, the B complex vitamins and vitamin C, as well as of minerals and amino acids, like the essential amino acid leucine and other sulphur-containing amino acids. The green pods are particularly rich in leucine and can be boiled and eaten like green beans (Fuglie, 2005). Recently, *Moringa oleifera* has attracted great attention to enhance plant growth and development, because of its phytochemical components, such as cytokinins, antioxidants, as well as many macro- and micro-nutrients (Abdalla and El-Khashiban, 2012).

Today, most farmers have become aware of the practice to aly organic fertilizer to improve crop production, while also trying to protect the environment (Gopalakrishnan et al., 2016). The applications of the extract made from moringa leaves (moringa leaf extract, MLE) is

considered as a cheap and environmentally friendly organic fertilizer alternative (Makkar et al., 2007). The effect of MLE is similar to that of a cytokinin applications, because the ethanolic extract (80%) obtained from the leaves of *Moringa oleifera* contains cytokinines (Makkar and Becker, 1996), such as zeatin. As a cytokinin, zeatin enhances vegetative growth, but also has antioxidant properties and protects the human cells from aging (Foidl et al., 2001). This leaf extract can be applied as a foliar spray to accelerate growth and development of young plants; it causes plants to be strong, firm and more resistant to pests and diseases (Makkar and Becker, 1996). Studies have proven that plants, such as tomatoes (*Solanum lycopersicum*), plums (*Prunus domestica*), maize (*Zea mays*), peers (*Capsicum*), Swiss chard (*Beta vulgaris* var. *cicla*), cowpeas (*Vigna unguiculata*) and beans (*Phaseolus vulgaris*), displaying an increase in yield following MLE applications. Crops treated with MLE generally also bear larger fruit and have heavier roots and stems (Foidl et al., 2001).

Radish *(Raphanus sativus)* is a quick-growing, cool-season, root vegetable, which belongs to the Brassicaceae family and is believed to be native to China. Radish is seed-propagated as seeds germinate quickly, usually within 3-4 days after sowing (Guitierrez and Perez, 2004). Optimal growing temperatures lie between 18°C to 25°C. Radish roots contain high nutrient concentration and the leaves can also be consumed raw, as a part of a salad (Zhou et al., 2013). There are numerous cultivars of radish categorized according to shape, size, colour, flavour and height. The plant requires only little irrigation (305-381 mm/ growing season), with the bulk applied during stages of rapid plant growth and development (Guitierrez and Perez, 2004). Radish roots are a rich source of sulphur-containing glucosinolates (also known as β-thioglucoside-N-hyroxysulfates) which impart a pungent aroma and bitter taste (Ishida et al., 2014). Glucosinolates are classified according to their chemical structure as derived from either aliphatic, indole or aromatic amino acid precursors (Ishida et al., 2014). Glucosinolate hydrolysis products (isothiocyanates, simple nitriles, epithionitriles or thiocyanates) play an

important role in disease prevention by triggering antioxidant production and anti-inflammatory responses and contributing to the maintenance of cell homeostasis in the human body (Ishida et al., 2014). Health benefits of radish are, however, mainly attributed to its high folate, dietary fibre and vitamin C (ascorbic acid) (Steinbrecher and Linseisen, 2009). Fresh storage roots are a good source of the water-soluble antioxidant ascorbic acid, an important antioxidant that is also required by the human body for collagen synthesis. Lastly, radish also contains other antioxidants, such as indoles, which can act as detoxifying agents, as well as xanthophylls (zeaxanthin and lutein), flavonoids and carotenoids (Zhou et al., 2013).

Green beans (*Phaseolus vulgaris*) are a warm-season, dicotyledonous crop in the Fabaceae family. The plant originated in Peru and was distributed to South and throughout Central America by migrating Indian tribes (Drost and Wytsalucy, 2014). Green beans are a seed-propagated, warm-season crop. The edible part, the unripe, immature legume, is produced from seed during the warm season. Year-round production can be achieved in frost-free areas and in tunnels (Drost and Wytsalucy, 2014). A soil pH of 5.0 – 6.5 is optimal for green beans, with preferred growing temperatures ranging from 14°C to 27°C. The plant grows best in well-drained, sandy loam soils; crusty soils should be avoided, as seedlings may then emerge slowly and seeds can suffer from cotyledonal cracking (Plumb et al., 2013). It is essential to prevent moisture stress during flowering, pod set and growth, as such stress influences pod shape and quality. Lastly, beans are very sensitive to manganese, zinc and iron deficiencies (Petry et al., 2015).

Green beans contain high levels of phytochemicals, such as quercetin, a flavonol responsible for the anti-inflammatory properties that may reduce the risk of heart disease and cancer, as well as the carotenoids β-carotene, lutein and zeaxanthin (Plumb et al., 2013). Beans are also a good source of folate (folic acid), a water-soluble B group vitamin especially important for the healthy development of the human foetus (Lanza et al., 2006); additionally, green beans

contain 10-20 mg ascorbic acid per 100 g FM (USDA, 2005). The health benefits of green beans are attributed to the pods' high nutrient, dietary fibre, vitamin, antioxidant and mineral content (Petry et al., 2015).

2 Rationale

Moringa leaves are rich in minerals, such as calcium, potassium, zinc, magnesium, iron and coer. Moringa leaves also contain vitamins, like the vitamin A precursor β-carotene, the B vitamins folic acid and pyridoxine, as well as vitamin C, D, and E (Kasolo et al., 2010). Table 1.1 illustrates the different phytochemicals found in *M. oleifera* and moringa leaves contain a variety of phytochemicals, such as tannins, sterols, flavonoids, saponins, terpenoids and certain anti-cancer agents, such as glucosinolates, isothiocyanates and specific glycoside compounds (Berkorich et al., 2013); further the leaf extracts have anti-inflammatory properties used for healing and nutrition (Hegaze et al., 2016). The pods of moringa are rich in fibre, lipids, non-structural carbohydrates, protein and fatty acids such as linoleic acid and oleic acid. Immature pods contain aroximately 62% fibre, 21% protein and 30% amino acids (Choudhary et al., 2013).

Studies conducted by Jhilik et al. (2017) have indicated that MLE contains zeatin, a natural plant growth hormone, along with other micronutrients (zinc, iron and manganese) that are involved in vital plant physiological processes. Yasmeen (2011) showed that MLE contains the growth activating plant hormones cytokinins, auxins and gibberellic acids. Moringa leaf extract also has a high concentration of abscisic acid; hence, it contains all four classical hormone groups that control plant growth and development (Thanaa et al., 2017). Foliar MLE applications has proven to enhance plant growth, leaf chlorophyll content, seed germination and root development, improve yield, pest and disease resistance and to delay fruit senescence (Abdalla, 2013). Although various parts of *M. oleifera* extracts are known to influence human and animal metabolism, little is known scientifically regarding the effects of MLE as a plant

bio-stimulant. The reported effects of MLE on plants are possibly due to the hormonal and antioxidant properties of MLE (Abdalla, 2013). Hence, this study aimed to evaluate the effects of foliar applications of MLE extracts on growth, development, quality, yield and nutritional value of radish and green beans.

Table 1. 1 Phytochemicals present in different plant organs of *Moringa oleifera* (Adapted from Godinez-Oviedo et al., 2016)

Plant organ	Phytochemicals present	References
Leaf	Tannins, Saponins, Alkaloids, Phenols, Flavonoids and Glycosides.	Mensah et al., 2012
Flowers	Tannins, Steroids, Flavonoids, Alkaloids, Glycoside, Quercetin and Terpenoids.	Alhakmani et al., 2013
Seeds	Gallic acid, Catechin, Epicatechin, Ferulic acid, Vanillin, Caffeic acid, Protocatechuic acid, Cinnamic acid, Quercetin, Phytosterol, Glycosides and Phenols	Singh et al., 2013 Hukkeri et al., 2006
Roots	Procyanidin, Aurantiamide, Acetate, 3-dibenzyl urea and Quercetin.	Atawodi et al., 2010
Stem bark	Procyanindin, Sterols, Triterpenoids, Glycosides, Tannins, Alkaloids (moringine and moringinine), Sitosterol and Sitostenone.	Anwar et al., 2007

3 Research problem

In conventional agriculture, chemical fertilizers, pesticides and plant growth regulators are applied to increase yield and quality of crops (Eman and El-sis, 2011). The main intention of the introduction of chemical foliar applications was to prevent and control pests and diseases in field crops (Bhanti, 2007). The simultaneous use of inorganic fertilizers and insecticides, fungicides and herbicides has resulted in the contamination of the environment and caused long-term effects on soil and human health (Bhanti, 2007). The frequent, and often excessive, use of inorganic fertilizers can disrupt the ecological balance, making cultivated plants more susceptible to pests and diseases (Eman and El-sis, 2011). Long-term use of inorganic

fertilizers can gradually change soil pH, since many fertilizers are highly acidic (Bhanti, 2007), thereby resulting in an increase in soil acidity, reducing the presence of beneficial microbial organisms and resulting in stunted plant growth (Bhanti, 2007). Over-applications of inorganic fertilizer can result in a build-up of chemicals in the soil to such a level that the soil micro-fauna is disturbed (Flander, 2015). Soil health has become a 'hot' topic in recent years and is defined as "the continued capacity of the soil to function as a vital living ecosystem that sustains plants, animals and humans" (van Es et al., 2016); therefore, it is important to manage soils sustainably for future generations (van Es et al., 2016). Plant agricultural products that contain high inorganic fertilizer levels can negatively affect human health (cancer and methemoglobinemia), since many inorganic fertilizers used in farming practices have long term effects on living organisms (Flander, 2015). Some of these inorganic fertilizers (nitrates, phosphates) can have negative environmental effects due to run-off into rivers, lakes or dams. Compounds, such as ammonia and nitrates from inorganic fertilizer, are the main pollutants in rivers and lakes, and their presence in waterbodies can lead to eutrophication and ground water pollution (Song et al., 2017). Furthermore, certain inorganic fertilizers have been associated with a wide spectrum of negative effects on human health, such as headaches, nausea and chronic diseases, like cancer and endocrine disruption (Thabet et al., 2016). Various studies have shown that, while vegetables are important in the human health diet, they can contain residues of inorganic fertilizers above their respective maximum residue limit (MRL) which may pose health hazards to consumers. These MRLs have been established for agricultural products in many countries to avoid health hazard caused by inorganic fertilizer residues (Thabet et al., 2016).

Many studies have raised concerns about increasing the usage of inorganic fertilizer to increase and enhance yields; this has led to increased consumer awareness and the shift towards the purchase of organically produced food products. In organic farming, growers refrain from the

use of chemical fertilizers, pesticides, herbicides or other synthetic chemicals during production, processing and storage (Bilal et al., 2015). Omidire et al. (2015) noted that organically produced food helps to reduce health risks of farm workers and consumers by minimizing exposure to inorganic fertilizers. Organic vegetables have also been shown to contain significantly higher antioxidant levels (17%) compared with conventional crops (Omidire et al., 2015). Natural foliar sprays and microbial inoculants, which are mainly plant growth-promoting bacteria, fungi and protozoa, such as *Rhizobium*, *Trichoderma* and *Azospirillum* (Toyota and Watanable, 2013), have been introduced to modern agriculture as a means to produce food of high quality that is safe, also in the long-term, for human consumption (Eman and El-sis, 2011).

4 Research aims and objectives

Two horticultural crops, radish (*Raphanus sativus*), a fast-growing root crop and green beans (*Phaseolus vulgaris*), a vegetable fruit crop, were used as experimental crops, to be able to derive the effect of MLE on growth and development, mineral composition and antioxidant properties of the root and fruit vegetable crops.

4.1 Research aims

- To enhance plant growth and development of radish and green beans using MLE.
- To improve the quantity and nutritional value of radish and green beans using MLE as an organic foliar plant growth enhancer.

4.2 Research objectives

- To determine the effect of MLE foliar applications as a growth enhancer by measuring physical and physiological plant responses to such applications.
- To determine the nutritional value of radish and green beans treated with foliar MLE applications compared with the control (untreated plants).

- To determine the antioxidant properties of root (radish) and fruit (beans) of treated crops compared with control plants.

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CHAPTER 2

THE IMPORTANCE OF PLANT BIOSTIMULANTS AND THE EFFECTIVENESS OF FOLIAR NUTRITION ON PLANT GROWTH AND DEVELOPMENT OF VEGETABLE CROPS – A REVIEW

Abstract

Agricultural growing practices are more and more directed towards organic, sustainable and environmentally friendly production systems. The aim of modern agriculture is to reduce inputs without reducing yield and quality of products. One such avenue is the use of plant biostimulants in the form of plant extracts that contain a wide range of bioactive compounds, the nature of which is mostly still unknown. This review concentrates on the effectiveness of five categories of bio-stimulants: microbial inoculants, humic acids, fulvic acids, protein hydrolysates and amino acids and seaweed extracts. It also discusses the potential of foliar nutrition for plant growth stimulation, the aropriate timing of foliar nutrient applications as well as various factors affecting foliar nutrition. Multiple authors have demonstrated the growing scientific evidence suorting the usefulness of biostimulants as agricultural inputs on diverse plant species. Furthermore, the cited literature also reveals some commonalities in plant responses to different biostimulants, such as enhanced nutrient uptake, increased root growth and improved stress tolerance.

Keywords: Plant biostimulants, foliar nutrition, sustainability.

1 Soil quality and soil health

Soil, like air and water, is regarded as a major natural resource suorting diverse ecosystems, goods and services to the benefit of mankind (Laishram et al., 2012). Soil quality can be defined as “the capacity of a soil to function within the ecosystem and land-use boundaries to sustain biological productivity, promote plant and animal health and maintain environmental quality” (Doran and Parkin, 1994). From an environmental perspective, Sims et al. (1997) defined soil quality as “the capacity of the soil to promote growth of plants; prevent water and air pollution by buffering potential pollutants, such as agricultural chemicals, organic wastes and industrial chemicals and, lastly, protect watersheds by regulating the infiltration and partitioning or precipitation”. A healthy soil suorts the functions of an ecosystem by enhancing the health of plants and animals and, furthermore “a healthy microbial community is vital to productivity, fertility and sustainability of an ecosystem” (Kennedy and Stubbs, 2006).

1.1 Quality parameters of a healthy soil

Kennedy and Stubbs (2006) define soil as a substance that serves as a medium for plant growth by providing physical suort, essential nutrients, water and oxygen for roots. Soil quality is determined by the soil’s chemical, physical and biological components as well as their interactions (Marinkovic et al., 2018). The suitability of a soil to sustain plant growth and biological activity is a function of the combination of physical properties (water-holding capacity, porosity, tilth and structure) and chemical properties (pH, nutrient supplying ability and mineral concentration) many of which are a function of soil organic matter content (Kenndey and Stubbs, 2006). The biological components of a soil are mainly represented by microorganisms and earthworms which play an important role in soil formation and fertility (Marinkovic et al., 2018). Soil contains a multitude of microbes, particularly bacteria, but also actinomycetes, fungi, algae and protozoa (Laishram et al., 2012). Soil microorganisms participate in decomposition of soil organic matter and cycling of nutrients (Marinkovic et al.,

2018). The soil profile plays a key role in the cycling of major elements required by biological systems (C, N, P, S) and further decomposing of organic wastes and detoxifying certain hazardous compounds (Kenndey and Stubbs, 2006). Microorganisms also affect the physical properties of the soil via production of extracellular polysaccharides and other debris, thereby sustaining the soil structure (Marinkovic et al., 2018).

2 Foliar applications of nutrients for crop enhancement

2.1 Means to supply nutrients to plants

Soil and foliar nutrient applications can be used to provide plants with the required nutrients. Foliar applications is considered one of the most common supply methods which can be used to deliver the required nutrients, particularly micro-nutrients, to plants in adequate concentrations, while improving the plant nutritional status, as well as increasing crop yield and quality (Oosterhuis and Weir, 2010). Foliar applications can be further characterized as a technique of feeding plants by applying liquid fertilizer directly to the leaves. The uptake of nutrients through leaves and stomata is usually faster than nutrient uptake via the root system (Fernandez et al., 2013). When applied foliarly, nutrients reach leaf cells after penetrating the cuticle and can be further transported to other parts through plasmodesmata (Kannan, 2010). Foliar applications of nutrients can also provide a more rapid material utilization and permit the correction of observed deficiencies in less time compared with soil applications, provided the leaf does not possess a thick, waxy cuticula as this can hinder penetration into the leaf tissue (Alshaal and El-Ramady, 2017). The advantage of foliar nutrient applications is the immediate nutrient uptake by plants (Alshaal and El-Ramady, 2017). Foliar nutrition has the ability to improve the efficiency and speed of utilization of nutrients urgently required by plants. Foliar fertilization is, therefore, a very effective way of applying fertilizer to plants, particularly when only smaller quantities of certain nutrients are required; compared with soil applications, foliar applications elicits a faster response in the plant (Alshaal and El-Ramady, 2017). The most

prominent use of foliar nutrition is the applications of micronutrients, as only small amounts are required (Oosterhuis and Weir, 2010).

2.2 Effects of foliar nutrient applications

Foliar applications of agrochemicals, including fertilizers, should be increased when seeking sustainability of agriculture (Marschner, 2012). Plants require minerals in addition to carbon-dioxide and water for growth and development; however, most of these minerals exist in the soil and can, therefore, get depleted (Alshaal and El-Ramady, 2017). The availability of phosphorus, potassium and most micronutrients in soil solution is relatively low because these elements are often fixed onto soil particles, becoming insoluble. The more soluble nutrients, such as nitrogen, are, on the other hand, easily leached deeper into the soil profile (Kannan, 2010). The simultaneous applications of foliar nutrition with plant growth and development enables the improvement of quality, nutrition and yield of many agricultural crops (Alshaal and El-Ramady, 2017).

2.3 Environmental factors affecting foliar applications

The effectiveness of foliar nutrition is determined by numerous endogenous, exogenous, as well as environmental factors (Alshaal and El-Ramady, 2017). Environmental factors, which influence the physical and biological aspects of foliar applications, include time of day, humidity, temperature and wind speed (Fernandez et al., 2013) as well as light and photoperiod (Table 2.1). Plant tissue permeability is an important factor in absorption of nutrients into the plant, with warm and moist conditions favouring high tissue permeability (Table 2.1). Such conditions are normally found in the late evening hours (due to the open stomata) and, occasionally, in the early morning hours (Alshaal and El-Ramady, 2017).

The response of plants to foliar nutrition varies not only amongst species and cultivars, but also depends upon the plant's phenology, its physiological status and the environment in which it

is planted in (Marschner, 2012; Kannan, 2010). Physical and physiological characteristics of a plant can alter the efficiency of foliar nutrition in two ways: Differences in canopy surface area and the characteristics of the plant surface have a quantitative impact on the amount of applied nutrients that can penetrate surface barriers, while differences in physiological processes (mineral uptake, storage, translocation) alter both the immediate and long-term biological efficacy of the nutrient once it has entered the plant (Fernandez et al., 2013).

Table 2. 1 Meteorological conditions favouring efficacy of foliar applications (Alshaal and El-Ramady, 2017)

Time of day	Late evening, after 6 pm or early morning before 9 am
Temperature	18 - 29°C, ideally 21°C
Humidity	> 70% relative humidity
Temperature/ Humidity Index	140-160
Wind Speed	Less than 8 kmh

3 Nutrient mobility and transport of foliar nutrient sprays

The efficiency of foliar nutrition applications depends not only on the absorption of the nutrients applied, but also on the transport of these nutrients to other plant parts (Fernandez et al., 2013). Marschner (2012) grouped nutrients into three categories according to phloem mobility: high mobile elements (N, P, K, Mg, S, Cl, Ni), followed by intermediate or conditionally mobile ones (Fe, Zn, Cu, B, Mo) and, lastly, low mobility elements (Ca, Mn). Epstein and Bloom (2005) are in agreement with Marschner (2012) classifying nutrients with regard to phloem mobility into three categories (Table 2.2). Boron is classified as a high or low mobility element, depending on plant species.

Mobility of micronutrients within plants is an important characteristic which determines plant growth and survival under conditions of limited nutrient availability. Factors which determine the overall phloem mobility of a nutrient are:

- ability of the nutrient to enter the phloem
- ability of the nutrient to move within the phloem
- ability of the nutrient to move out of the phloem into plant tissue cells

Table 2. 2: Classification of nutrients with regard to their phloem mobility (Epstein and Bloom, 2005)

High mobility	**Intermediate/ conditional mobility**	**Low mobility**
Nitrogen	Sodium	Calcium
Potassium	Iron	Silicon
Sulphur	Zinc	Manganese
Magnesium	Coer	Boron (species-dependent)
Phosphorus	Molybdenum	
Boron (species-dependent)		
Chlorine		

4 Importance of plant bio-stimulants in agricultural plants

A plant bio-stimulant is defined as "any substance or micro-organism applied to plants with the aim of enhancing nutritional efficiency, abiotic stress tolerance and/or crop quality, regardless of their nutrient content" (Jardin, 2015). Bio-stimulants can also be referred to as "substances, other than organic commercial fertilizers, promoting plant growth when applied in low quantities" (Kauffman et al., 2007). Brown and Saa (2015) describe plant biostimulants as "substances and micro-organisms whose function, when applied to plants or the rhizosphere,

is to stimulate natural processes, such as enhancing nutrient uptake, nutrient efficiency, tolerance to abiotic stress and general crop quality". Bulgari et al. (2015) further describe plant bio-stimulants as "plant extracts which contain a wide range of bioactive compounds which are mostly still unknown and are able to improve nutrient use efficiency of plants and enhance their tolerance to biotic and abiotic stresses". Bio-stimulants are defined as "extracts obtained from organic raw materials containing bioactive compounds which contain traces of natural plant hormones" (Bulgari et al., 2015).

