EFFECTS OF SOIL TYPE, SALINITY AND VESICULAR ARBUSCULAR MYCORRHIZA ON GROWTH AND FOLIAR NUTRIENT ELEMENTS IN *MIMUSOPS ZEYHERI* (SOND.) INDIGENOUS FRUIT TREES

THABO SELBY NKUNA

DISSERTATION SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE MASTER OF AGRICULTURAL MANAGEMENT (PLANT PRODUCTION), DEPARTMENT OF PLANT PRODUCTION, SOIL SCIENCE AND AGRICULTURAL ENGINEERING, SCHOOL OF AGRICULTURAL AND ENVIRONMENTAL SCIENCES, FACULTY OF SCIENCE AND AGRICULTURE, UNIVERSITY OF LIMPOPO, SOUTH AFRICA

SUPERVISOR : PROF. P.W. MASHELA (UL)

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TABLE OF CONTENTS

DECLARATION

I, Nkuna Thabo Selby, declare that the dissertation hereby submitted to the University of Limpopo, for the degree Master of Agriculture Management has not been submitted previously by me or anybody for a degree at this or any other University. Also, this is my work in design and in execution and related materials contained herein had been duly acknowledged.

Candidate: Thabo Selby Nkuna _____ Signature _____ Date

DEDICATION

To the family I have always wanted to be my family

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LIST OF TABLES

LIST OF FIGURES

LIST OF LEGENDS

LIST OF APPENDICES

ABSTRACT

The evergreen Red Milkwood (*Mimusops zeyheri* Sond.) is being targeted as a rural and urban-greening tree in Limpopo Province, due to its excellent nutritional, pharmaceutical and aesthetic attributes. Slow growth of *M. zeyheri* seedlings is the main drawback in the potential domestication and commercialisation of this tree species. The objectives of this study were to determine (1) the influence of soil type on root growth and foliar nutrient elements in *M. zeyheri* seedlings, (2) the degree of salt tolerance in *M. zeyheri* seedlings and (3) the influence of vesicular arbuscular mycorrhiza (VAM) fungi on growth of *M. zeyheri* seedlings. Objective 1 was achieved in clay, calcareous, loam and sandy soils. At 60 days after the treatments, soil type effects were significant on plant height, leaf number, chlorophyll content of primary leaves (dicots), chlorophyll content of secondary leaves, root length, number of root branches and dry root mass, contributing 60, 72, 84, 85, 74, 80 and 40% in total treatment variation (TTV) of the respective variables. Objective 2 was achieved by exposing seedlings to 0, 2, 4, 8, 16, 32, 64 and 128 NaCl + $CaCl_2$ mM/m^3 at 3:1 ratio. At 90 days after the treatments, salinity effects were significant on leaf number, dry shoot mass, root length and leaf length, contributing 73, 60, 50 and 64% in TTV of the respective variables. Leaf number, dry shoot mass, root length and leaf length each against increasing concentration of salinity exhibited positive curvilinear quadratic relations, with 74, 91, 95 and 66% associations, respectively. Responses of essential nutrient accumulation in leaf tissues of *M. zeyheri* to salt treatments had significant effects on K and Na, contributing 28 and 19% in TTV of the respective variables. Potassium over increasing salt concentrations exhibited positive quadratic relations and Na over increasing salt concentrations, exhibited negative quadratic relations. The models for each relation were explained by 95 and 86%, respectively.

Objective 3 was achieved by exposing seedlings to 0, 10, 20, 30, 40, 50, 60 and 70 g VAM per plant. At 90 days after the treatments, VAM effects were significant on plant height, leaf number, stem diameter and chlorophyll content contributing, 