Bio-stimulants function by virtue of the presence of essential mineral elements and plant hormones (Brown and Saa, 2015). Bio-stimulants benefit plant productivity by interacting with plant signalling processes (Calvo et al., 2014). Brown and Saa (2015) have reported that bio-stimulants may either directly interact with plant signalling cascades or act through stimulation of endophytic and non-endophytic microorganisms (bacteria, yeast and fungi) to produce molecules which will enhance plant growth and development. Calvo et al. (2014) noted that the benefit of bio-stimulants is derived from the reduction in assimilates that are diverted to the unproductive stress response metabolism. Many bio-stimulants contain simple or complex carbohydrates that, when applied to the plant, alter its metabolism by directly acting as a source of energy for beneficial microbial populations (Jardin, 2015). The complexity of the roles of carbohydrates in 'plant immunity' has been reviewed by Trouvelot et al. (2014) who suggested that carbohydrates activate defence reactions to pathogens associated molecular patterns (PAMP's), microbe associated molecular patterns (MAMP's), and damage molecular patterns (DAMP's).

Bio-stimulants have no direct effect on pests and, therefore, do not fall within the regulatory framework of pesticides; therefore, they are often marketed as 'fertilizers' in mixture with nutritive chemicals (Colla and Rouphael, 2015). Bio-stimulants differ from fertilizers acting

on the plant's metabolism, often through an increase in root development; they can be applied through soil drench or via foliar nutrition (Bulgari et al., 2015).

Plant bio-stimulants are normally applied to high-value, mainly greenhouse, crops, fruit trees, open-field vegetables, flowers and ornamentals to increase yield and product quality in a sustainable way (Colla and Rouphael, 2015). Bio-stimulant products were initially used in organic production; however, recently they have been introduced to conventional crop production to respond to economic and sustainability demands (Colla and Rouphael, 2015). Bio-stimulants have been reported to be effective in improving plant productivity, often by increasing the synthesis of secondary compounds and enhancing the activity of enzymes (Bulgari et al., 2015). Bio-stimulants can be used in vegetable production to improve plant performance, yield and general plant health, as well as to enhance plant tolerance to various stresses (Bulgari et al., 2015). Abdalla (2013) has reported that bio-stimulants darken the colour of leaves by stimulating chlorophyll synthesis in the leaves of cowpeas (*Vigna unguiculata*). Plant bio-stimulants can be divided into microbial stimulants, humic acids, fulvic acids, protein hydrolysates and amino acids, seaweed extracts and moringa extracts (Calvo et al., 2014).

4.1 Microbial stimulants

In agriculture the use of microbial stimulant, organisms that produce substances beneficial to plant agriculture, has greatly increased during the past two decades, both in the private and public sector. Microbial stimulants are commonly classified as biocontrol agents (also called biopesticides) or biofertilizers (Calvo et al., 2014). According to Vejan et al. (2016), microbial stimulants play a crucial role in maintaining soil fertility and enhancing plant growth and development. In agreement with these authors, Singh et al. (2016) have noted that microbial stimulants play an important role in sustainable crop production due to their immense plant-growth-promoting attributes, such as improved environmental adaptability and reducing pesticide and fertilizer applications to plants. Beneficial microbial inoculants are plant growth-

promoting bacteria and fungi that function through various mechanisms, such as enhancing the supply of nutrients to plants, production of plant hormones and suression of various crop pests (Toyota and Watanable, 2013).

Biofertilizers are regarded as natural products containing living micro-organisms that, when applied to plants, promote growth by a variety of mechanisms. Such mechanisms include the increase in supply of nutrients, in root biomass and root area and, therefore, in nutrient-uptake capacity (Calvo et al., 2014). In Adesemoye et al.'s (2017) view, beneficial microbes interact with plants and suort plant health in various ways, such as enhancing plant growth, and, thereby, yield, but also controlling pests, contributing to the survival in and recovery from diverse environmental conditions, including drought. Calvo et al. (2014) have reported that microbial stimulants mainly include free-living bacteria, and arbuscular mycorrhizal fungi. The presence of beneficial microbes in the rhizosphere minimizes the susceptibility of crops to diseases because of the ability to produce molecules, enhancing plant growth and products of the secondary metabolism; hence, beneficial microbes are widely used as commercial bio-inoculants. Calvo et al. (2014) noted that some microorganisms increase the availability of selected soil nutrients through enhanced solubilisation of these nutrients, thereby allowing plants to take up nutrients more efficiently.

Plant growth-promoting rhizobacteria (PGPR) are a group of bacteria which can be found in the rhizosphere; these microbes colonize the rhizosphere and include bacteria, fungi, protozoa and algae (Toyota and Watanable, 2013). The rhizosphere, as the zone of maximum microbial activity, contains a confined nutrient pool of essential macro- and micro-nutrients that can be extracted by plant roots (Vejan et al., 2016). Adesemoye et al. (2017) confirm that the major PGPR genera studied include *Azotobacter* s, *Azospirillum* s., *Pseudomonas* s., *Achromobacter* s., *Burkholderia* s. and *Bacillus* s. Based on the interactions with plants, PGPR can be separated into symbiotic bacteria with directly or indirectly beneficial mechanisms. Symbiotic bacteria

live inside plants, exchanging metabolites with these plants directly, while free-living rhizobacteria live outside the plant (Vejan et al., 2016). A direct beneficial mechanism of microbes refers to either biofertilization, stimulation of root growth and/or plant stress control. Furthermore; indirect mechanisms reduce the impact of disease, which include induction of systemic resistance and competition for nutrients (Vejan et al., 2016).

According to Trabelsi and Mhamdi (2013) microbial stimulants play an important role in agricultural systems, particularly plant growth-promoting micro-organisms (PG-PMS); benefits of these organism are attributed mainly to three mechanisms: firstly, PG-PMS act as biofertilizer (such as nitrogen- fixing bacteria and phosphate-solubilizing bacteria) which assist plant nutrient uptake by providing nitrogen and other plant nutrients; secondly, as phytostimulants (microbes producing phytohormones, such as *Azospirillum*) can directly promote plant growth by making plant hormones available during plant growth stage, and lastly, biological control agents, such as *Trichoderma*, *Pseudomonas* and *Bacillus* that can protect plants against phytopathogenic organisms. The different soil microorganism which have been extensively used as stimulants include bacteria such as *Rhizobium*, *Azospirillum*, other mycorrhizal organisms such as Glomeromycota and arbuscular mycorrhiza (Trabelsi and Mhamdi, 2013).

4.2 Humic acids

Humic acids are end-products of microbial decomposition and chemical degradation of biota in soils and are considered the most abundant, naturally occurring organic molecules on Earth as major components of soil organic matter (Calvo et al., 2014). Nardi et al. (2009) also noted that the difference between humic and fulvic acids is that humic acids are characterised by high molecular mass, while fulvic acids are of low molecular mass. Trujillo (2017) describes humic acids as ‘naturally occurring compounds, resulting from the decomposition and transformation of plant, animal and microbial residues. In the view of Nardi et al. (2015) humic acids ‘consist

of organic material resulting from concerted reactions of various biotic and abiotic processes'. The complex assemblage of molecules into humic acids is derived from plant and animal debris and results in one of the most abundant organic substances on Earth; furthermore, humic acids are present in both, aquatic environments and the atmosphere (Nardi et al., 2015). Piccolo and Spiteller (2003) have reported that humic acids play key roles in various soil and plant functions, such as controlling nutrient availability, carbon and oxygen exchange between the soil and the atmosphere, as well as the transformation and transport of chemicals. Humic acids present in the soil affect plant physiological processes, as well as the composition and function of rhizosphere microorganisms (Calvo et al., 2014).

Humic acids have been reported to enhance some aspects of plant growth in over 16 plant species, including agronomic crops, such as soybean (*Glycine max*), wheat (*Triticum*), rice (*Oryaz sativa*), and maize (*Zea mays*), vegetables crops, such as potatoes (*Solanum tuberosum*), tomatoes (*Solanum lycopersicum*), cucumber (*Cucumis sativus*) and pepper (*Capsicum*), and fruit crops, such as citrus (*Citrus limon*) and grape (*Vitis vinifera*) as well as *Arabidopsis* (Calvo et al., 2014). According to Trevisan et al. (2010) humic acids have beneficial effects on plant physiological processes by improving soil structure and fertility, thereby affecting nutrient uptake and root architecture, since fractions of humic acids interact directly with root structures. The promotion of root development by humic acids is the most commonly reported effect of humic acids on plant growth. This promotion of root growth includes the enhancement of seedling and lateral root growth (Calvo et al., 2014).

Additionally, applications of humic acids also increases yield and crop quality (Calvo et al., 2014). Nardi et al. (2009) noted that most humic acids bind tightly to plant cell walls and can be absorbed by roots, while some are transported to the shoots. Humic acids have auxin-like effects on plants, the main reason for their diverse beneficial effects on plants (Calvo et al., 2014). Some of the benefits of humic acids in soil include improved soil aggregation and

structure, increased pH-buffering and enhanced cation exchange capacity, as well as increased water-holding capacity, bioavailability of phosphorus, iron and zinc. Humic acids also decrease the toxicity of aluminium and heavy metals (Trujillo, 2017). In agreement with Trujillo (2017), Nardi et al. (2015) noted that humic acids increase soil fertility by influencing soil structure and porosity through an effect on particle aggregation; furthermore, humic acids allow for a more constant nutrient supply through chelating minerals. Humic acids also represent the main source of available organic carbon in the soil (Trujillo, 2017).

4.3 Fulvic acids

Fulvic acids are a family of natural compounds, they are organic acids and components of humus. The difference between humic and fulvic acids lies in the degree of polymerization, (fulvic acids have a greater total acidity, greater numbers of carboxyl groups, and higher adsorption and cation exchange capacities than humic acids.) (Canellas et al., 2015). Fulvic acids are organic acids that arise naturally in decomposing elements as part of their molecular complexes (Mahoney et al., 2016). These acids are created in extremely small amounts by millions of beneficial microbes, with the aim to decay plant matter (Canellas et al., 2015). Fulvic acids are considered as a soil organic fraction soluble in both alkaline and acidic soil water (Calvo et al., 2014) and said to have greater total acidity, greater numbers of carboxyl groups and higher adsorption and cation exchange capacity than humic acids (Bocanegra et al., 2006). Fulvic acids can act as chelating agents and metabolize metal ions, including iron and zinc. Given their small molecular size, fulvic acids can pass through pores of biological or artificial membrane systems (Calvo et al., 2014). According to Zimmerli et al. (2008), fulvic acid can remain in soil solution even at high salt concentrations and at a wide range of pH due to the low molecular mass; therefore, they have the ability to consistently interact with plant roots (Varanini and Pinton, 2001).

Fulvic acid has been reported to enhance some growth aspects of plants, including agronomic crops, such as soybean, wheat, maize and rice, and vegetable crops, such as green beans, tomato, cucumber, peer as well as tree species including wild olive (*Olea europaea*) (Calvo et al., 2014). Studies conducted by Poapst and Schnitzer (1971) reported enhanced root growth and an increase in number of initial roots of green bean, six days after treatment with fulvic acid. Eyheraguibel et al. (2008) observed a significant increase in root elongation of maize seedlings treated with fulvic acid and a general increase in above-ground plant growth, while an enhancement in shoot growth has been noted in fulvic-acid-treated tomatoes by Calvo et al. (2014). Anjum et al. (2011), furthermore, described an increase in shoot dry mass following fulvic acid treatment of maize. Foliar applications of fulvic acid was also evaluated in pot experiments and field trials, investigating effects of such treatment on plant growth and yield of wheat under drought conditions. Applications of fulvic acid reduced stomatal conductance and resulted in higher relative water content, higher water potential and chlorophyll content in treated plants at the end of a 9 day drought cycle. In a glasshouse study, seed treatment with fulvic acid significantly increased leaf area and above-ground dry mass of tomato seedlings (Calvo et al., 2014); moreover, fulvic acid enhanced multiple fruit quality parameters, including total soluble solids, antioxidant activity, total phenolics, carbohydrates and carotenoids in avocado (*Persea americana*) (Eyherguibel et al., 2008).

4.4 Protein hydrolysates and amino acids

Natural bio-stimulants, like protein hydrolysates, are gaining attention as a source of nutrients, as they not only contain minerals, but also contain substances that display hormone-like functions and influence plant metabolism by interacting with basic biochemical processes and physiological mechanisms, such as glycolysis and nitrogen assimilation (Ertani et al., 2014). Protein hydrolysates are a group of plant bio-stimulants containing a mixture of amino acids and peptides, which are mainly produced by enzymatic or chemical hydrolysis of proteins from

animal- (leather, viscera, feathers and blood) or plant-derived raw materials (Colla et al., 2014). Protein hydrolysates can be classified on the basis of the protein source and the method of protein hydrolysis (Fig. 2.1).

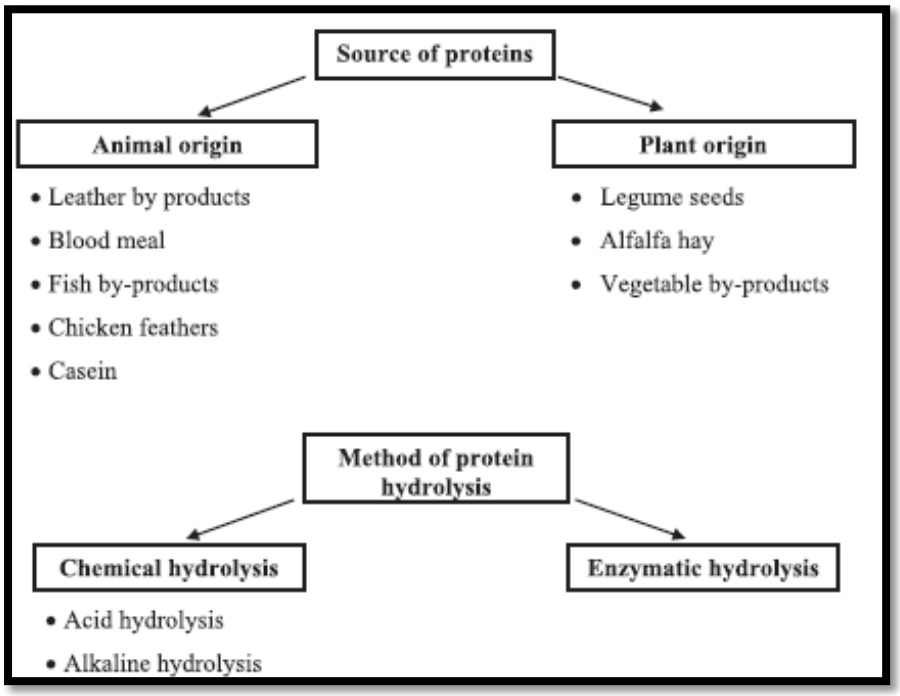

Figure 2. 1: Classification criteria of protein hydrolysates on the basis of protein source and method of protein hydrolysis used in the hydrolisation process. (Adapted from Colla et al., 2015)

Besides amino-acids and peptides, protein hydrolysates contain other compounds that contribute to their bio-stimulant action. These compounds include fats, phenols, carbohydrates, mineral elements, phytohormones and other organic compounds (Ertani et al., 2014); moreover, plant-based protein hydrolysates contain phenolics and soluble carbohydrates which play a role in energy metabolism and oxidative stress defences, while animal-derived protein hydrolysates lack phenolics and phytohormones.

Lisiecka et al. (2011) identified that protein hydrolysates improve the performance of several horticultural crops, increasing root and shoot biomass as well as quality. According to Ertani et al. (2014) applications of plant hydrolysates to leaves and roots of tomato increased Fe and

N translocation, water and nutrient uptake and nutrient use efficiency of both macro- and micro-nutrients. Studies conducted by Garcia-Martinez et al. (2010) demonstrated higher nutrient uptake by protein- hydrolysate-treated plants and attributed to an increase in soil microbial, enzymatic activity and improvement of micronutrient mobility and solubility, in particular of Fe, Zn, Mn and Cu. Protein hydrolysates have shown not only to improve plant nutrition, but also the quality of vegetables and fruit in terms of enhancing presence of phytochemicals, such as carotenoids and polyphenols (Parrado et al., 2007). Studies conducted by Morales- Pajan and Stall (2003) on papaya (*Carica papaya*) demonstrated that monthly foliar applications of animal-derived protein hydrolysates increased marketable yield by 22% in comparison to untreated plants.

4.5 Seaweed extracts

Seaweed is a diverse assemblage of close to 10 000 species of brown, red and green algae (Battacharyya et al., 2015); however, of these, brown seaweed is the one commonly used commercially. Brown seaweeds are normally brown in colour and they belong to the Phaetophyceae family (Pal et al., 2014). Brown seaweeds include some of the largest and most complex seaweeds; such as kelps, wracks and sargassums and are particularly common in temperate zones of the world (Pal et al., 2014). Seaweed extracts are, therefore, a complex mixture of component that may vary according to seaweed species and source, season of collection and extraction process used (Khan et al., 2009; Rioux et al., 2009). Most commercial seaweed extracts are made from brown seaweed genera including *Fucus, Laminaria, Sargassum, Turbinaria* and *Ascophyllum* (Calvo et al., 2014).

Seaweed extracts can be used on horticultural crops to enhance plant performance and are available as liquid extracts or in a soluble powder form. Liquid extracts are normally applied via the drip irrigation water (Battacharyya et al., 2015). Seaweed extracts can also be applied foliarly to a variety of flowers, vegetables and tree crops, including tomato, potato, cherry

(*Prunus avium*), plum (*Prunus domestica*), mango (*Mangifera indica*) and almond (*Prunus dulcis*). The biostimulant effect of seaweed has been attributed to the presence of plant growth hormones, namely auxins, cytokinins, gibberellins, abscisic acid and ethylene, as well as to hormone-related low molecular weight compounds present in the extracts (Tarakhavskaya et al., 2007).

According to Rayorath et al. (2009) one of the major components of commercial extracts of all seaweeds are polysaccharides, as they account for up to 30-40% of the extract on a dry mass basis. The common polysaccharides found in brown seaweed extracts are fuccidan, alginates, laminarin and fucose-containing glucans. Seaweed, particularly brown seaweed, is rich in phenolic compounds. Seaweed extracts can alter physical, biochemical and biological properties of the soil and may also affect the architecture of plant roots facilitating efficient nutrient uptake (Calvo et al., 2014). Studies conducted by Khan et al. (2009) revealed a wide range of beneficial effects of seaweed extract applications to plants, such as early seed germination and establishment, improved crop performance and yield, elevated resistance to biotic and abiotic stress and, lastly, enhanced postharvest shelf-life of perishable products (Fig. 2.2).

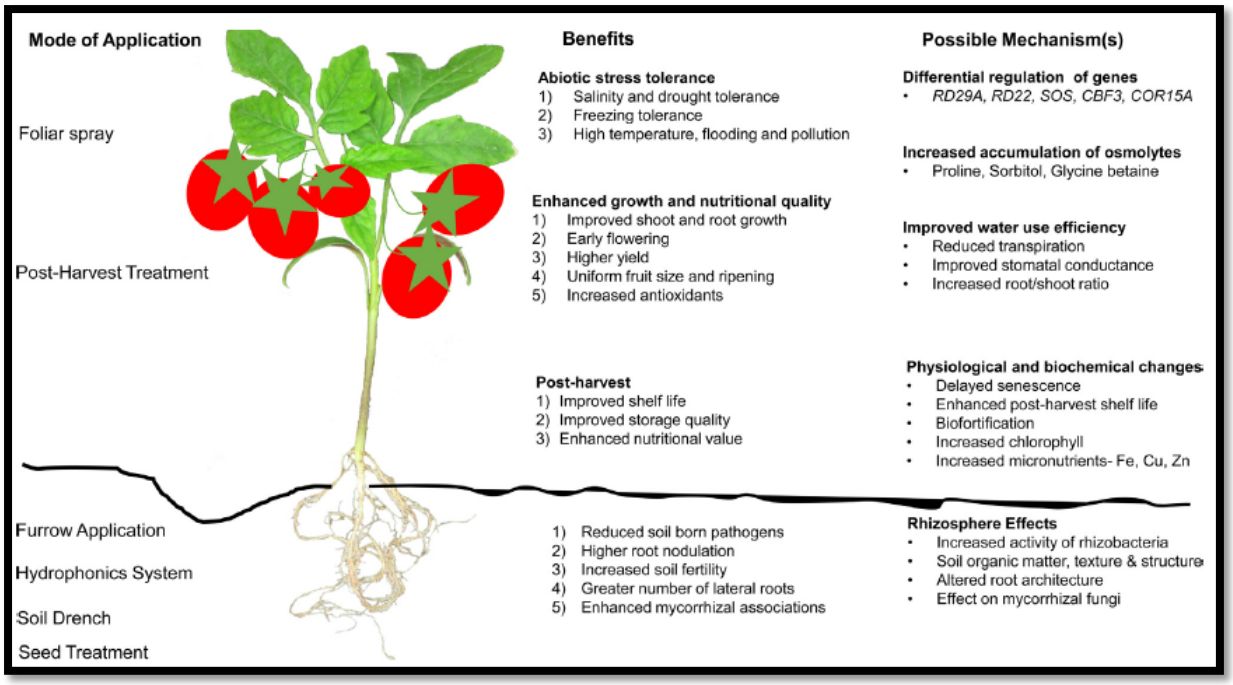

Figure 2. 2: Methods of applications of seaweed extracts and their effects on plant and mechanisms of action. (Adapted from Battacharyya et al., 2015).

5 Conclusion

There is great potential of various categories of biostimulants to improve crop production as well as the effectiveness of foliar nutrition, making minerals available for plant growth and development. The applications of bio-stimulants in vegetable crop cultivation allows higher levels of sustainability through the reduction of fertilizers and environmental contamination, while increasing plant tolerance to abiotic and biotic stresses enhancing internal and external quality. The effect of biostimulants is, however, not always consistent among plant species. Many scientific papers have reported the effects of bio-stimulant applications on plants, but few have investigated the effects of such compounds on the plant's physiology and biochemistry. Moreover, recent papers have focused on the mechanisms of action of bio-stimulants. Henceforth, this study will mainly focus on use of *Moringa oleifera* leaf extract as a foliar applications to enhance growth and development, mineral composition and antioxidant properties on horticultural root crops (using radish as example) and fruit crops (using green

beans as example). *Moringa oleifera* has been identified to contain natural antioxidants, particularly in its leaves, due to the presence of various compounds such as phenolics, carotenoids, ascorbic acid and flavonoids.

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CHAPTER 3

EVALUATION OF ANTIOXIDANT PROPERTIES IN *MORINGA OLEIFERA* LEAF POWDER

Abstract

It is believed that two-thirds of the world's plants have medicinal properties and almost all of these plants have high antioxidant potential. Natural antioxidants, such as flavonoids, ascorbic acid (vitamin C), tocopherols and other phenolic compounds are known to be present in many plants. *Moringa oleifera* is one of such plants that has been identified to contain a wealth of natural antioxidants. The leaves of moringa are a good source of natural antioxidants, such as phenolics, carotenoids, ascorbic acid and flavonoids; therefore, the presence of various types of antioxidant compounds enables plant leaves to become a valuable source of natural antioxidants. In this study the antioxidant compounds (ascorbic acid, phenolics, carotenoids and total chlorophylls) of moringa leaf extract (MLE) were evaluated. Findings revealed that MLE contains high amounts of antioxidants (0.14 $mg*g^{-1}$), ascorbic acid (0.75 $mg*g^{-1}$) and total phenolics (0.80 $mg*g^{-1}$). Therefore, MLE is a good source of natural antioxidant compounds that can have beneficial effects on other plants. Furthermore; findings show that MLE contains high amounts of total chlorophyll (10.10 $mg*g^{-1}$) and further potent antioxidant, indicating that MLE could be used to enhance plant performance.

Keywords: Antioxidants, Moringa leaf extract, Ascorbic acid (Vitamin C), Phenolics, Carotenoids, Chlorophylls

1 Introduction

Antioxidants play important roles as inhibitors of oxidation process and are, therefore, crucial in preventing plant disease and damage caused by pollutants (Akbarirad et al., 2016). Natural antioxidants are commonly present in plants; therefore, the basic source of these compounds for humans is plant-based. Food rich in antioxidants may enhance the protection against free radical damage, not only in food but also in the human body, protecting it against cardiovascular disease and nucleic acids (Halliwell, 2013). According to Kasote et al. (2015), two-thirds of the world's plant species have medicinal properties, and extracts of almost all of these plants have a high antioxidant capacity. The antioxidants found in plants have received wide attention because increased oxidative stress has been identified as a major factor causing the development and progression of several life-threatening diseases, such as neurodegenerative ones (Krishnaiah et al., 2011). Kumar (2014) further explained that natural antioxidants occur in all parts of plants, with fruit and vegetables containing various antioxidant compounds, such as carotenoids, vitamins, flavonoids and phenolics.

There is a wide variety of naturally occurring antioxidants which differ in their physical and chemical properties, composition, mechanisms and site of action (Gupta and Sharma, 2005). Certain enzymes Superoxide dismutase (SOD), Catalase (CAT), Glutathione peroxidase (GPx) are present in the plasma and act as antioxidants by transforming reactive oxygen species and nitrogen species into stable compounds (Gupta and Sharma, 2005). Certain low molecular mass compounds are lipo-soluble antioxidants or water-soluble antioxidants (Gupta and Sharma, 2005). Tocopherol and quinines form part of the lipo-soluble antioxidants, while ascorbic acid, and phenolics form part of water-soluble antioxidants. Lastly, minerals and vitamins, such as Vit A, C and E are antioxidants that play a crucial role in preventing peroxidation damage in plants and animals (Gupta and Sharma, 2005).

Plants have efficient, complex enzymatic and non-enzymatic antioxidant defence systems to avoid the toxic effects of free radicals (Fig. 3.1). Enzymatic systems include SOD, (CAT) and (GR); while non-enzymatic systems consist of low molecular mass antioxidants (ascorbic acid, proline, glutathione, phenolic, carotenoids, flavonoids) and high molecular mass secondary metabolites, such as tannins (Kasote et al., 2015). Plants require antioxidants to protect themselves against oxidative stress (Fig. 3.1); therefore, plants have evolved the ability to synthesize antioxidant components. Under biotic and abiotic stress, plants accumulate low molecular mass antioxidants, as well as antioxidants of high molecular mass, such as tannins; all these compounds confer antioxidant activity through functioning as free radical scavengers (Kasote et al., 2015).

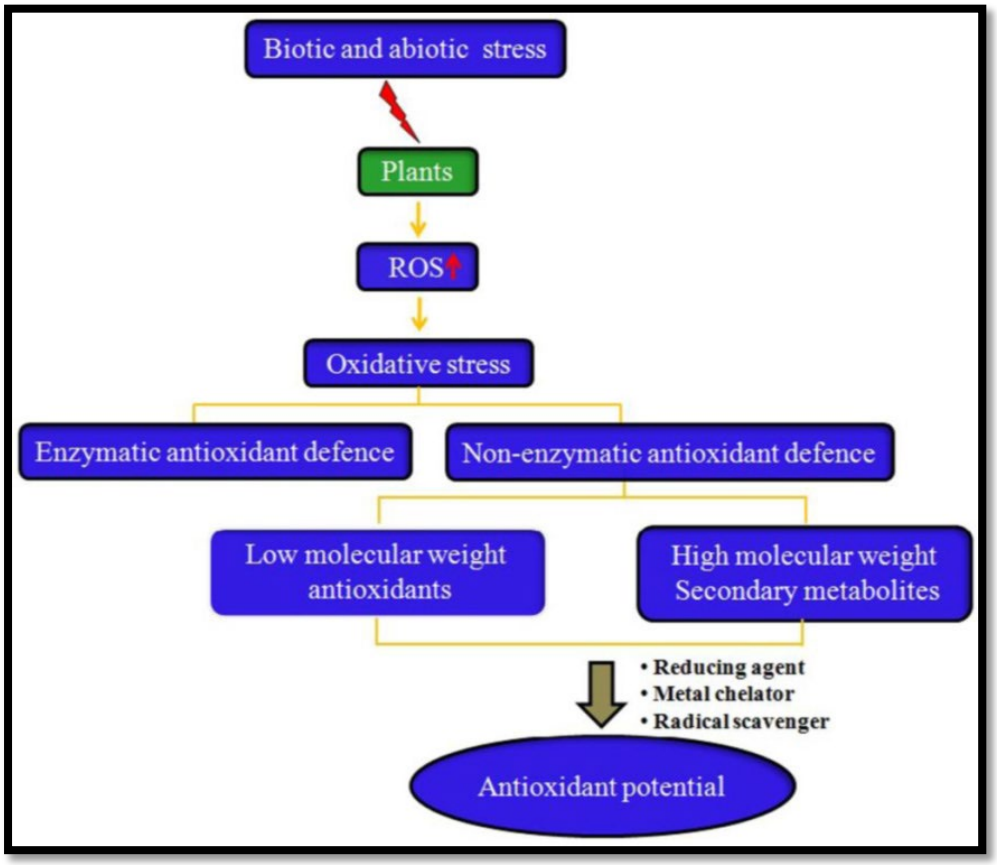

Figure 3. 1: Functions of antioxidants in plants (Adapted from Kasote et al., 2015).

Natural antioxidants, such as tocopherols, vitamin C, flavonoids and other phenolic compounds are known to be present in many plants and *Moringa oliefera* is one of the plants (Pakade et al., 2013). *Moringa oleifera* is a perennial plant known for its high antioxidant levels (Anwar et al., 2007) and is native to Asian, African and Central American countries (Welch and Tietjie, 2017). It is one of the most-cultivated species of the genus *Moringa* belonging to the Moringaceae family (Mohammed et al., 2015). *Moringa oleifera* is a highly valued plant found in different parts of the tropics and subtropics (Mohammed, 2015). All plant parts possess some functional and nutraceutical properties (El Sohaimy et al., 2015); hence, the plant has been used in traditional medicine for various medical conditions for an extended period of time (Singh et al., 2012). Many parts of the *M. oleifera* tree are highly nutritious, containing various minerals, vitamins, antioxidants, proteins, amino acids and phenolics (Anwar et al., 2007). The leaves of moringa are good sources of natural antioxidants, containing, phenolic compounds, carotenoids, ascorbic acid and flavonoids (Makkar and Becker, 1997; Dillard and German, 2000); these antioxidant compounds make moringa leaves a valuable source of natural antioxidants (El Sohaimy et al., 2015).

The recent focus on antioxidants in the human diet has been fuelled by the rise in diseases that seemingly can be suressed or partly counteracted by antioxidants (Mohammed, 2015). Antioxidants perform a number of functions, such as enzyme inhibitors, free radical scavengers, damage reduction caused by free radical activity and oxidation which aid in stress-prevention that otherwise might cause several degenerative diseases (Mohammed, 2015). Various antioxidants, such as flavonoids, vitamin C, tocopherols and other phenolic compounds are known to be present in *Moringa oleifera* (Anwar et al., 2007). Therefore, the experiment will focus on the evaluation of antioxidant properties in *Moringa oleifera* leaf powder.

2 Materials and Methods

2.1 Plant material

Moringa leaf powder was purchased from a local commercial retailer (RunX-KZN, Pietermaritzburg, South Africa). The powder was further arranged into three (5 g), resulting in a total of 15 samples.

2.2 Biochemical leaf powder analysis

2.2.1 Determination of Antioxidants

Samples of 0.5 g dried, fine powder was weighed and placed into a test tube; thereafter, 10 mL 80% methanol was added to the sample. The solution was homogenised using a top vortex mixer (Heidolph REAX 2000, Burladingen, Germany) and placed into an oven for 24 hr at 40°C.

Preparations of reagents was carried out according to Brand-Williams et al. (1995). An amount of 0.0024 g DH was dissolved in 100 mL methanol to prepare the DH working solution.

Total antioxidant activity was determined using the DH (2, 2 – diphenyl- 1 - pycrylhydrazyl) assay described by Miliauskas et al. (2004); DH is one of the few stable and commercially available organic nitrogen radicals. The antioxidant effect is proportional to the disappearance of DH in test samples (MacDonald-Wicks et al., 2006). Briefly, sample extract (1 mL) was transferred into test tubes, DH solution (1 mL) added and the volume made up to 4 mL with 95% methanol. The mixture was placed at ambient temperature for 30 min before the absorbance was read at 515 nm with a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

2.2.2 Determination of Vitamin C

Vitamin C was determined according to Mohammed et al. (2018) with slight modifications. An amount of 0.5 g DM was weighed out into centrifuge tubes and mixed with 4 mL 0.56 M metaphosphoric acid, shaken and centrifuged in a table-top centrifuge (PLC – 05 Centrifuge

PLC Gemmy, Berlin, Germany) at 3000 rpm for 10 min. Thereafter, the solution was mixed with 2 mL 0.3 M trichloro acetic acid and again centrifuged at 2000 rpm for 10 min. The supernatant was mixed with 2 mL 2, 4-dinitrophenyl-hydrazine reagent. Finally, samples were heated and maintained at to 60°C for 1hour, followed by cooling in an ice bath for 5 min. Addition of 4 mL 18 71 M sulphuric acid into the samples which resulted into a colour change to a blue hue and absorbance (spectrophotometer UV-1800 Shimadzu, Kyoto, Japan) was read at 520 nm after 20 min.

2.2.3 Determination of Phenolics

Samples of 0.1g dried fine powder was extracted with 10 mL of 1% (v/v) hydrochloric acid in methanol, followed by ultra-sonication (35 Hz) at ambient temperature for 20 mins. The mixture was then centrifuged in a table-top centrifuge (PLC – 05 Centrifuge PLC Gemmy, Berlin, Germany) at 400rpm for 10 mins.

Total phenolics was determined according to Boonkasem et al. (2015). An amount of 0.2 mL sample extract was transferred into a test tube, 1 mL 10% Folin- Ciocalteu reagent added, and the test tube thoroughly shaken using a top vortex mixer (Heidolph REAX 2000, Burladingen, Germany). After 3 min, 0.8 mL 7.5% Na_2CO_3 solution was added and the mixture allowed to stand for 30min at room temperature. Thereafter, absorbance was measured at 765 nm. Gallic acid was used as standard and the total phenolic content calculated as gallic acid equivalent (GAE) and expressed as g GAE per 100 g dry mass (% g DM).

2.2.4 Determination of Chlorophyll a, Chlorophyll b and Total Carotenoids

The concentration of chlorophyll a, chlorophyll b and total carotenoids (xanthophylls and carotenes) in green bean pods was determined spectrophotometrically. An amount of 1 g lyophilized, fine powder was weighed out into a centrifuge tube, 4 mL acetone/ water (4:1) added and the samples left to stand for 10 min, covered with aluminium foil. The samples were

then placed into a table-top centrifuge (PLC – 05 Centrifuge PLC series, Berlin, Germany) for 5mins at 1000 rpm. Thereafter, samples were transferred into clean test tubes. Absorption was read at various wavelength (663.3, 649.8 and 470 nm) using the equation by Lichtenthaler and Buschmann (2001) to determine chlorophyll a, chlorophyll b, total chlorophylls and total carotenoids.

2.3 Statistical analysis

The data were statistically analysed using GenStat (version 14.1) (VSN, Hemel Hempstead, England, UK.). Analysis of variance (ANOVA) was used to test the overall significance of the data and Duncan's multiple range tests at 5% level of significance was used

3 Results

3.1 Antioxidants

Antioxidant activity of the *Moringa oleifera* leaf extract was assessed using the 2, 2-diphenyl-1-picrylhydrazyl (DH) assay. The antioxidant activity of the leaf extract ranged between (0.92-1.23 $mg*g^{-1}$), with a mean value of 1.55 $mg*g^{-1}$. Table 3.1 further illustrates that there was no significant difference ($p> 0.05$) in antioxidant activity of the prepared moringa leaf samples between the replications.

3.2 Ascorbic Acid

There was a slight, but significant difference ($p> 0.05$) between moringa leaf samples in ascorbic acid concentration, ranging between 0.17 - 0.40 $mg*g^{-1}$ leaf powder, with a mean concentration of 0.45 $mg*g^{-1}$ ascorbic acid/g leaf powder (Table 3.1).

3.3 Phenolics

There was no significant effect ($p> 0.05$) on total phenolics found in moringa leaf powder; means were in the same range of total phenolics (0.75 – 0.83 $mg*g^{-1}$) (Table 3.1). The total mean of phenolic compounds found was 0.80 $mg*g^{-1}$.

Table 3. 1 : Antioxidant compounds found in moringa leaf powder.

Phytochemicals	**LSD**	**CV%**	**Range concentration (3 replicates) (mg*g^{-1})**	**Mean concentration ±SD (3 replicates) (mg*g^{-1})**
Antioxidants	0. 05	2. 8	0. 92 – 1. 23	1. 55 ± 0. 83
Vitamin C	0. 14	41. 3	0. 17 – 0. 40	0. 45 ± 0. 75
Phenolics	0. 04	4.1	0. 75 – 0. 83	0. 80 ± 0. 07

The values in the table are means of triplicates ± SD, *P> 0.05*.

3.4 Chlorophylls and Carotenoids

Table 3.2 illustrates that chlorophyll a and chlorophyll b concentrations found in moringa leaf powder were in the range of 5.49 – 7.11 and 3.3 – 4.55 mg *g^{-1}, respectively. The concentration of total chlorophylls was 8.79 -11.65 mg*g^{-1}, more than 4 times higher than the total carotenoid concentration (1.83 – 2.06 mg*g^{-1}). Furthermore, moringa leaf powder contained total chlorophyll concentration of 10.10 mg*g^{-1}, while less carotenoids (2.10 mg*g^{-1}) were present in the extract.

Table 3. 2: Total chlorophylls and carotenoids found in moringa leaf powder.

Pigments	**LSD**	**CV%**	**Range concentration (3 replicates) (mg*g^{-1})**	**Mean concentration ±SD (3 replicates) (mg*g^{-1})**
Chlorophyll a (C_a)	1. 25	16. 2	5. 49 – 7. 11	6. 21 ± 0. 26
Chlorophyll b (C_b)	0. 88	18. 2	3. 3 – 4. 55	3. 90 ± 0. 22
Total chlorophylls (C_{a+b})	2. 13	16. 9	8. 79 – 11. 65	10. 10 ± 0. 25
Total carotenoids (C_{x+c})	0. 45	17. 2	1. 83 – 2. 06	2. 10 ± 0. 27

Values are means of triplicate determinations ± SD, *P> 0.05*.

4 Discussion

4.1 Antioxidant properties present in *M. oleifera* leaf powder

Most antioxidant compounds are derived from plant sources and encompass a wide range of chemical compounds, and, therefore, display varying chemical properties (Fitriana et al., 2016). Confirming the present findings, literature clearly shows that antioxidant compounds are a common feature of moringa leaves. Findings from the current study shows that moringa leaf powder contains relatively high amount of antioxidants (Table 3.1). Yang et al. (2006) noted that the antioxidant concentration of moringa leaves (on a fresh and dry mass basis) is higher than that of fruit and vegetables known for their high levels of antioxidants, such as carrots (*Daucus carota subsp. sativus*) being high in β-carotene (1.8 µmol g^{-1} FM), hot peer being high in ascorbate (110 µmol g^{-1} FM), soya beans being high in α-tocopherol (1.8 µmol g^{-1} FM) and strawberries (*Fragaria * ananassa*) being high in phenolics (190 µmol GAE g^{-1} FM). Mohammed et al. (2018) further explained that moringa leaves outperform some vegetables as sources of antioxidants, as studies by Pakade et al. (2013) revealed that the total phenolic content of moringa leaves is twice that of peas (*Pisum sativum*), spinach (*Spinacia oleracea*),

cauliflower (*Brassica oleracea var. botrytis*) or broccoli (*Brassica oleracea var. italica*). *Moringa oleifera* is regarded as a plant of high nutritional value due to its high concentration in primary and secondary metabolites. Moringa leaves have been considered as a good source of natural antioxidants due to the prevalence of various polyphenolic compounds, such as flavonoids, phenolics and flavonols (Mohammed, 2015). Findings from the current study are in agreement with Yasmeen et al. (2013) who reported that the foliar applications of moringa extract acts as exogenous plant growth enhancer due to the presence of phenolics, carotenoids, ascorbates and other antioxidants (Table 3.1) as well as the presence of essential plant nutrients.

Ascorbic acid is a water-soluble organic compound which participates in many biological processes (Ahmed et al., 2016). *Moringa oleifera* leaves (FM) are well-known to be rich in vitamin C, possessing more than five times the amount present in orange juice (Mohammed, 2015). The analysis of moringa leaf powder revealed that it contained high levels of vitamin C (Table 3.1). Furthermore; Sankhyan et al. (2013), who reported that the drying process of moringa leaves adversely affects the amount of vitamin C, showed that dried moringa leaves contain 0.07 – 0.14 mg/g vitamin C, which was much lower in comparison to fresh leaves (0.17 mg/g - 0.40 mg/g).

Total leaf pigments, namely chlorophyll a, chlorophyll b and carotenoids, are necessary for a balanced photosynthetic activity (Hala and Nabila, 2017). Chlorophylls and carotenoids are very common pigments, not only participating in photosynthesis, but also giving colour to vegetables and fruits (Hala and Nabila, 2017). In the current study, acetone extraction of moringa leaves revealed a higher total chlorophyll than total carotenoid in moringa leaves (Table 3.2). Yameogoe et al. (2011) mentioned that moringa leaves contain several major elements, such as Mg, a constituent of chlorophyll a and chlorophyll b and other macro-elements required for growth and development. Hence, when applied foliarly, MLE is able to enhance the leaf chlorophyll concentration and, thereby, the photosynthetic activity of plants.

These findings are in agreement with Owusu (2008) who noted that moringa leaves contain a high concentration of plant pigments (carotenoids, particularly ß-carotene and lutein, and chlorophylls), therefore displaying potent antioxidant properties. Stohs and Hartman (2015) further noted that moringa leaves contain an extraordinary amount of lutein, which is linked to improving eye health, as it reduces the risk of macular degeneration and cataracts.

5 Conclusion

The leaves of moringa are known to be a good source of natural antioxidants due to the presence of several antioxidant compounds. The present study focused on the evaluation of antioxidant properties and plant pigments in MLE, revealing that MLE contains high amounts of antioxidants (1.55 $mg*g^{-1}$), vitamin C (0.45 $mg*g^{-1}$) and total phenolics (0.80 $mg*g^{-1}$). Therefore, MLE is a good source of natural antioxidant compounds that can have beneficial effect on plants. Furthermore, MLE contains high amounts of total chlorophylls (10.10 $mg*g^{-1}$) and other potent antioxidants that could be used to enhance crop performance.

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CHAPTER 4

THE EFFECT OF MORINGA LEAF EXTRACT (MLE) ON THE GROWTH AND DEVELOPMENT OF RADISH (*RAPHANUS SATIVUS*) AND GREEN BEANS (*PHASEOLUS VULGARIS*)

Abstract

Moringa oleifera leaf extract (MLE) has potential as a natural growth enhancer for many crops, but has only been researched little on root vegetables and fruit crops. The aim of this experiment was, therefore, to evaluate the effect of MLE on growth and development of radish (*Raphanus sativus*) and green beans (*Phaseolus vulgaris*). The experiment was laid out in a completely randomized design with five replications and comprised of three treatments, viz. (control, only inorganic fertilizer Calmag+B (5 g/plant) (T1), common fertilizer plus MLE 100% (T2) (20 g dried moringa powder and MLE 50% (T3) (T2 diluted to 50% with 100% methanol). Applications of MLE was carried out once during the flowering, pod formation stage of green beans and prior to harvest while radish was sprayed during development of the fourth leaf and prior to harvest. The obtained results demonstrated that MLE can increase growth parameters such as leaf size, pod size, number of flowers, number of mature leaves and root size compared with control plants. Among the various MLE treatments, MLE 50% produced superior growth and yield parameters on both, radish and green bean plants compared with the MLE 100% treated and control plants. It can, therefore, be concluded that MLE 50% should be applied at critical growth and developmental stages of green beans and radish to produce root and fruit vegetables of superior quality.

Keywords: Moringa leaf extract (MLE), radish, green beans, growth parameters.

1 Introduction

Over the past decades, interest in organically grown vegetables has been on the rise worldwide, as a result of growing emphasis of consumers on healthier and safer produce (De Pascale et al., 2017). As a result, agricultural growing practices have been evolving towards organic, more sustainable, environmentally friendly farming systems (Bulgari et al., 2014). Organic vegetable production has doubled in total production worldwide since 2008, accounting for 3.5 million/hectare of cultivated organic land in 2014, with more than 87 countries practicing organic agriculture (Willer and Lernoud, 2016). Dorais (2007) noted that organic vegetable production is considered an environmentally friendly production system, as it contributes minimal harm to the ecosystem (*i.e*., results in soil and water conservation) with minimal use of farm production inputs and labour. Furthermore, Phiri (2010) reported that there has been an increase in global inorganic fertilizer prices. The extensive use of inorganic fertilizers as a source of plant nutrients is not sustainable and often associated with high production costs due to the high production, and can be associated with land and soil degradation and as well as environmental pollution (Abdalla, 2013). According to Bulgari et al. (2014), the aim of modern, sustainable agriculture is to reduce inputs without reducing crop yield and quality; while these goals can be partially achieved by breeding programmes, success is species-specific and time-consuming. Moreover, the identification of organic molecules able to activate plant metabolism may allow an improvement in plant performance within a short period and in a cost-effective way (Bulgari et al., 2014).

Today farmers are well-aware of the applications of organic fertilizer to improve crop production as well as maintaining productivity and soil health of farming land (Yasmeen, 2011). Hence, there is a need to continuously search for alternative, safe, natural sources of plant nutrients (Yasmeen, 2011). *Moringa oleifera* is one such alternative investigated as a growth and yield enhancer of crops; thus, Moringa leaf extract (MLE) could be possibly used

as a biostimulant (Phiri, 2010). According to Jhilik et al. (2017) and Latif and Mohammed (2016), MLE contains zeatin, a natural adenine plant hormone. Fuglie (2000) also reported that MLE is rich in zeatin with concentrations between 5 µg and 200 µg/g leaves. Zeatin is a common, naturally-occurring cytokinin, which plays an essential role in cell division and cell elongation and is, therefore, involved in the promotion of plant growth (Abou-Sreea and Matter, 2016). Cytokinins also play a central role during the cell cycle and further influence numerous developmental processes, such as root growth and branching, leaf senescence, control of apical dominance and chloroplast development (Dandekar and Muhammad, 2017). Amino acids found in moringa leaves can play various roles in plant growth, such as being hormone precursors, stress-reducing agents and nitrogen sources (Teixeira et al., 2018). Miller et al. (2007) noted that amino acids are capable of triggering a variety of physiological processes, such as root development, regulation of nitrogen uptake and stimulating the antioxidant metabolism. Ascorbic acid, also found in MLE, is responsible for regulating various physiological processes, such as controlling growth and development and conveying stress tolerance (Smirnoff and Wheeler, 2000). Gallie (2012) describes that ascorbic acid is involved in the regulation of cell elongation and also serves as a major contributor to the cellular redox state, which is important in maintaining photosynthetic function. Additionally, moringa leaves contain various phenolics, amino acids, as well as micro-nutrients that are involved in essential plant physiological processes (Yasmeen, 2011; Fuglie, 2000; Phiri, 2010). Therefore, this study was conducted to evaluate the effect of MLE on growth, development and quality of a model root vegetable (radish) and a model fruit vegetable (green beans) from seeding to horticultural maturity.

2 Materials and Methods

2.1 Experimental site

The experiment was conducted at the University of KwaZulu-Natal - Pietermaritzburg campus, at the School of Agricultural, Earth and Environmental Sciences (29°37'34.8"S, 30°24'12.0"E) in a poly-ethylene tunnel.

2.2 Experimental design and treatment structure

A completely randomised design was used in this experiment. The experiment tested the response of two crops, radish and green beans and was replicated five times. The following treatments were used; control, only inorganic fertilizer Calmag+B (5 g/plant) (T1), common fertilizer plus MLE 100% (T2) (20 g/L dried moringa powder (obtained from Run KZN, Pietermaritzburg, South Africa)) and MLE 50% (T3) (T2 diluted to 50% with 100% methanol).

2.3 Plant material

Seeds of the garden radish cultivar 'Sparkler' and the garden bushbean 'Contender' were purchased from Starke Ayres, Pietermaritzburg, South Africa.

2.4 Growing medium analysis

The medium used for the experiment (Gromor potting medium, obtained from Gromor®, Cato Ridge, South Africa) was analysed for essential nutrients before planting in a tunnel at the Controlled Environment Facility (CEF), University of KwaZulu-Natal - Pietermaritzburg campus. The medium was analysed for total nitrogen using a Leco (Tru Mac CNS, St. Joseph, Michigan, USA), whilst phosphorus, potassium, calcium and magnesium were determined with Atomic Absorption Spectroscopy (AAS), using the AMBIC-2 method described by Hunter (1974).

Briefly, reagents were prepared in the following manner:

Ambic-2 extraction solution:

Superfloc (N100) solution was prepared by slowly adding 5 mL superfloc (N100) into 500 mL lukewarm de-ionised water, while stirring at 400 rpm to ensure the final solution to be viscous and gel-like. Thereafter, 9.88 g ammonium bicarbonate, 1.86 g di-sodium EDTA and 0.185 g ammonium fluoride were dissolved in 500 mL de-ionised water. The solution was mixed well and after allowing to stand overnight, the pH was adjusted to 8.0 with concentrated ammonia solution.

Concentrated colour reagent:

An amount of 2 g antimony potassium tartrate was added into a 2 L volumetric flask, followed by an addition of 800 mL de-ionised water to the volumetric flask. Concentrated H_2SO_4 (300 mL) was added slowly to the solution which was left to cool overnight. Separately, 15 g ammonium molybdate was dissolved in 600 mL de-ionised water. Once the acid antimony potassium tartrate solution had cool down, the ammonium molybdate solution was added followed by de-ionised water to a final volume of 2 L.

Diluted colour reagent:

On the day of use, 75 mL of concentrated colour reagent was diluted into 1 L of a solution containing 0.5 g of gelatin (the gelatin was dissolved in warm, de-ionised water). Then, 1 g ascorbic acid was added and the solution mixed.

Extraction procedure:

The quantity of P that can be extracted from soil is temperature-dependent (Hunter, 1974); hence, the laboratory air and the extracting solution temperature were kept at 20°C. An amount of 2.5 mL fresh potting medium was scooped into a sample cup and 25 mL of the extraction

solution added to the medium. The mixture was stirred for 10 min at 400rpm and filtered into a clean sample cup through Whatman no.1 filter paper.

Mineral determinations:

To analyse samples for phosphorus, 2 mL extract, 8 mL de-ionised water and 10 mL diluted colour reagent were combined. After 40 min the absorbance values were read at 670nm. For potassium, calcium and magnesium analysis, 5 mL extraction solution was diluted with 25 mL de-ionised water adding up to 30 mL and all elements were determined on an atomic absorption spectrometer (AA280FS, Agilent Technologies, Santa Clara, CA, USA).

2.5 Preparations of moringa leaf extract (MLE)

The preparation of MLE was performed according to Makkar and Becker (1996), with slight modifications. Moringa leaf powder was purchased from a commercial retailer (RunX KZN), Pietermaritzburg, SA). Dried moringa leaf powder (20g) was placed into a 1L volumetric flask, followed by addition of 225 mL 99.5% methanol. Two drops of glycerin were added and the flask filled to volume with distilled water. The solution was then stirred for 40 min; thereafter, the extract was allowed to stand overnight in a cold room at 1˚C. Methanol was used to obtain MLE instead of ethanol, due to ethanol being a cell poison and in plant hormone extraction 80% methanol is commonly employed, despite recent studies indicating that methanol can stimulate plant growth (Nicholson, 2018).

The moringa leaf suspension was filtered through glass wool into a 1 L volumetric flask to obtain a precipitate-free extract. About 100 mL of the extract was lost through the extraction process due to the moringa leaf particles found in the powder. An amount of 450 mL extract was poured into a 1L volumetric flask and distilled water was added to volume. This solution was termed ‘MLE 100%’. This extract was diluted (1:1, v: v) with 99.5% methanol and termed ‘MLE 50%’.

2.6 Growth and development

Radish and green bean seeds were planted individually into 30 cm diameter plastic pots filled with composted pine bark (Gromor®, Cato Ridge, SA). The pots were placed on the floor of the tunnel and irrigated using a Netafilm 8 L drip pipe, delivering a volume of 33.3 mL water per minute. Irrigation was applied twice a day, 6 min/per cycle for radish and 8 min/per cycle for green beans. Temperatures in the tunnel were set at 15°C min and 25°C max to enhance adequate plant growth and development. Radish and green beans were planted mid-March 2018 and harvesting carried out from late May 2018 to early June 2018. Growth starter fertilizer (agchem Easygro® water-soluble 2:1:2 (43), Silverton, Gauteng, RSA) was applied to the pots through a fertigation system twice a day.

The applications of granular fertilizer (Ca, Mg and B as Vitassol CaMg + B; SQM VITAS Southern Africa, Fourways, SA) commenced two weeks after planting and each plant receiving 5 g granular fertilizer, twice a week, until harvest. The first applications of MLE to green beans plants occurred six weeks after planting, during the flowering stage, and a second dose was applied eight weeks after planting, during pod formation, and the third dose was applied prior to harvest and lastly the fourth dose was applied after the first harvest. Furthermore, MLE was applied to radish plants in the second week after planting, as well as during development of the fourth leaf and prior to harvest. Bean pods were first harvested during week 8, and a second pod harvest was carried out at week 10 after planting, while radishes were harvested six weeks after planting. At harvest the plants were pulled out from the growing pots and the medium was removed from the plants by slightly shaking plants until the roots were free of pine bark.

The following parameters were determined weekly:

For radish, leaf size (length and width, using a ruler), number of leaves and leaf chlorophyll concentration, using a hand-held chlorophyll meter (C CM-200 plus, Opti-sciences, Hudson,

CA, USA) were determined. This chlorophyll meter provides a relative indication of the chlorophyll in plant leaves measuring the chlorophyll content of a certain area with outputs of an estimate of actual chlorophyll concentration in μmol total chlorophyll per m^2 of leaf surface area (Thanaa et al., 2017).

At harvest, leaf fresh and dry mass, as well as leaf colour, using a chromameter CR-400 (Konica Minolta, Ramsey, NJ, USA), were recorded.

For radish leaf colour determination, one mature leaf from each plant was used to determine various colour co-ordinates [L*, a*, b* and hue angle (h°)]. Leaf colour parameters were measured at oosite sides of each leaf and described using the CIELAB colour system. In this system a three-dimensional colour space is used, which is interpreted as follows: L* indicates lightness with values ranging from 0° (complete black) to 100° (complete white), a* values indicate green (+a*) to red (-a*) and b* values indicate blue (+b*) to yellow (-b*) (Gonnet, 1993). The hue angle (h°) expresses the qualitative attribute of colour and uses a 360° circle, where 0° is red, 90° is yellow, 180° is green, 270° is blue and 360° is red.

For radish, size (fresh and dry mass) of the storage root and total biomass per plant were determined, while in green beans the number of flowers and fruit, the number of pods, pod length and chlorophyll content index (CCI) were determined. At harvest, fresh and dry mass of individual pods and yield per plant were also recorded.

2.7 Statistical analysis

The data were statistically analysed using GenStat (version 14.1) (VSN, Hemel Hempstead, England, UK.). Analysis of variance (ANOVA) was used to test the overall significance of the data, while treatment means compared by Duncan's multiple range tests at 5% level of significance.

3 Results

3.1 Gromor® potting medium

The used potting medium contained high amounts of the plant nutrients Ca, K, Mg and P, with only very low quantities of N (0.04 ppm, Table 4.1). The mineral analysis of the growing medium (Table 4.1) demonstrated that the potting medium required further incorporation of certain fertilizers to enhance crop growth. It was, therefore, decided to amend the medium with high N-containing fertilizer (Calmag +B).

Table 4. 1 : Potting medium analysis for N, P, K, Ca and Mg

Elements	**N**	**P**	**K**	**Ca**	**Mg**
ppm	0.03826	8.068	1938.6	3257.2	440.3

3.2 Radish

3.2.1 Growth and development of radish plants

3.2.1.1 Leaf size

Statistically, treatments applied had a significant influence ($p < 0.05$) on the growth and development of radish plants. Overall, leaf length increased weekly over the first three weeks after planting (Fig. 4.1). During week 1, it was observed that MLE 100% attained the longest leaves, with a mean value of 8.51 cm, while MLE 50% plants had an average leaf length of 6.35 cm and control plants of 4.88 cm. In week 2, it was found that leaves of all three treatments were similar in length, with mean values ranging from 9.3 cm (MLE 100%), to 9.1 cm (MLE 50%) and lastly 8.1 cm (control plants). Furthermore, during the final week of measurement, (week 3), moringa-treated plants displayed a significant effect on radish leaf length, with MLE

50% resulting in a leaf length of 11.6 cm, while MLE 100% plants had a length of 10.7 cm. Control plants had the shortest leaves at 8.5 cm.

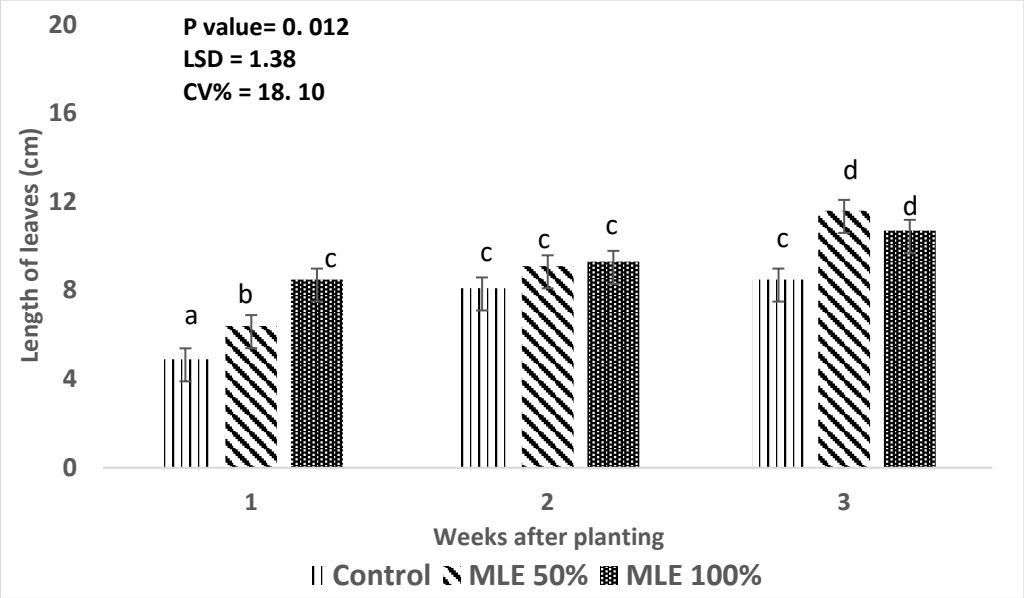

Figure 4. 1: Alterations in radish leaf length (first mature leaf) over the growing period as affected by MLE treatment

3.2.1.2 Number of mature leaves

Statistically, there was a significant difference *($p < 0.05$)* between the treatments applied on the number of radish leaves over the 3-week observation period. During week 1 (Fig. 4.2), all three treatments had the same number of leaves, on average control plants had 2.2, MLE 50% 2.6 and MLE 100% 2.6 leaves per plant, with only a trend of an increase in leaf number in the MLE treatments. A significant influence of MLE was observed in week 2 and particularly week 3, when moringa-treated plants had a higher number of leaves than the control.

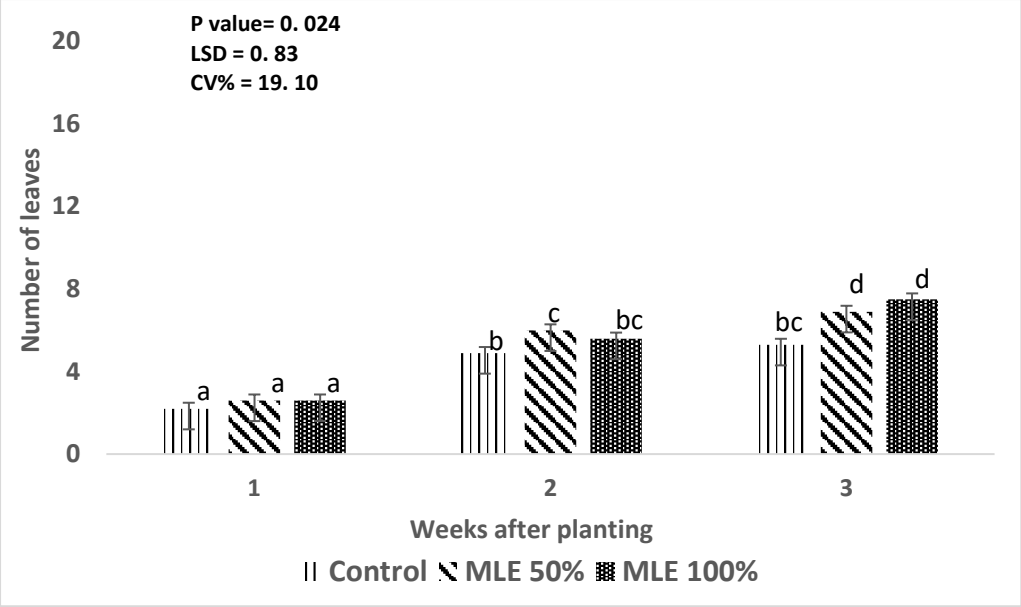

Figure 4. 2: Radish leaf number obtained over the growing period.

3.2.1.3 Leaf chlorophyll

The treatments applied to radish plants had a significant ($p < 0.05$) effect on radish leaf chlorophyll. Weeks after planting had, non-surprisingly, a significant effect on growth and development of radish leaves. In week 1, leaves treated with MLE 100% treatment had a tendency towards the highest CCI values (11.00), with MLE 50% (9.7) and control plants (9.3) having a tendency towards lower CCI (Fig. 4.3). The highest CCI values were recorded in week 3, with both MLE treatments attaining a CCI value of 13.8. Control plants had the least CCI values during week 3 (12.6).

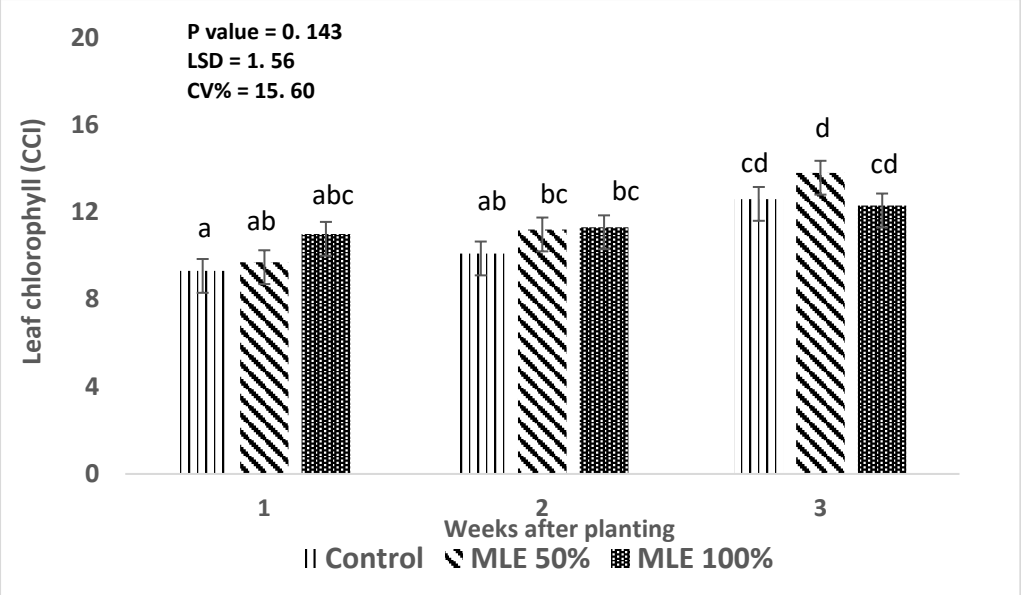

Figure 4. 3: Radish leaf chlorophyll concentration observed over the growing period.

3.2.2 Yield parameters

3.2.2.1 Fresh mass

The treatments applied to radish plants had a highly significant influence *($p < 0.05$)* on the above-ground and below-ground fresh mass of radish leaves and storage roots. On the day of harvest, both MLE treatments resulted in a higher foliage fresh mass than that of control plants (Table 4.2). MLE 50% treatment significantly increased above-ground and below-ground fresh mass (Table 4.2).

3.2.2.2 Dry mass

The treatments applied to radish plants had a significant *($p < 0.05$)* effect on above-ground and below-ground dry mass. During the drying period radish leaves experienced aroximately 90% water loss. Control plants and MLE 100% had the lowest above-ground and below-ground dry mass, while both MLE 50% resulted in higher above-ground dry mass (Table 4.2). All radish roots experienced aroximately 90% water loss, with MLE 50% plants having the highest dry mass (2.5 g).

Table 4. 2: Effect of foliar MLE applications on radish yield parameters.

Treatment	Fresh leaf mass (g)	Fresh root mass (g)	Dry leaf mass (g)	Dry root mass (g)
Control (T1)	7. 8 a	18. 6 a	0. 7 a	1. 6 a
MLE 50% (T2)	11. 9 b	26. 1 b	1. 3 b	2. 5 b
MLE 100% (T3)	11. 7 b	22. 4 ab	0. 9 ab	2. 2 b
P= 0,05	<. 001	0. 016	0. 005	<. 001
LSD	2. 19	4. 93	0. 34	0. 35
CV%	22. 6	23. 8	39. 7	18. 0

Mean values in the same column for each trait followed by the same letter are not significantly different. MLE = Moringa leaf extract

3.2.2.3 Radish root colour

Lightness

At harvest, radish plants treated with MLE displayed an overall deeper red root colour than control plants. The treatments applied had a highly significant effect *($p < 0.05$)* on the lightness of radish roots. Roots of plants treated with MLE were darker on the day of harvest, obtaining mean values of 35.9 (MLE 50%) and 35.1 (MLE 100%), while control plants had lighter roots, resulting in a mean L* value of 42.7.

a* co-ordinate

It was observed that the moringa treatments had a significant influence ($p < 0.05$) on radish root colour. Radish plants treated with MLE had a deeper red root colour with MLE 50% attaining the highest a* mean value of 39.6, followed by MLE 100% with 37.7. Roots of control plants were less red attaining a mean a* value of 32.7 (Table 4.3).

b* co-ordinate

Statistically, the treatments applied to the radish plants had no significant influence *(p > 0.05)* on the b* colour coordinate of radish storage organs. Table 4.3, however, demonstrates a tendency of MLE 100% plants displaying a less yellow root colour, obtaining a mean value of 7.1, followed by MLE 50% treated plants with a main value of 7.6. Control plants were of a more yellow root colour, as the mean value obtained was 8.7.

Hue angle

Statistically, the treatments applied to radish plants had a significant influence on root hue angle values. Control roots displayed a lighter red and duller appearance on the day of harvest while moringa-treated roots were of redder colour (Table 4.3).

Table 4. 3: Radish root colour at harvest

Treatment	Lightness (L*)	a* co ordinate	b* co ordinate	Hue angle
Control (T1)	42. 7 b	32. 7 a	8. 7 a	17.7 b
MLE 50% (T2)	35. 9 a	39. 6 b	7. 6 a	13. 9 a
MLE 100% (T3)	35. 1 a	37. 7 b	7. 1 a	15. 3 ab
P= 0,05	<. 001	0. 004	0. 241	0. 006
LSD	2. 50	2. 21	2. 03	2. 21
CV%	7. 1	18. 6	28. 1	15. 2

Mean values in the same column for each trait followed by the same letter are not significantly different. MLE = Moringa leaf extract

3.3 Green beans

3.3.1 Growth and development of green beans

3.3.1.1 Number of leaves

Statistically the treatments applied to green bean plants had a significant effect *(p < 0.05)* on the number of leaves over the growing period. Prior to MLE applications (week 1 and 2) plants contained the same number of leaves; however; from week 3 onwards new leaf development

was observed in the plants, with moringa-treated plants obtaining higher mean values than control plants (Fig. 4.4). Overall, plants treated with MLE 50% obtained greater leaf number, with mean values of 6.4-18.9 compared with MLE 100% and control plants.

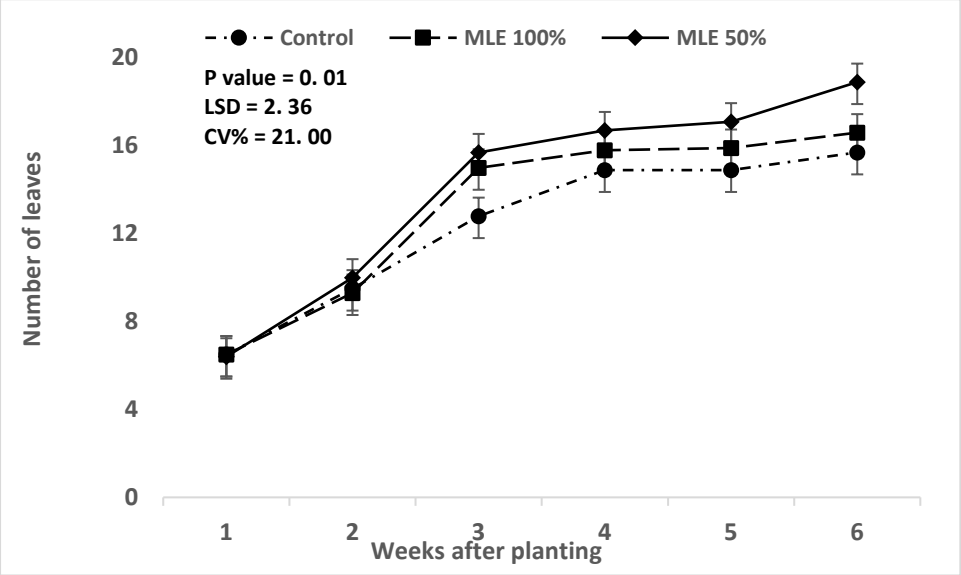

Figure 4. 4: Number of leaves on green bean plants over the six-week growing period.

3.3.1.2 Number of flowers

Statistically, there was a highly significant difference *($p < 0.05$)* on the different treatments applied to the green bean plants; MLE 50% plants attained the highest number of flowers over the from week 3-6., followed by MLE 100 and least was found control plants (Fig. 4.5)

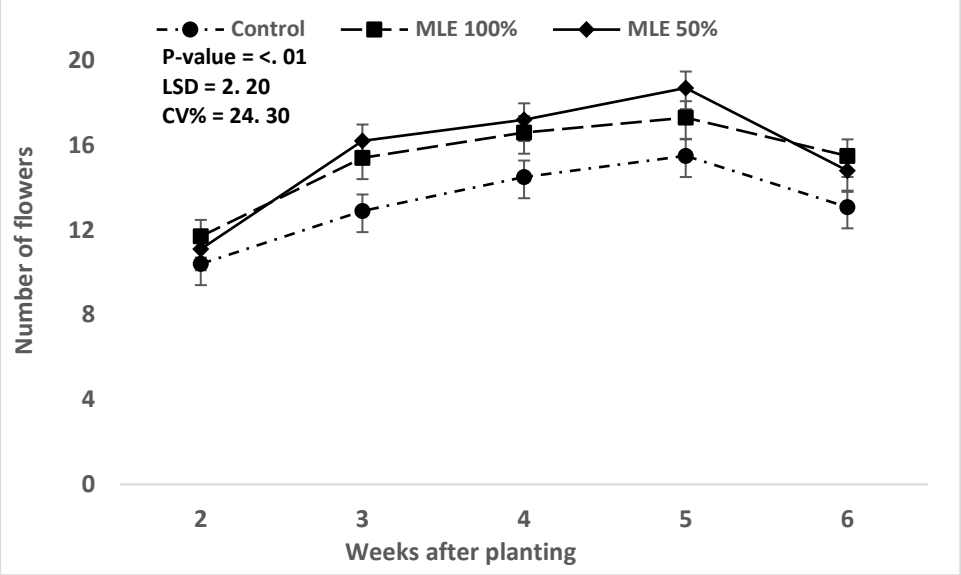

Figure 4. 5: Total number of green bean flowers obtained over the six-week growing period.

3.3.1.3 Number of pods

Statistically, the treatments applied significantly *($p < 0.05$)* affected the number of pods per green bean plants, as figure 4.6 illustrates that moringa-treated plants bore a higher number of pods per plant. Pods were found from 3 weeks after planting (WAP), with MLE 50% plants showing a higher number of pods per plant in week 4-6 compared with MLE 100% and control plants.

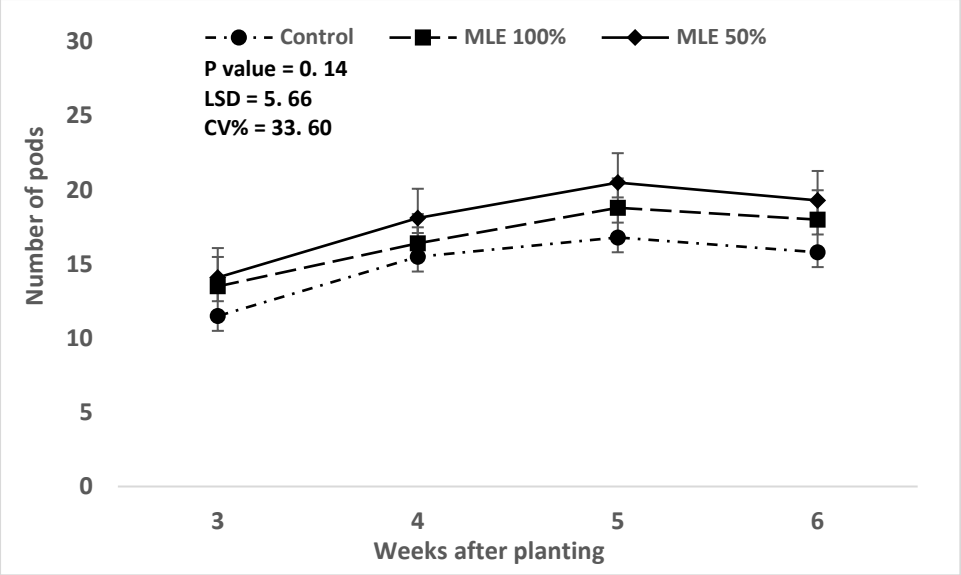

Figure 4. 6: Total number of green bean pods (newly matured) attained over the six-week growing period.

3.3.1.4 Length of pods

The treatments applied to green bean plants had a highly significant effect ($p < 0.05$) on the pod length over the growing period. From week 4-6, plants treated with MLE 50% attained the longest pods (11.73-14.8 cm), whereas, on plants treated with MLE 100% pods were slightly longer (10.01-13.96 cm) compared with control plants which had bear the shortest pods (11.3-12.93 cm) (Fig. 4.7).

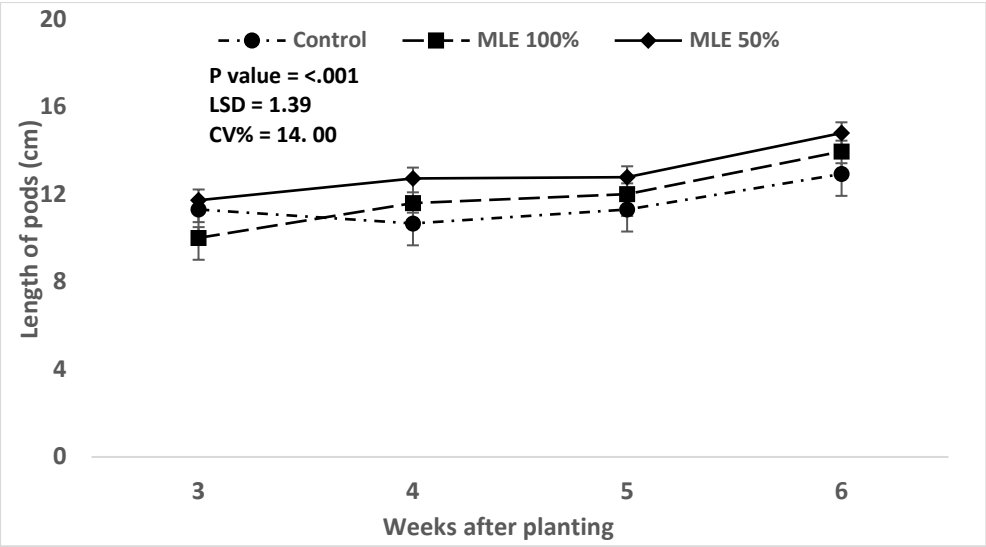

Figure 4. 7: Length of green bean pods over the observed six-week growing period.

3.3.1.5 Leaf chlorophyll

The treatments applied to green bean plants had a highly significant effect *($p < 0.05$)* on the leaf chlorophyll concentration. From week 3 to 6, moringa-treated plants had the highest leaf chlorophyll (Fig. 4.8), with leaves on the MLE 50% plants displaying the highest CCI mean values (13.29 – 20.36), followed by MLE 100% (CCI: 14.29-19.28) and control plants with a CCI of 15.39-17.46.

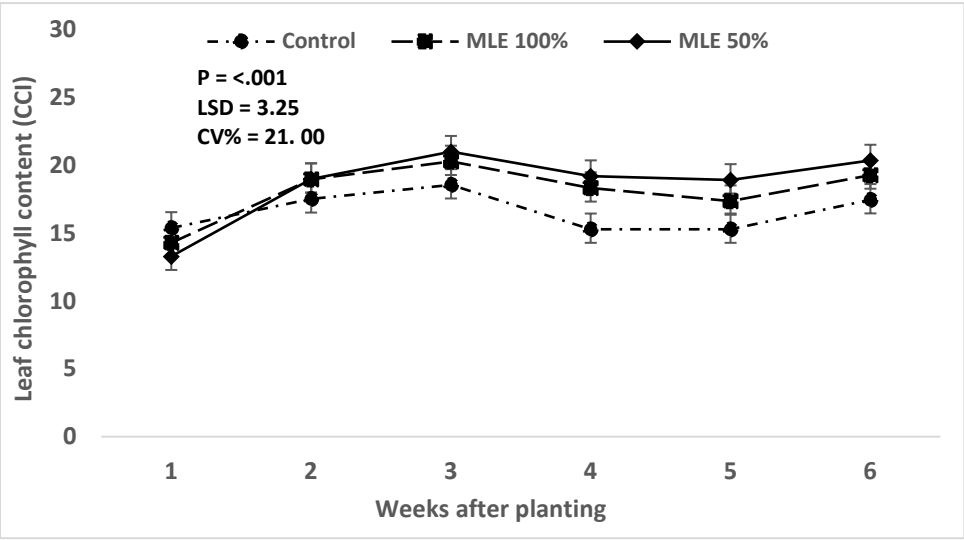

Figure 4. 8: Green bean leaf chlorophyll content index over the six-week growing period.

3.3.2 Yield parameters during the first pod harvest (harvested from the first flower flush)

3.3.2.1 Fresh mass

Statistically, pod fresh mass was significantly influenced ($p < 0.05$) by the treatment (Table 4.4). Applications of MLE 100% (930 g) and MLE 50% (888 g) resulted in the highest pod fresh mass of the first pod harvest and the control yielding 634 g per plant.

3.3.2.2 Dry mass

The treatments applied to green bean plants had no significant effect ($p < 0.05$) on pod dry mass. Green bean pods lost approximately 90% water during the freeze-drying process. Pods of MLE 100% treated plants experienced the highest water loss and displayed a mean dry mass value of 6.43 g compared with MLE 50% and control plants. Table 4.4 shows that MLE 50% treated plants had a total dry mass mean value of 5.88 g, which is tended to be higher than that of the control plants (5.68 g).

3.3.2.3 Number of pods/plant

Statistically, number of pods per plant was significantly affected ($p < 0.05$) by the treatment (Table 4.4), with MLE having a positive effect on pod number resulting in higher fresh mass per plant than the control. Overall, plants treated with MLE 50% obtained the highest value of 18.06 pods, followed by MLE 100% (15.90 pods/ plant) and control plants (12.22 pods/ plant).

3.3.2.4 Length of pods

Treatments applied significantly affected ($p < 0.05$) pod length, with MLE 100% and MLE 50% plants had the overall highest pod length (Table 4.4). Control plants attained the lowest pod length of 11.2 cm.

Table 4. 4: Yield parameters obtained during the first pod harvest.

Treatment	Fresh mass (g)	Dry mass (g)	No. of pods/plant	Length of pods (cm)
Control (T1)	634 a	5. 68 a	12. 22 a	11. 22 a
MLE 50% (T2)	888 b	5. 88 a	18. 60 b	14. 79 b
MLE 100% (T3)	930 ab	6. 43 a	15. 90 a	13. 03 b
P= 0,05	0. 019	0. 755	0. 029	0. 003
LSD	215. 23	2. 12	4. 56	1. 40
CV%	28. 50	38. 20	30. 90	12. 00

Mean values in the same column for each trait followed by the same letter are not significantly different. MLE = Moringa leaf extract

3.3.3 Yield parameters during the second pod harvest (harvested from the second flower flush after an additional MLE foliar applications)

3.3.3.1 Fresh mass

Above-ground biomass of the second pod harvest was significantly ($p < 0.05$) affected by treatment. While MLE 100% plants had the highest above-ground fresh mass (167.24 g), followed by MLE 50% plants (155.38 g), the lowest fresh mass (121.06 g) was achieved by the control plants (Table 4.5).

There was a highly significant difference ($p < 0.05$) in below-ground fresh biomass of green bean plants (Table 4.5). Plants treated with MLE100% and MLE 50% obtained the highest below-ground fresh mass.

There was a significant difference *($p < 0.05$)* in fresh mass attained during the second pod harvest. Table 4.5 demonstrates that plants treated with MLE 100% attained a greater fresh mass (163.5 g) in the second pod harvest, while MLE 50% produced a mass of 147 g and control plants only attained a pod mass of 105 g.

3.3.3.2 Dry mass

Statistically, the treatments applied had a significant influence *($p < 0.05$)* on above-ground dry mass in the second pod harvest. Green beans experienced approximately 90% water loss throughout the 4-day oven-drying process. Control plants (19.6 g) and MLE 50% (23.42 g) attained the lowest above-ground dry mass (Table 4.5). Plants sprayed with MLE 100%, however, obtained the highest dry mass of 30.88 g/plant.

There was a significant difference *($p < 0.05$)* between the treatments applied to green bean plants. Table (4.5) illustrates that MLE 50% plants obtained the highest mass (14.8 g), followed by MLE 100% plants, attaining a below-ground dry mass of 10.79 g. Control plants attained the lowest below ground dry mass of 3.01 g.

Statistically, there was no significance difference *($p > 0.05$)* on the treatments applied on the pod dry mass. Plants treated with MLE 50% had a tendency towards the highest pod dry mass (16.5 g/plant), followed by MLE 100% plants with a mass of 14.72 g, while control plants obtained the lowest dry mass (14.02 g) (Table 4.5).

3.3.3.3 Number of pods/plant

Statistically the treatments applied to green bean plants had a significant influence ($p < 0.05$) on the number of pods per plant obtained at the second pod harvest. Table 4.5 demonstrates that MLE 50% plants attained the highest number of pods with a mean value of 21.9 pods/plant, followed by MLE 100% plants with 19.9 pods/plant and the lowest mass was found in control plants, producing of 15.9 pods/plant.

3.3.3.4 Length of pods

Statistically, treatments applied had a highly significant effect *($p < 0.05$)* on the length of green bean pods in the second harvesting period. The longest pods were found on MLE-treated plants, but overall no significant difference ($p > 0.05$) was found between both MLE-treated plants.

Treatment with MLE 100% (16.06 cm) and MLE 50% (16.16 cm) resulted in the longest pods. Table 4.5 demonstrates that control plants had the shortest pods with a length of 12.6 cm.

Table 4. 5: Yield parameters of green bean pods attained during the second harvest.

Treatment	Above ground FM (g)	Above ground DM (g)	Below ground FM (g)	Below ground DM (g)	Pod FM (g)	Pod DM (g)	Number of pods/plant	Length of pods (cm)
Control (T1)	121. 06a	19. 6 a	16. 55 a	3. 01 a	104. 98 a	14. 02 a	15.9 a	12. 6 a
MLE 50% (T2)	155. 38 ab	23. 42 ab	57. 24 b	14. 87 b	147. 4 ab	16. 56 a	21.9 b	16. 16 b
MLE 100% (T3)	167. 24b	30. 88 b	45. 09 b	10. 79 b	1630 5 b	14. 72 a	19. 9 ab	16. 06 b
P= 0,05	0. 031	0. 023	<. 001	0. 001	0. 042	0.633	0. 225	<. 001
LSD	34. 91	7. 97	17. 99	5. 80	46. 35	4. 96	6. 134	1.70
CV%	25. 50	35. 00	49. 10	65. 60	36. 10	35. 20	35. 10	12. 30

Mean values in the same column for each trait followed by the same letter are not significantly different. MLE = Moringa leaf extract, FM = Fresh mass, DM = Dry mass

4 Discussion

4.1 Gromor potting medium

The growth and development of any plant grown not in soil, but in a growing medium, highly depends on the chemical and physical properties of the medium (Massey et al., 2011). The current study revealed that potting medium used for radish and green bean cultivation was free from pests and was able to retain sufficient moisture for plant growth. All plants were fertigated from sowing to the day of harvesting. Granular fertilizer (Calmag + B) was applied to the plants on a weekly basis, while irrigation was supplied twice a day. It was observed that both crops performed well throughout the observed growth and development period, as fertilizer was incorporated into the medium and MLE applied to the plants (Fig. 4.1 - 4.8).

4.2 Enhanced growth and development of radish plants by MLE foliar applications

Plant extracts, such as the one prepared from *M. oleifera* leaves, and crop residues have been reported to influence crop growth and yield (Maishanu et al., 2017). It has been reported that foliar applications of MLE enhances growth of the early vegetative development of tomato (*Solanum lycopersicum*), peanut (*Arachis hypogaea*), corn (*Zea mays*) and wheat (*Triticum aestivum*), while also improving resistance to pests and diseases and generally increasing yield by 20 – 35% (Culver et al., 2012). In this study, the applications of MLE also improved growth and development of radish plants, as applications of MLE triggered an increase in leaf size from week 1 to week 3 (Fig. 4.1). It can be concluded that, since radish leaves have a flat base leaf structure, the plant tissue cells were able to effectively absorb the MLE applied.

Findings from Moore (2008) revealed that MLE significantly increased plant height as well as lateral shoot development of the tomato plants, when compared with untreated plants. Abdalla (2013) also reported an increase in leaf size, following MLE foliar applications to rocket plants, resulting in an overall increase in rocket plant height. Several researchers have indicated that MLE contains the plant hormone zeatin, which is involved in various vital plant physiological processes (Schafer et al., 2017). It can be concluded that this group of natural plant growth hormones found in MLE had a positive effect on the number of radish leaves by allowing MLE-treated plants to develop more radish leaves throughout the growing period (Fig. 4.2). Findings by Culver et al. (2012) demonstrated that MLE foliar applications significantly increases tomato fruit mass and plant height, as well as yield and other quality components. Studies by Bashir et al. (2014) concur with the current study (Fig. 4.2), as those authors demonstrated that MLE foliar applications increases average plant height, leaf number, number of branches and the yield of tomato plants.

Chlorophyll is the single most critical pigment giving green colour to plants and allowing to convert this light to usable energy (Hala and Nabila, 2017). The current study demonstrates

that radish plants respond positively to growth enhancing fertilizers (e.g. Ca, Mg and B) and MLE applications which acts as a biostimulant increasing leaf chlorophyll concentrations (Fig. 4.3). Similarly, Hala and Nabila (2017) reported that MLE applications leads to a significant increase in leaf area and photosynthetic activity as well as leaf chlorophyll content in peer (*Capsicum annuum*). Abdalla (2013) also reported that MLE applications significantly increased the chlorophyll concentration in rocket.

Plants treated with MLE attained a higher above ground fresh mass and had denser foliage compared with control plants (Table 4.2). The current results (Fig. 4.1, 4.2, and 4.3) are in agreement with Fuglie (2000) who reported that MLE accelerates growth of young plants, strengthens plants and increases leaf area. Studies by Bashir et al. (2014) also found that vegetative growth parameters of tomato increased significantly following applications of different concentrations of MLE. Moringa-treated plants had a higher below ground fresh mass than control plants (Table 4.2). As a result, MLE-treated plants were characterized by a larger root system. Findings from Jhilik et al. (2017) revealed that MLE can accelerate growth of young plants, improve resistance to pests and diseases, and increase leaf duration on the plant and root number per plant. Studies by Yasmeen et al. (2012) and Rady et al. (2015) concur with the present study in that MLE applications was found to maintain an optimal tissue water status and membrane stability, enhance antioxidant levels and activate plant defence system, while also increasing the level of plant secondary metabolites leading to vigorous seedling growth and maximizing crop performance.

In the current study, moringa-treated plants obtained a higher above-ground dry mass (Table 4.2), in line with studies conducted by Culver et al. (2012) demonstrating that MLE applications significantly increases dry matter (yield) and root dry mass of tomato plants. Biswas et al. (2016) reported that the spraying of MLE onto most field crops can strengthen plants, promote vegetative growth and increase root and shoot fresh mass.

Moringa-treated radishes attained a higher storage fresh root mass, compared with the control plants (Table 4.2). These findings are in accordance with Biswas et al. (2016), who reported that applications of MLE enhanced the number of leaves, plant height, shoot and root length, fresh and dry mass of maize shoots and roots. The current study is also in agreement with Azooz et al. (2004) who reported that different concentrations of MLE were able to enhance the photosynthetic capacity of treated plants, leading to increased plant productivity and fruit dry matter.

Produce colour, such as that of fruit, plays an important role in consumer attraction (Thanaa et al., 2017). Radish is a unique root vegetable associated with the accumulation of anthocyanins; therefore, radish contains large amounts of these red pigments (Chen et al., 2016). Anthocyanins are one of the largest and most important group of water-soluble pigments. They accumulate in cell vacuoles and are largely responsible for diverse pigmentation from red to blue and purple (Horbowicz et al., 2008). Overall, MLE-treated plants displayed a deeper red root colour compared with the control plants. The treatments applied to radish plants all resulted in a red colour (Table 4.3); however, MLE 50% treated plants had a denser red root colour, illustrating that MLE contains compounds that stimulate plant pigment biosynthesis.

Results of the current study confirm findings by Hala and Nabila (2017), who reported that the presence of zeatin-like cytokinins in MLE resulted in higher leaf area and thereby enhanced photosynthetic activity and leaf chlorophyll in moringa-treated pepper leaves (*Capsicum*). Radish plants treated with MLE were of less yellow colour compared with the control and plants treated with MLE 50% (Table 4.3). Findings by Thanaa et al. (2017) noted that moringa foliar extract is rich in minerals, possibly enhancing the activity of certain enzymes that result in the biosynthesis of certain pigments.

4.3 Effect of MLE foliar applications on growth and development of green bean plants

Leaves of *M. oleifera* are highly nutritious and are a source of essential vitamins such as Vit. A, B and C; these leaves are also a key source of natural antioxidants, vitamins and several elements, making MLE a potent natural growth stimulant (Yasmeen et al., 2012). Findings from the present study show that MLE applications had a positive response on the number of leaves of green bean plants (Fig. 4.4), confirming findings by Aluko (2016) who reported that MLE applications increases the height of peer plants significantly ($p < 0.05$), when applied at two-week intervals after transplanting.

Plants treated with MLE also produced a higher number of flowers (Fig. 4.5). It is likely that this enhancement in flower number was due to the zeatin found in MLE. The present study is in line with Hala and Nabila (2017) who reported the zeatin found in MLE enhances growth and increases yields by 25% - 30% for many crops. Moringa leaf extract also had a positive effect on the number of pods on green bean plants (Fig. 4.6), confirming findings by Singh (2014) that an increase in pod yield of snap beans (*Phaseolus vulgaris*) following MLE applications was due to the presence of cytokinins in MLE. Moringa-treated green bean plants also had a greater pod length (Fig. 4.7). This is in agreement with Makkar and Beckar (1996) who noted that the possible reason of the acceleration of the plant growth by MLE might be the high content of not only cytokinins but also auxins in moringa leaves, with both plant growth hormones increasing cell division and enlargement. Plants treated with MLE contained more chlorophyll in their leaves (Fig. 4.8), a finding similar to that of Abdalla (2013) who attributed this effect to the presence of several macro-elements, such as Mg, in MLE and the fact that Mg is a constituent of chlorophylls; therefore its presence 'greens up' plants.

Moringa leaf extract positively enhanced pod fresh and dry mass of green beans during the first pod harvest (Table 4.4). These findings correspond with Singh (2014) who reported that MLE significantly enhances fresh and dry mass of pea pods. Green bean plants treated with MLE

produced pods of superior quality and in greater number per plant during the pod first harvest (Table 4.4). The results obtained from this study are in agreement with Safí-naz and Mostafa (2015) who reported that MLE improves crop performance, resulting in vigorous seedling growth, improved membrane stability, enhanced antioxidant levels and, thereby, an activated plant defence system. Makkar and Becker (1996) further reported that plants treated with MLE display higher pest and disease resistance, have a longer life-span, heavier roots, stems and leaves, larger fruit and, overall, show an increase in yield. Moringa leaf extract had a significant ($p < 0.05$), positive effect on green bean total biomass, as well as above-ground and below-ground fresh mass (Table 4.4 and 4.5). Moringa leaf extract also enhanced the fresh and dry mass of the pods. Findings from the current study are in agreement with Emongor (2015) who reported that MLE applied to beans significantly increases total leaf area and enhances the growth of stems and roots. Brockman and Brennan (2017) reported that MLE sprays increased the biomass of wheat. Phiri (2010) noted that MLE improved seedling growth traits (i.e. shoot length, number and area of leaves per plant and plant dry mass).

Observations from the current study demonstrate that MLE applied to green beans positively affects the length of pods and the number of pods per plant, a phenomenon evident in the first and second harvesting periods (Table 4.4 and 4.5). Fuglie (2000) highlighted that MLE improves plant growth and development in different crops, which is also evident from the results of the present study (Table 4.4 and 4.5). Safí-naz and Mostafa (2015) reported that MLE can be considered a plant biostimulant that, when sprayed onto plants, has positive effects on plant growth and development.

5 Conclusion

The present study suggests that the use of MLE as a foliar spray to radish and green bean plants has significant positive effects on growth parameters, such as leaf size, number of leaves, number of flowers, number of pods, length of pods and fresh root and dry mass. Furthermore,

MLE applications significantly improved various yield components of both, radish and green bean plants. It can also be concluded that plants treated with MLE 50% were of good quality compared with MLE 100% plants. Findings from the present study further indicate that MLE should be applied at critical growth stages (3rd leaf development, flowering and pod formation, prior to harvest) for better growth and higher yields of radish and green beans.

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CHAPTER 5

EFFECT OF MORINGA LEAF EXTRACT ON THE MINERAL COMPOSITION OF RADISH (*RAPHANUS SATIVUS*) STORAGE ORGANS AND GREEN BEAN (*PHASEOLUS VULGARIS*) PODS

Abstract

One of the most important challenges in agriculture, besides enhancing food production, is to provide essential minerals and nutritional compounds to humans for maintenance of health and proper organ function. Humans require more than 22 mineral elements, with some of them required in large amounts (Ca, K, Na, Mg, P and K) and others, such as Fe, Zn, Cl, Cu, I, Se, F and Mn in trace amounts, being harmful at higher concentrations. Vegetables play a significant role in human nutrition, especially as a source of minerals, vitamins, dietary fibre and phytochemicals. Applications of *Moringa oleifera* leaf extract (MLE) has been investigated as means to affect the nutritional composition of various crops; MLE is rich in health-promoting phytochemicals, vitamins and minerals; therefore, the effects of MLE on the mineral composition of various parts of radish (*Raphanus sativus*) and green bean (*Phaseolus vulgaris*) plants were investigated. Radish leaves and roots, as well as green bean pods, were analysed for their mineral content. Findings showed that applications of MLE significantly influenced ($p < 0.05$) the mineral concentration of radish and green bean plants, with MLE-treated plants obtaining better growth and higher mineral concentrations than control plants.

Keywords: Mineral elements, Moringa leaf extract, Radish, Green beans.

1 Introduction

One of the greatest challenges in agriculture, besides enhancing food production, is to provide essential minerals and organic compounds necessary for humans to maintain good health and proper organ function (Martinez-Ballesta et al., 2009). There is an increasing awareness among the general public of the advantages of a diet rich in vegetables, as it ensures adequate intake of dietary fibres, vitamins, micronutrients and phytochemicals that contribute to a healthy human body (Dias, 2012). Aslam et al. (2015) highlighted that the production of safer and healthier food, possibly by using sustainable and environmentally friendly agricultural practices, plays a vital role in determining the nutritional benefits of food, as well as its market value. Humans require more than 22 mineral elements (Welch and Graham, 2004), some in large amounts, such as calcium, potassium, magnesium and phosphorus and others, elements such as iron, zinc, chloride, copper, iodine, selenium, fluoride and manganese, in trace amounts with higher concentrations being potentially harmful.

Vegetables are edible portions of herbaceous plants and are considered highly beneficial for the maintenance of human health and prevention of diseases (Hanif et al., 2006). Culver et al. (2012) describes vegetables as high-value crops which have a high nutritive value. Vegetables play a significant role in human nutrition, especially as a source of minerals, vitamins, dietary fibre and phytochemicals (Hanif et al., 2006). Minerals are essential components of a healthy, balanced diet required for normal metabolic activities of the human body (Hanif et al., 2006). Out of 92 naturally occurring elements, 25 are present in living organisms and are constituent of teeth, bones, hair, blood, nerve cells and muscles (Litwack, 2017). Furthermore, vitamins cannot be properly assimilated without the correct balance of minerals and occur in foods, especially in vegetables (Sonni-Alvarez, 2002). Chatterja and Shinde (1998) further highlight that vitamins help the body absorb calcium and phosphorus required for bone growth and maintenance and are involved in the normal function of the nerve system and endocrine glands.

According to Dias (2012), consumers' interest in vegetables with high nutritional quality is at an all-time high, so that more and more consumers are choosing to purchase and consume vegetables on the basis of their health benefits. As a result, MLE is continuously investigated to ascertain its effect on the nutritional composition of various crops, as MLE is rich in phytochemicals, such as antioxidants, vitamins and minerals (Biel et al., 2017). Therefore, the current study was conducted to evaluate the effect of MLE on the nutritional composition of two commonly consumed vegetables, green beans *(Phaseolus vulgaris)* and radish (*Raphanus sativus*).

Green beans are one of the most important vegetables in the Fabaceae family (Safí-naz and Mostafa, 2015) and play a vital role in human nutrition representing a cheap source of protein, vitamins (vitamin A, C and K) and minerals (Hamaiel et al., 2016). Green beans have a high fibre content which helps stabilize blood sugar, boast satiety and improve digestive health (Culver et al., 2012). Green beans contain a relatively low glycemic index (GI) (about 15), meaning, they are considered foodstuff that contributes minimally to enhancing the effect of blood sugar level (Safí-naz and Mostafa, 2015).

Radish is a root vegetable which is grown and consumed in most parts of the world, as a result it is not common among some populations, but considered part of the human diet. Radish is normally consumed raw, as a crunchy vegetable, mainly in salads (Banihani, 2017). Khattak (2011) noted that the importance of radish as foodstuff is derived from its fibre, carbohydrate, protein and fluoride content. Radish is a low-calorie vegetable that has no fat or cholesterol; for its size, radish contains a relatively high amount of vitamins, such as vitamin K, vitamin C and other water-soluble vitamin B-complex group ($B_1, B_2, B_3, B_5, B_6, B_9$) ; minerals (calcium, iron, magnesium, manganese, zinc, potassium and phosphorus); folate and antioxidants (N.C. Cooperative Extension, 2012).

2 Materials and Methods

2.1 Experimental Site

Mineral analysis of radish leaves as well as storage roots and green bean pods was carried out by an independent laboratory at Cedara College of Agriculture in Hilton, South Africa.

2.2 Experimental design and treatment structure

A completely randomised design was used in this experiment. The experiment consisted of crops, radish (Raphanus sativus) and green beans (*Phaseolus vulgaris L.*) and was replicated five times. The following treatments were used; inorganic fertilizer Calmag+B (5 g/plant) (T1), 100% MLE (T2) (20 g/L) and 50% MLE (T3) (T2 diluted to 50% with 100% methanol). After harvest, the plants were freeze-dried for three days, followed by blending the plant material into fine powder for the biochemical analysis.

From the fine powder, 5 g was weighed out and a total of 54 samples (18 radish leaves; 18 radish storage root and 18 green bean pods) was prepared and sent to an independent laboratory for mineral analysis.

2.3 Plant material

The garden radish cultivar ‘Sparkler’ and garden bushbean ‘Contender’ were purchased from Starke Ayres, Pietermaritzburg.

2.4 Procedure for determination of minerals in MLE treated plants

Elements were analysed on ICP-OES [Al, Fe, Mn, Zn, Cu, Ca, Mg, Na, P and K] according to Hunter’s method (1984) by an independent laboratory at Cedara College of Agriculture in Hilton, South Africa.

Briefly, analysis was carried out as follows: Extraction procedure: Sample aliquots (0.5 g) were weighed into beakers and placed in an oven set at 110°C for 2 hr. After two hours, the samples were removed and placed in a desiccator for cooling. After 30 min of cooling, the samples were

removed using a pair of tongs and weighed again. Thereafter, samples were taken to the furnace, set at 450°C, for ashing for 4 h. On the following day, the furnace was opened to allow samples to cool off and be taken for digestion.

Digestion and filtration procedure: Samples were ashed, cooled and wetted with a few drops of distilled water. Concentrated HCl (2 mL) was added to each sample. Thereafter, samples were allowed to evaporate slowly to dryness in a water bath in a fume cupboard equipped with an extractor fan. Freshly prepared 1:9 (HCl: Water) solution (25 mL) was added to the samples. Prior to analysis by ICP, the samples were diluted with de-ionized water (1:4 ratio) (Solution: De-ionized water).

2.5 Procedure for determination of C, N, S in moringa-treated plants

Mineral elements were determined using Duma's method (Matejovic, 1996) on a CNS analyser (LECO Africa, Kempton Park, SA). Briefly, samples were oven-dried at 110°C overnight; thereafter, 0.125 g sample was placed into the analyser for determination of the elements (CNS Instrument procedure: Oxygen pressure was set at 3000 kPa, while temperature of the furnace was set at 950°C and after-burn temperature was set to 850°C).

2.6 Statistical analysis

The data were statistically analysed using GenStat (version 14.1) (VSN, Hemel Hempstead, England, UK.). Analysis of variance (ANOVA) was used to test the overall significance of the data, while treatment means were compared by Duncan's multiple range tests at 5% level of significance.

3 Results

3.1 Mineral concentration of radish plant material

3.1.1 Radish leaves

Applications of MLE to radish plants had a significant effect ($p < 0.05$) on the mineral concentration of radish leaves, such that plants treated with MLE were characterised by an overall higher mineral concentration than control plants (Table 5.1). Basic macro-elements (N, P, K, Ca, Mg) required for plant growth and development were found to be higher in MLE 50%. Plant macro-element (N, P, K, Ca, Mg) concentrations were found higher in MLE 50% plants and mean values ranged from N -7.46%, P- 0.58%, K- 8.19% to Ca- 3.79%, followed by MLE 100% treated plants and the lowest concentrations were found in control plants. Plant micro-element (Na, Cu, Mn, Fe, Al, Zn) concentrations were also found to be higher in MLE 50% plants than MLE 100% plants and control plants. Lastly, Na concentrations were found to be higher in all treatments. Overall, nitrogen, magnesium and iron concentrations were found to be higher in MLE-treated plants.

Table 5. 1: Mineral nutrients (% DM and mg/kg DM) of radish leaves from the first harvest.

Treatment	N %	P %	K %	Ca %	Mg %	Na mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Al mg/kg	Zn mg/kg
Control	5,59a	0,13a	5,03a	2,59a	0,69a	11867a	2,40a	40,67a	66,30a	36,33a	46,67a
MLE 50%	7,46b	0,58b	8,19b	3,73b	0,91b	11130a	3,50b	60,67a	123,30a	52,67b	72,00ab
MLE 100%	7,03b	0,54b	8,04b	3,51b	0,96b	9351a	3,17ab	57,00a	117,70a	53,33b	84,00b
LSD	0,98	0,18	1,13	0,09	0,04	5329,42	0,74	19,65	79,53	8,62	21,66
P-value	0,013	<,001	0,002	<,001	<,001	0,473	0,033	0,094	0,206	0,009	0,020
CV%	6,40	8, 90	6, 90	2, 00	2, 20	21, 80	10, 80	16, 40	34, 20	8, 00	14, 10

Mean values in the same column for each trait followed by the same letter are not significantly different; MLE = Moringa leaf extract.

3.1.2 Radish storage roots

Treatments applied to radish plants had a significant influence ($p < 0.05$) on the mineral concentration of storage roots (Table 5.2). Both MLE treatments resulted in enhanced mineral levels compared with control plants, however, MLE 100% had a tendency towards the highest mineral concentrations. Plant macro-element (N, P, K, Ca, Mg) concentrations were found to be higher in MLE 100% plants with mean values ranging from N- 3.92 %, P- 0.59%, K- 8.40% to Mg- 0.88% DM; followed by MLE 50% treated plants and the lowest concentrations were found in control plants. Plant micro-element (Cu, Mn, Fe, Al) concentrations were found to be greater in MLE 50% plants and mean values ranged from Cu- 2.90 mg/kg, Mn- 7.67 mg/kg, Fe- 46.00 mg/kg to Al- 44.33 mg/kg DM; followed by MLE 100% plants and lowest concentrations were found in control plants. The latter plants also attained the highest Na (7682 mg/kg DM) concentrations compared with MLE plants.

Table 5. 2: Mean values of radish storage mineral concentration (% DM and mg/kg DM) of radish storage from the second harvest.

Treatment	N %	P %	K %	Ca %	Mg %	Na mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Al mg/kg	Zn mg/kg
Control	2,54a	0,40a	5,410a	0,10a	0,10a	7680a	1,47a	4,67a	24,00a	15,67a	21,67a
MLE 50%	3,69b	0,54ab	8,430a	0,65b	0,82b	6605a	2,90a	7,67b	46,00b	44,33b	28,33a
MLE 100%	3,92b	0,59b	8,400a	0,24ab	0,88b	6793a	2,83a	6,33b	43,67b	29,33ab	34,00a
LSD	0,24	0,13	2,62	0,31	0,10	3713,54	1,43	1,51	4,31	18,22	14,89
P-value	<,001	0,029	0,052	0,017	<,001	0,713	0,083	0,013	<,001	0,030	0,185
CV%	3,10	10,90	15,60	42,00	7,30	23,30	26,20	10,70	5,00	27,00	23,50

Mean values in the same column for each trait followed by the same letter are not significantly different; MLE = Moringa leaf extract.

3.2 Mineral concentration of green bean pod material

3.2.1 First pod harvest (harvested from the fruit developed from the first flower flush)

Statistically, the treatments applied to green bean plants had a significant effect ($p < 0.05$) on the mineral concentration of the first pods harvested. Moringa treatment increased mineral elements in bean pods (Table 5.3), with MLE 50% plants recording the highest macro-element (N, P, K, Ca, Mg) concentrations containing: N- 3.73%, P- 0.58%, K- 3.90%, Ca- 1.85%, Mg- 0.81%; followed by MLE 100% plants, while the lowest concentrations were recorded for control plants. Plant micro-element (Na, Cu, Mn,) concentrations were found to be greater in both MLE treatments compared with control plants; MLE 50% treated plants had higher concentrations of Na (427. 40 mg/kg), Mn (51.33 mg/kg), Al (7.33 mg/kg), Zn (45.00 mg/kg), while MLE 100% plants recorded highest Cu (4.00 mg/kg) and Fe (85.33 mg/kg) concentrations.

Table 5. 3: Mineral nutrients (% DM and mg/kg DM) of green bean (*Phaseolus vulgaris*) pods from the first harvest.

Treatment	N %	P %	K %	Ca %	Mg %	Na mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Al mg/kg	Zn mg/kg
Control	1,97a	0,36a	2,29a	0,74a	0,36a	198,90a	1,53a	35,33a	54,33a	4,00a	31,33a
MLE 50%	3,73b	0,58c	3,90b	1,85b	0,81b	427,40b	3,70b	51,33b	78,67ab	7,33a	45,00a
MLE 100%	3,70b	0,55b	3,57b	1,84b	0,69b	393,00b	4,00b	50,00b	85,33b	6,00a	43,33a
LSD	0,59	0,02	0,65	0,12	0,31	102,87	0,56	6,59	19,60	2,39	11,32
P-value	0,002	<0,001	0,005	<,001	0,036	0,007	<,001	0,004	0,025	0,043	0,053
CV%	8,50	1,80	8,80	3,70	22,30	13,40	8,00	6,40	11,90	18,20	12,50

Mean values in the same column for each trait followed by the same letter are not significantly different. MLE = Moringa leaf extract.

3.2.2 Second pod harvest (harvested from the second flower flush with and additional MLE foliar applications)

Treatments applied to green bean plants had a significant influence ($p < 0.05$) on the mineral concentration of pods from the second harvest, as most MLE-treated plants had higher mineral concentrations than control plants. It is evident from Table 5.4 that MLE-treated plants contained higher mineral concentrations in the pods. Overall, MLE- treated plants had higher pod macro-element concentrations compared than the control; MLE 50%-plants recorded the highest in P (1.18%) and K (4.22%) concentrations, while pods on MLE 100% plants recorded the highest N (5.51%), Ca (1.27%) and Mg (0.82%) percentages. Plant micro-elements (Na, Cu, Mn, Fe, Al, Zn) concentrations were found to be greater in pods of MLE-treated plants compared with those on control plants; MLE 50% plants contained pods with the highest concentration in Zn (55.67 mg/kg) and MLE 100% treated plants recorded highest in Na (375.7 mg/kg) Cu- 4.57 mg/kg; Mn- 44.33 mg/kg and Fe (84.33 mg/kg).

Table 5. 4: Mineral nutrients (% DM and mg/kg DM) of green bean pods from the second harvest.

Treatment	N %	P %	K %	Ca %	Mg %	Na mg/kg	Cu mg/kg	Mn mg/kg	Fe mg/kg	Al mg/kg	Zn mg/kg
Control	2,43a	0,34a	2,44a	0,42a	0,17a	158,40a	1,06a	27,33a	66,33a	2,66a	34,33a
MLE 50%	4,70b	1,18a	4,22b	0,79b	0,46b	350,20b	4,07b	44,00b	78,67b	5,66b	55,67b
MLE 100%	5,51b	0,80a	3,82b	1,27b	0,82b	375,5b	4,57b	44,33b	84,33b	5,67b	50,00b
LSD	1,19	0,76	1,36	1,05	0,79	86,48	0,75	5,37	7,49	1,60	9,92
P-value	0,003	0,067	0,046	0,059	0,055	0,004	<,001	0,001	0,006	0,010	0,009
CV%	11,40	43,30	17,20	56,10	72,20	12,90	9,70	6,10	4,30	15,20	9,40

Mean values in the same column for each trait followed by the same letter are not significantly different. MLE = Moringa leaf extract.

4 Discussion

Various parts of *Moringa oleifera* are highly nutritious and contain important antioxidants, vitamins, minerals, proteins and amino acids (Anwar et al., 2007). Applications of MLE has been shown to improve concentrations of various minerals, phenolics and antioxidants in plants (Mohammed, 2015). Furthermore, various parts of *M. oleifera* are excellent sources of minerals necessary for human health (Mohammed, 2015). In the described current experiment, MLE applications also affected the mineral composition of radish leaves, storage root and green bean plants. MLE applications significantly ($p < 0.05$) increased both plant macro- and micro-elements concentrations in radish and green bean plants.

4.1 Effect of MLE foliar applications on radish mineral concentration

Table 5.1 reveals that MLE 50% treated radish plants contained a slightly higher leaf Mg and Fe concentration. These results are in agreement with Kasolo et al. (2010) who noted that MLE contains a variety of minerals such as Ca, Fe, K, Zn, Mg and Cu. Studies conducted by El Sohaimy et al. (2015) revealed that Mg levels in moringa leaves were 25, 64 mg ± 0.35 mg/100 g DM. The same authors further mentioned that Fe levels in moringa leaves are 9,45mg per 100 g DM. Sodamade et al. (2013) stated that moringa leaves contain high Na levels (289 mg per 100 g DM), as confirmed in Table (5.1 and 5.2) resulting in an increase in Na concentrations in radish leaves.

The supply of minerals is an important factor influencing the growth of plant roots and, henceforth, the development of storage roots, ultimately resulting in healthy plants and higher yields (Sainju et al., 2005). *Moringa oleifera* has been reported as a valuable source of both macro- and micro-nutrients, needed by the plant as well as the human body. Moringa leaves are a significant source of protein, vitamin C, β-carotene, potassium, iron and calcium (Yasmeen et al., 2013). The present study revealed that radish contains remarkable macro- and micro-nutrient concentrations in the storage roots; furthermore, MLE 50% and MLE 100%

treatments resulted in plants with higher K concentrations in radish storage roots compared with those of control plants. These findings are in accordance with Yasmeen et al. (2013) who reported that exogenous applications of MLE to wheat plants increased leaf K concentrations. Sivakumar and Ponnusami (2011) revealed that exogenous foliar applications of MLE increased uptake and accumulations of some elements, such as N, P, Ca, Mg, and as well as K and Fe, into roots and shoots of several plants. Hanafy (2017) reported that MLE contains a significant level of Ca which can prevent injuries and leakages to membranes and can stabilize membrane structures. Mohammed et al. (2018) further concluded that plant hormones, such as cytokinins, contained in MLE can result in growth stimulation due to MLE acting as a natural plant biostimulants.

4.2 Effect of MLE foliar applications on green bean mineral concentration

Applications of MLE 50% and MLE 100% increased the mineral concentrations in bean pods, with later harvested pods even displaying slightly higher mineral concentrations than earlier harvested pods. This could possibly be explained as a transport or penetration phenomenon, with pods removed during the first harvest and the final spray prior to harvest had not penetrated well into the plants. Sodamade et al. (2013) revealed that moringa leaves are constituent of various nutrients and a good source of dietary minerals from plants. Mohammed et al. (2018) also highlighted that MLE is a source of several mineral elements, such as Ca, Mg, P, K, Mn, Zn, Fe and Cu. Table 5.3 and 5.4 results ascribed to the high level of macro- and micro-nutrients in MLE, mainly the high potassium and zinc levels. Potassium improves fruit quality by enhancing the formation and translocation of carbohydrates from the shoots to storage organs (Ramezani and Shekafandeh, 2011). Zinc is precursor of tryptophan which is involved in synthesis of indole acetic acid required for cell fruit growth and development (Zekri and Obreza, 2009).

5 Conclusion

Every part of the *Moringa oleifera* plant contains minerals and vitamins. Applications of MLE has been shown to improve various minerals, phenolics and antioxidants in plants; following two levels of moringa applications, the mineral concentration of radish leaves and roots as well as green bean pods was analysed. Findings showed that applications of MLE significantly increased ($p < 0.05$) the mineral concentration of radish and green bean plants, with MLE-treated plants in general obtaining better growth and higher mineral concentrations in leaves, pods and roots compared with control plants.

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CHAPTER 6

EVALUATION OF MORINGA LEAF EXTRACT ON ANTIOXIDANT PROPERTIES AND PLANT PIGMENTS OF RADISH (*RAPHANUS SATIVUS*) AND GREEN BEANS (*PHASEOLUS VULGARIS*)

Abstract

Plant pigments belong to various categories such as anthocyanins, carotenoids, betalains and chlorophylls. Increasing attention has been given to these pigments due to their health benefits as these compounds can serve as antioxidants. *Moringa oleifera* leaf extract (MLE) has been identified to contain a variety of natural antioxidants such as phenolics, ascorbic acid, carotenoids and flavonoids. It is, however not known, if applications of leaf extract can confer the properties of MLE onto the plant it has been applied to. Therefore, the aim of this experiment was to determine if MLE applications can enhance antioxidant properties (presence of ascorbic acid, anthocyanins, carotenoids and chlorophylls) of various parts of radish (*Raphanus sativus*) and green beans (*Phaseolus vulgaris*). The experiment was laid out in a completely randomized design with five replications and comprised of three treatments *viz*.: (control, only inorganic fertilizer Calmag+B (5 g/plant) (T1), common fertilizer plus MLE 100% (T2) (20 g/L dried moringa powder and MLE 50% (T3) (T2 diluted to 50% with 100% methanol). The findings illustrate that *M. oleifera* leaves are a rich source of antioxidants and also contain numerous beneficial nutrients, likely to be aligned with the enhanced pigment concentrations in various plant parts following MLE applications. Plants treated with MLE recorded a higher concentration of antioxidants, anthocyanins, ascorbic acid and total chlorophylls ($p < 0.05$) than control plants. Overall, leaves and fruit of plants treated with MLE 50% showed a higher overall antioxidant concentration than the MLE 100% applications and the control.

Keywords: Moringa leaf extract (MLE), antioxidants, anthocyanin, ascorbic acid, plant pigment

1 Introduction

Antioxidants found in vegetables play an important role in the maintenance of health and prevention of diseases (Shetty et al., 2013). It has been estimated that every serving of vegetable reduces the risk of cardiovascular disease by 30% and cancer by 15%; this is attributed to the antioxidants, such as carotenoids, ascorbic acid, vitamin E, lycopene and polyphenols found in these commodities (Shetty et al., 2013). Natural antioxidants include a vast number of plant phenolic compounds that occur in almost all plant parts (Akbarirad et al., 2016). Some of these antioxidants, such as phenolics, are also pigments, e.g. the anthocyanins and betalains, although non-phenolic anti-oxidant pigments such as carotenoids and chlorophylls are also of great importance as antioxidants (Jensen et al., 2011). Increasing attention has been given to the various groups of pigments due to their health benefits as antioxidants and or as anti-inflammatory substances (Cruz et al., 2013). The presence of pigments gives colour to vegetables, with the pigment composition depending on species and cultivar (Costache et al., 2012). Plant pigments can further be separated into fat-soluble pigments, found in plastids, such as the chlorophylls and carotenoids in chloroplasts and/ or chromoplasts (Boo et al., 2012), and the water-soluble pigments, dissolved in the cell sap, including flavonoids, such as anthocyanins (Zhoh et al., 2010).

Anthocyanins are the largest group of water-soluble pigments in plants, responsible for the red, purple and blue colours found in many fruit, flowers and vegetables (Kim et al., 2012). These pigments occur in all tissues of higher plants, providing colour to leaves, stems, roots, flowers and fruit (Jensen et al., 2011). Although anthocyanins are used by plants as attractants of pollinators and fruit or seed dispersers, they are also compounds involved in a wide range of plant biological activities (Kong et al., 2003) as well as being substances that enhance the health of humans by decreasing the risk of cancer and reducing inflammation (Kim et al., 2012). Betalains are red and yellow pigments, similar to anthocyanins, as they are water-soluble, but

are only found in certain groups of the plant kingdom, where they fulfil functions similar to anthocyanins, such as being responsible for the deep red colour of beets; commercially, they are important as food-colouring agents (Boo et al., 2012). Carotenoids are lipo-soluble pigments found in plants, algae, photosynthetic bacteria and animals, where they are responsible for the yellow, orange and red colour of various fruit and vegetables and animal organs (Saini et al., 2015). The types of carotenoids present in fruit and vegetables can be predicted by the commodity's colour; yellow-orange vegetables and fruit are generally rich in carotenes and xanthophylls found in subtropical fruit, such as orange, mandarin and papaya; lycopene, on the other hand, is a pigment of bright red colour and the major constituent of tomatoes. Lutein and β-carotene are yellow carotenoids predominantly commonly found in green leafy vegetables (Haskell, 2013). Lastly, chlorophylls are the most abundant plant pigments on Earth, giving green colour to plants (Boo et al., 2012).

Natural antioxidants, such as tocopherols, vitamin C, flavonoids and other phenolic compounds are known to be present in many plants (Pakade et al., 2013). *Moringa oleifera* is one such plant that has been identified to contain high concentrations of natural antioxidants (Anwar et al., 2007). Moringa leaves have been described to contain high concentrations of minerals, vitamins, fatty acids and amino acids (Teixeira et al., 2014). Additionally, moringa leaves have been reported to contain various antioxidant compounds such as phenolics, ascorbic acid, carotenoids and flavonoids (Stohs and Hartman, 2015). These antioxidants can be applied to plants and enhance their antioxidant concentration, either by directly adding to the antioxidants available or triggering reactions in the plant that up the antioxidant pool. Therefore, the aim of this study was to determine the effect of moringa leaf extract on the antioxidant properties of radish and green beans.

2 Materials and Methods

2.1 Experimental site, design and treatment structure

Radish (*Raphanus sativus*) and green bean (*Phaseolus vulgaris*) plants were grown in a temperature-controlled glasshouse from mid-March to early June 2018 at the University of KwaZulu-Natal - Pietermaritzburg campus, at the School of Agricultural, Earth and Environmental Sciences (29°37'34.8"S, 30°24'12.0"E). A completely randomised design was used in this experiment. Treatments used: (control, only inorganic fertilizer Calmag+B (5 g/plant) (T1), common fertilizer plus MLE 100% (T2) (20 g/L dried moringa powder (obtained from Run KZN, Pietermaritzburg, South Africa)) and MLE 50% (T3) (T2 diluted to 50% with 100% methanol). Plants were harvested once they had attained the 10 fully-mature-leaf-stage; plants were separated into leaves and roots and freeze-dried, followed by blending the plant material into a fine powder that was kept at -20ºC until biochemical analysis - except for the radish storage root, where anthocyanins were determined from fresh material.

2.2 Plant material

Seeds of the garden radish cultivar 'Sparkler' and the garden bushbean 'Contender' were purchased from Starke Ayres, Pietermaritzburg.

2.3 Biochemical analysis of plant material

2.3.1 Determination of anthocyanins

The total anthocyanin concentration in radish roots was determined according to Lange et al. (1970), with slight modifications. Briefly, 2 g of fresh radish root cambium was weighed out, and the plant material cut into pieces using a razor blade. Each sample was placed in a mortar and 3 mL 3 M HCl (pH 0.2) was added together with a pinch of sand, to assist in the grinding of the plant material. Thereafter, the mixture was poured into centrifuge tubes and homogenised in 10 mL 3 M HCl (pH 0.2), homogenized using an Ultra- Turrax (IKA T25 digital, Staufen, Germany) at 8000 rotations/min for 30 sec. The homogenate was then placed into a centrifuge

(Avanti J-26 XPI Beckman Coulter, Brea, CA) at 5000 rpm for 10 min at 4°C. Thereafter, the sample was transferred into an evaporation vial, in order to reduce volume to less than 10 mL in a Genevac sample evaporator (EZ- 2plus, New York, USA) for 30 min.

Sample absorbance was read at 530 and 653 nm using a spectrophotometer (UV- 1800 Shimadzu Corp., Kyoto, Japan) according to the formula provided by Lange et al. (1970) (A_{530}-0.24 A_{653}) = μg anthocyanin / g FM^{-1}. The solution was diluted at ratio of 1:8 prior to reading the absorbance.

2.3.2 Determination of antioxidants

Samples of 0.5 g DM fine plant powder was analysed according to Brand-Williams et al. (1995); the material was weighed out into a test tube and 10 mL 80% methanol were added. The solution was homogenised using a top vortex mixer (Heidolph REAX 2000, Burladingen, Germany) and thereafter placed in an oven for 24 hr at 40°C. Samples were extracted in DH (2, 2-diphenyl-1-picryl-hydrazyl-hydrate, 0.0024 g dissolved in 100 mL methanol to make a working solution of DH).

Total antioxidant activity was determined using the DH (2, 2 – diphenyl- 1 – pycrylhydrazyl) assay described by Miliauskas et al. (2004). This free radical scavenging method evaluates the antioxidant activity of an extract and is based on the measurement of scavenging capacity of antioxidants expressed as μmol Trolox equivalent/g of dry matter. Briefly, a sample extract amounting to 1 mL was transferred into test tubes, DH solution (1 mL) added and the volume made up to 4 mL with 95% methanol. The mixture was placed at ambient temperature for 30 min before reading the absorbance at 515 nm using a UV-1800 spectrophotometer (Shimadzu, Kyoto, Japan).

2.3.3 Determination of ascorbic acid

Ascorbic acid was determined according to Mohammed et al. (2018) with slight modifications. Samples of 0.5 g DM were weighed out, placed into centrifuge tubes and mixed with 4 mL of 0.56 M metaphosphoric acid, shaken and centrifuged (PLC – 05 Centrifuge PLC series, Berlin, Germany) at 3000 rpm for 10 min. Thereafter, the solutions were mixed with 2 mL 0.3 M trichloroacetic acid and centrifuged at 2000 rpm for 10 mins. The supernatant was mixed with 2 mL 2, 4-dinitrophenyl-hydrazine reagent. Finally, samples were heated at 60°C for 1 hr, followed by an ice bath cooling for 5 min. The addition of 4 mL 18.71 M sulphuric acid to the samples resulted in a colour change from colourless to a blue hue and absorbance was read at 520 nm after 20 min (UV-1800 Shimadzu, Kyoto, Japan).

2.3.4 Determination of chlorophyll a, chlorophyll b and total carotenoids

The concentration of chlorophyll a, chlorophyll b and total carotenoids (xanthophylls and carotenes) was determined spectrophotometrically in extracts of the green bean pods. Samples of 1 g DM fine powder were weighed out into centrifuge tubes, 4 mL acetone: water (4:1) was added to each sample which was then left to stand for 10 min, covered with aluminium foil. Following homogenisation, samples were placed into a table-top centrifuge (PLC – 05 Centrifuge PLC series, Berlin, Germany) for 5 min at 1000 rpm. Thereafter, samples were decanted into clean test tubes to read the absorbance at various wavelengths (663.3, 649.8 and 470 nm) using the equation by Lichtenthaler and Buschmann (2001) to determine chlorophyll a, chlorophyll b, total chlorophylls and total carotenoid concentrations.

2.4 Statistical analysis

Data was statistically analysed using GenStat (version 14.1) (VSN, Hemel Hempstead, England, UK.). Analysis of variance (ANOVA) was used to test the overall significance of the data, while treatment means were compared by Duncan's multiple range tests at 5% level of significance.

3 Results

3.1 Biochemical parameters of radish plants

3.1.1 Root anthocyanin concentration

Statistically, the treatments applied to radish plants had a significant influence ($p < 0.05$) on the anthocyanin concentration in the root cambium. Overall (Fig 6.1), MLE 100% applications resulted in the highest root cambium anthocyanin concentration (0.79 mg g^{-1}), followed by MLE 50% (0.70 mg g^{-1}) and control plants (0.60 mg g^{-1}).

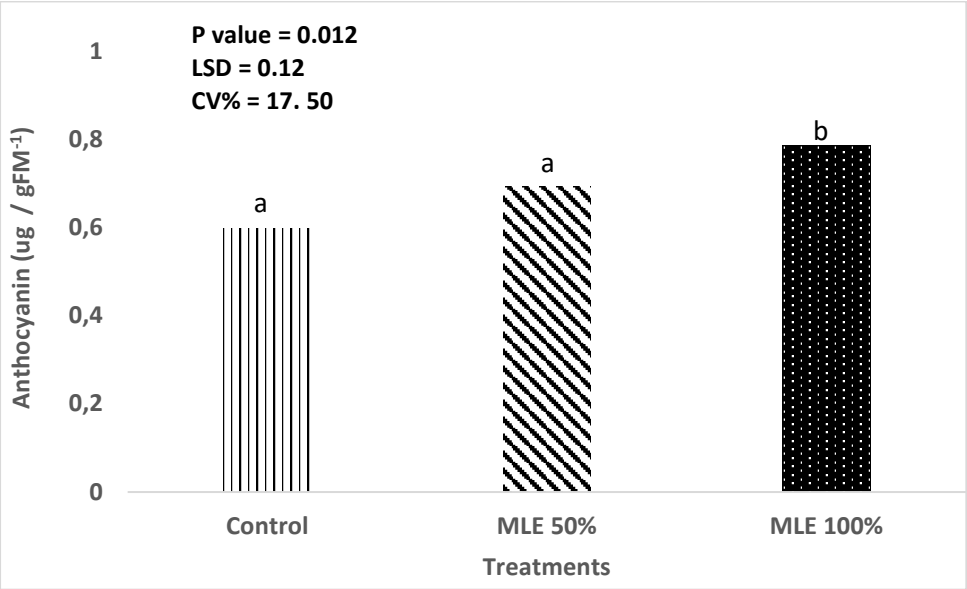

Figure 6. 1: Radish root cambium anthocyanin concentration (Control = 0.60 mg g^{-1}, MLE 50% = 0.70 mg g^{-1}, MLE 100% = 0.79 mg g^{-1}).

3.1.2 Root antioxidant concentration

The entire root antioxidant concentration of moringa-treated plants was significantly ($p < 0.05$) higher than the control; overall both MLE treatments resulted in a significantly higher antioxidant concentration than the control (Fig. 6.2). MLE 50% obtained a mean value of 0.19 mg g^{-1} DM (TEAC), followed by MLE 100% with a mean value of 0.17 mg g^{-1} DM (TEAC) and the lowest antioxidant concentration was recorded in control plants, obtaining a mean value of 0.14 mg g^{-1} DM (TEAC) (Fig. 6.2).

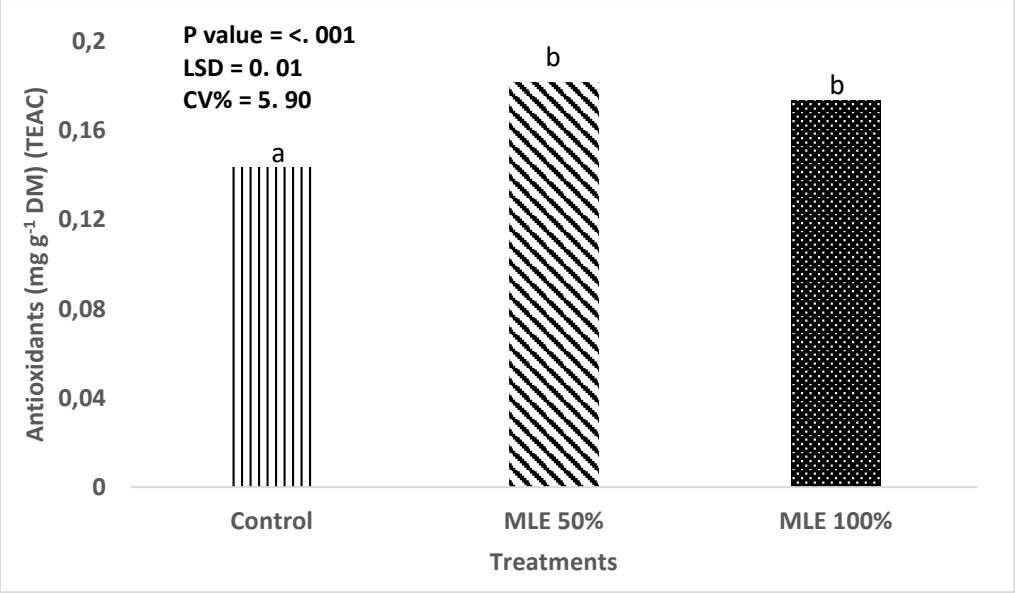

Figure 6. 2: Radish (entire root) antioxidant concentration (Control= 0.14 mg g^{-1} DM (TEAC), MLE 50% = 0.19 mg g^{-1} DM (TEAC), MLE 100% = 0.17 mg g^{-1} DM (TEAC). TEAC – Trolox equivalent antioxidant capacity.

3.1.3 Chlorophyll a, chlorophyll b and total carotenoids in radish leaves

The total chlorophyll concentration in radish leaves was significantly ($p < 0.05$) higher in moringa-treated than in control plants. Table 6.1 indicates that MLE 50% and MLE 100% treatments resulted in a similar range of chlorophyll concentrations, with both treatments displaying higher chlorophyll a, chlorophyll b and total chlorophyll concentrations than the control. Furthermore, the treatments applied had no significant effect on the carotenoid concentrations of radish leaves (Table 6.1).

Table 6. 1: Total chlorophylls and carotenoids concentrations of radish leaves

Treatment	Chlorophyll a (Ca) (mg*g^{-1})	Chlorophyll b (Cb) (mg*g^{-1})	Total chlorophylls (Ca+b) (mg*g^{-1})	Total carotenoids (Cx+c) (mg*g^{-1})
Control	4. 415 a	1. 492 a	5. 909 a	2. 333 a
MLE 50%	5. 917 b	3. 709 b	9. 626 b	2. 472 a
MLE 100%	5. 592 b	3. 405 b	8. 997 b	2. 486 a
P value	<. 001	<. 001	<. 001	0. 582
LSD	0. 44	0. 38	0. 64	0. 33
CV%	8. 40	13. 70	8. 00	14. 00

Mean values in the same column for each trait followed by the same letter are not significantly different. MLE = Moringa leaf extract.

3.2 Biochemical parameters of green beans from the 1st pod harvest (harvested from the first flower flush)

3.2.1 Total antioxidant concentration of bean pods

Statistically, MLE treatments had a significant influence ($p < 0.05$) on the antioxidant concentration of pods from the 1st harvest. Commercially mature, fully green fruit of moringa-treated plants recorded a higher antioxidant concentration than control plants, with MLE 100% and MLE 50% achieving more than 1.5 times higher antioxidant concentrations than the control (Fig. 6.3).

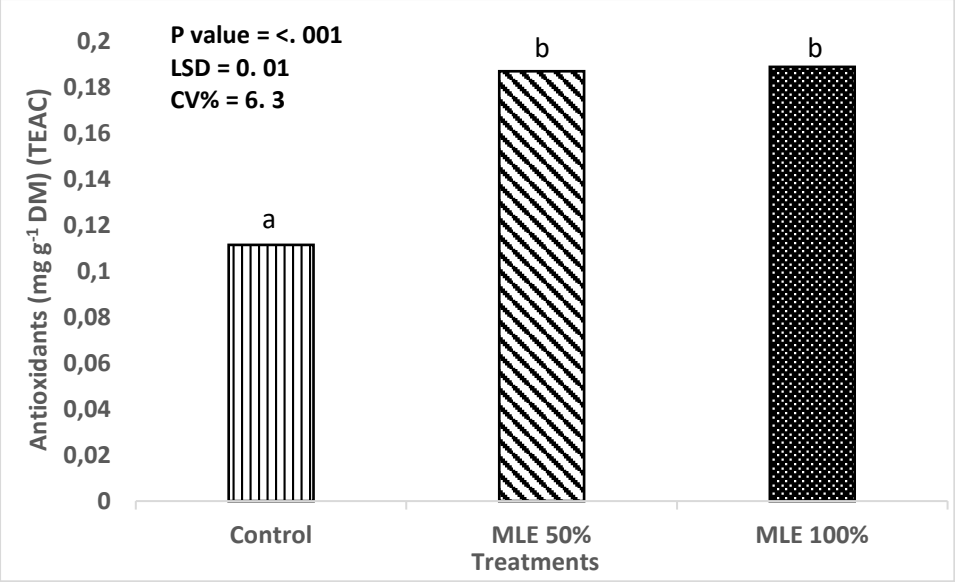

Figure 6. 3: Antioxidant concentration of green bean pods treated with MLE from the 1st green bean harvest. TEAC – Trolox equivalent antioxidant capacity

3.2.2 Pod ascorbic acid concentration

The treatments applied to green bean plants had a significant effect ($p < 0.05$) on the ascorbic acid concentration of the pods from the 1st harvest. Beans from moringa-treated plants had a higher ascorbic acid concentration than those from control plants (Fig. 6.4). Control plants only achieved an ascorbic acid concentration less than half of the moringa treatments.

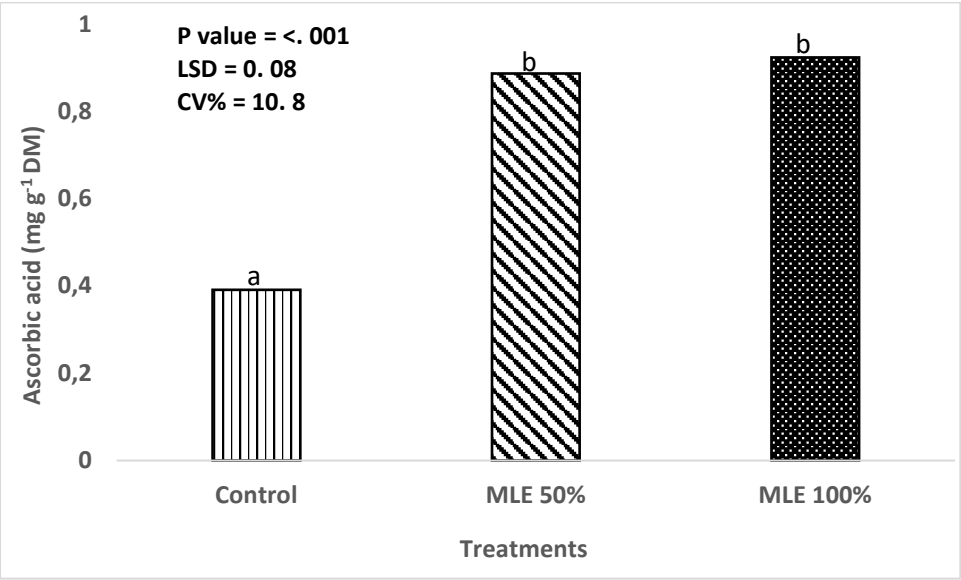

Figure 6. 4: Ascorbic acid concentration of pods from the 1st green bean harvest.

3.2.3 Chlorophyll a, chlorophyll b and total carotenoids in pods of green beans

Statistically, there was a highly significant difference ($p < 0.05$) in total chlorophylls and total carotenoids of pods from the first harvest. It was observed (Table 6.2) that pods of both moringa treatments had a higher chlorophyll a and chlorophyll b concentrations than those of control plants. Furthermore, control plants recorded a higher pod total carotenoid concentration than both moringa treatments.

Table 6. 2: Total chlorophylls and total carotenoids concentrations in pods from the first harvest

Treatments	Chlorophyll a (C_a) (mg*g^{-1})	Chlorophyll b (C_b) (mg*g^{-1})	Total chlorophylls (C_{a+b}) (mg*g^{-1})	Total carotenoids (C_{x+c}) (mg*g^{-1})
Control	5. 55 a	1. 67 a	7.21a	3. 17 b
MLE 50%	8. 03 b	3. 47 b	11. 91 b	2. 21 a
MLE 100%	8. 45 b	3. 26 b	11. 29 b	2. 17 a
P value	<. 001	0. 008	<. 001	0. 007
LSD	1. 37	1.18	2. 30	0.66
CV%	19. 10	43. 10	23. 30	27. 00

Mean values in the same column for each trait followed by the same letter are not significantly different. MLE = Moringa leaf extract.

3.3 Biochemical parameters of green beans from the 2nd pod harvest (harvested from the second flower flush, following an additional MLE 50% and MLE 100% treatment applications

3.3.1 Total antioxidants concentration of green bean pods

The antioxidant concentration of the pods from the second harvest was significantly ($p < 0.05$) higher in moringa-treated than in control plants; both applications, MLE 50% and MLE 100%, recorded antioxidant concentrations similar to the first harvest in pods from the second harvest. 'Generation of pods' had, therefore no significant influence on the antioxidant concentration

of the pods, as MLE treatments gave higher antioxidant concentration than the control (Fig. 6.5).

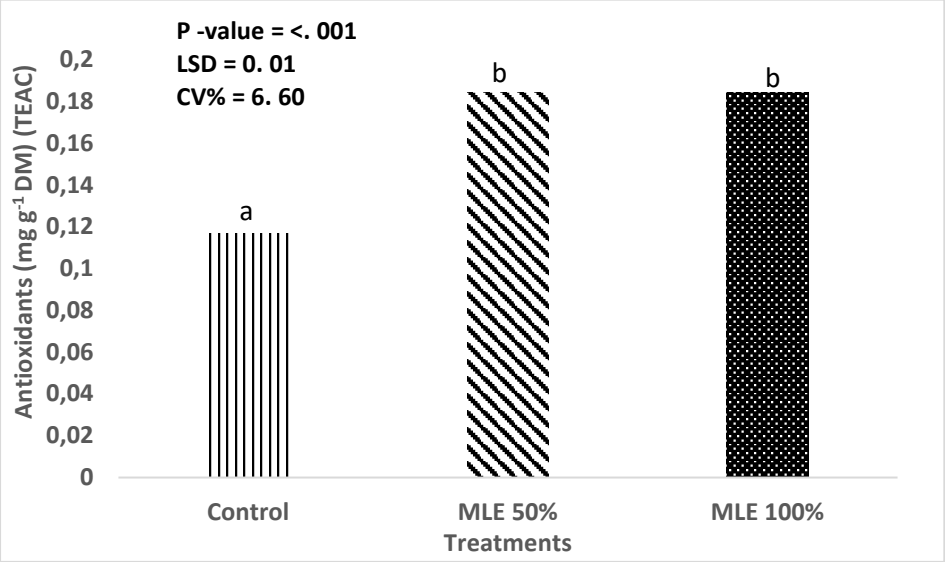

Figure 6. 5: Antioxidant concentration obtained during the second pod harvest. TEAC – Trolox equivalent antioxidant capacity.

3.3.2 Pod ascorbic acid concentration

The pod ascorbic acid concentration differed significantly ($p < 0.05$) between treatments applied to green bean plants. Moringa-treated plants attained higher ascorbic acid concentrations than pods from control plants (Fig. 6.6). Ascorbic acid concentrations of pods from the first and the second harvests showed similar trends.

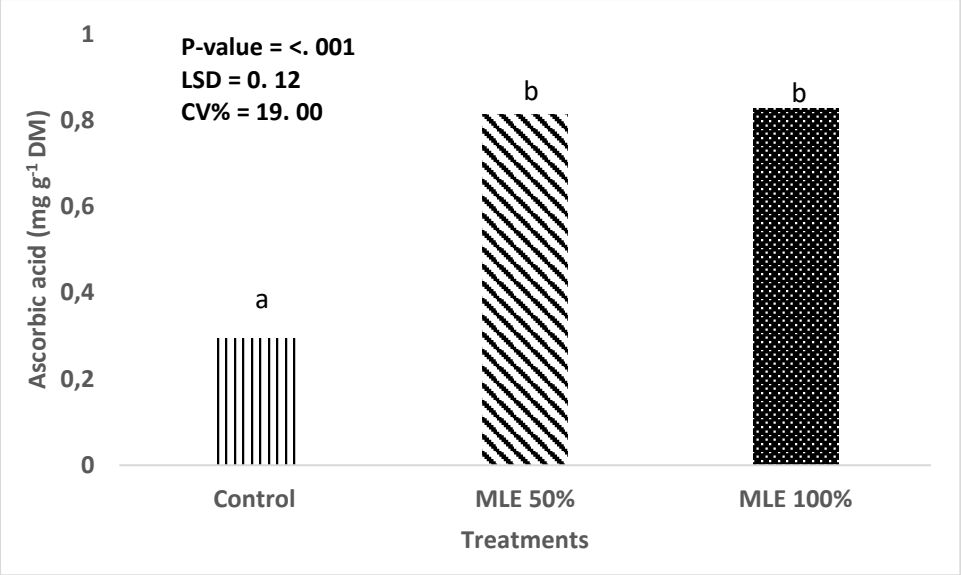

Figure 6. 6: Ascorbic acid concentration of pods from the 2nd harvest.

3.3.3 Chlorophyll a, chlorophyll b and total carotenoid concentration in pods of green beans

Statistically, treatments significantly influenced ($p < 0.05$) the total chlorophyll and total carotenoid concentrations of pods from the 2nd harvest. There was a similar result as pods from the first harvest, with no significant difference between the two MLE treatments (Table 6.3). The lowest chlorophyll a and chlorophyll b concentration were recorded in control plants. Moreover, total carotenoids were higher in pods from MLE treatments than from control plants. Total chlorophyll and total carotenoid concentrations seem to be influenced by 'pod generation', as pods from the first harvest tended towards a higher total chlorophyll and total carotenoid concentration compared to the second harvest (Table 6.2 and 6.3).

Table 6. 3: Total chlorophyll and total carotenoid concentrations of pods from the second pod harvest.

Treatments	Chlorophyll a (Ca)	Chlorophyll b (Cb)	Total chlorophylls (Ca+b)	Total carotenoids (Cx+c)
Control	3. 83 a	2. 14 a	5. 924 a	1. 58 a
MLE 50%	7. 72 b	2. 978 a	10. 70 b	2. 21 b
MLE 100%	7. 76 b	3. 28 a	11. 04 b	2. 16 ab
P value	<. 001	0. 049	<. 001	0. 029
LSD	1. 23	0.97	2.13	0.47
CV%	19. 50	35. 70	23. 70	24. 90

Mean values in the same column for each trait followed by the same letter are not significantly different. MLE = Moringa leaf extract.

4 Discussion

In recent years, the use of MLE as a biostimulant employed to improve the performance of many agricultural crops has gained significance (Jhilik et al., 2017). *Moringa oleifera*, also known as the "drumstick tree", has been described as a 'nutritiously rich' food, owing to the presence of a variety of phytochemicals in its leaves, pods and seeds (Gopolakrishnan et al., 2016). Ahmed et al. (2016) reported that *M. oleifera* is a highly nutritive, multipurpose plant that can be consumed as a fresh vegetable or spice, be used for livestock fodder and as green manure, as well as a cosmetic oil, biogas or medicine. Every part of the plant is edible, the leaves, in particular, can be eaten fresh, similar to other leafy green fresh commodities, such as lettuce (Fahey, 2005). Mohammed (2015) further explained that *M. oleifera* plays an important role in the provision of antioxidants, particularly the leaf is a rich source of natural antioxidants, such as flavonoids, phenolics, ascorbic acid and carotenoids. Other authors (Fuglie, 2000; Yasmeen, 2011) also reported that phenolics, vitamin C and other antioxidants, as well as photosynthetic pigments are present in high concentrations in moringa leaf extracts. Following

the applications of MLE 50% and MLE 100% (Fig. 6.2, 6.3 and 6.5), radish roots and green bean pods had high antioxidant concentrations. Saini et al. (2015) highlighted that the potent antioxidant activity in moringa leaves is particularly attributed by the high concentration of phenolics and pigments. There is no literature on MLE foliar applications enhancing root antioxidant concentration on storage organs. However; the high antioxidant activity present in moringa leaves could have possibly enhanced the antioxidant concentration in the radish roots, either transported by other plant organs or the foliar applications triggered the production of new antioxidants in the radish roots.

The present study demonstrated that treatment of green bean plants with MLE 50% and MLE 100% resulted in higher ascorbic acid concentrations than the control (Fig. 6.4 and 6.6). This finding is in alliance with Mohammed (2015) who noted that moringa leaves can act as the major source of nutrients and antioxidant compounds, such as ascorbic acid. Since moringa contains ascorbate, its exogenous applications might trigger the endogenous production of ascorbate (Thanaa et al., 2017). Ascorbate is a potent antioxidant, which is normally involved in sugar metabolism and also directly related to the production of vitamin C. The applications of MLE adds ascorbate (vit. C) giving the plant tissue even more antioxidants than it can produce by itself (Thanaa et al., 2017). These results are also in agreement with Nasira et al. (2016) who reported that 'Kinnow' mandarins treated with MLE displayed an increase in vitamin C concentrations. Zaki and Rady (2015) also found that spraying *Phaseolus vulgaris* plants with MLE resulted in an increase of vitamin C concentration of green beans

In the present study, the foliar applications of MLE 50% and MLE 100% significantly ($p < 0.05$) increased the radish anthocyanin concentration (Fig. 6.1) and the presence of photosynthetic pigments (Table 6.1, 6.2 and 6.3), namely chlorophyll a, chlorophyll b and carotenoids, in both, radish and green bean. This results support Ashraf et al. (2016) who

reported that foliar MLE applications resulted in higher concentration of photosynthetic pigments, ultimately enhancing the leaf chlorophyll concentration.

5 Conclusion

The outcome of this experiment revealed that both, MLE 50% and MLE 100%, significantly ($p < 0.05$) influenced the concentration of antioxidants, ascorbic acid and photosynthetic pigments of radishes and green beans. The present data clearly show that both MLE treatments have enhanced the antioxidant concentration and pigments in various plant parts of radish and green beans. Treating plants with MLE significantly ($p < 0.05$) enhanced the concentration of antioxidants, anthocyanins, ascorbic acid and total chlorophylls compared with control treated plants. Overall, applications of MLE 50% resulted in the highest antioxidant properties. Therefore, it can be recommended as a valuable source of natural plant biostimulant.

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CHAPTER 7

GENERAL DISCUSSION, CONCLUSION AND RECOMMENDATIONS

The present study aimed at evaluating the effect of moringa leaf extract (MLE) on the growth and development, mineral composition and antioxidant properties of radish (*Raphanus sativus*) and green beans (*Phaseolus vulgaris*). The applications of MLE effectively improved growth and development of radish (leaf size, number of leaves and, at final harvest, fresh and dry mass of storage root) and green bean plants (leaf size, pod size, number of flowers, number of mature leaves and, at final harvest, above and below ground fresh and dry mass). It has been reported that foliar applications of MLE can enhance the early vegetative growth of tomatoes (*Solanum lycopersicum*), peanuts (*Arachis hypogaea*), maize (*Zea mays*) and wheat (*Triticum aestivum*), improve the resistance of plants to pests and diseases and can generally increase yield by 20 to 35% (Culver et al., 2012). Among the two MLE treatments, MLE 50% resulted in superior development and yield of both radish and green bean plants compared with the MLE 100% and control plants. It can be concluded that the MLE 50% (diluted solvent) was easily and immediately absorbed by the plant tissue, thereby enhancing growth and development of radish and green beans at a faster rate compared with MLE 100%, which was undiluted and the concentration was possibly not immediately absorbed by plants after the MLE foliar applications.

Moringa oleifera has been reported as a valuable source of both macro- and micro-nutrients, valuable to plants as well as the human body. Moringa leaves are a significant source of protein, vitamin C, β-carotene, potassium, iron and calcium (Yasmeen et al., 2013). The results of the present findings showed that foliar applications of the treatments had significant influence ($p < 0.05$) and that MLE-treated plants obtained higher mineral concentrations compared with the control plants in both radish (N, P, K, Ca, Mg, Cu, Mn, Al, Fe) and green bean (N, P, K, Ca, Mg, Na, Mn, Al, Fe, Zn, Cu). These results are in agreement with Kasolo et al. (2010) who

noted that MLE contains a variety of minerals essential for plant growth and development, such as calcium, iron, potassium, zinc, magnesium, and coer.

Natural antioxidants, such as tocopherols, flavonoids, vitamin C and other phenolic compounds are known to be present in many plants (Pakade et al., 2013). Mohammed (2015) explained that *Moringa oleifera* plays an important role in the provision of antioxidants, particularly the leaves are a rich source of natural antioxidants, such as flavonoids, phenolics, ascorbic acid and carotenoids. In this study it was shown that treatments with MLE 50% significantly ($p < 0.05$) increased antioxidant properties of treated plants, particularly concentrations of total antioxidants, anthocyanins, ascorbic acid and total chlorophylls. It can be concluded that, since *M. oleifera* leaves are a rich source of antioxidants and also contain numerous beneficial nutrients, MLE applications are likely to be aligned with enhanced pigment concentrations and antioxidants properties in various plant parts.

Future research and recommendations

- More research is needed on how various MLE concentrations may influence the growth and development, antioxidant properties and also ascertain which concentration is best suitable for root and fruit crops.
- More intense studies are required on the frequency of MLE applications to enhance growth and antioxidant compounds of root and fruit crops.
- The results have shown that MLE contains various types of antioxidant compounds; therefore, it can be recommended as a valuable source of natural plant biostimulant.

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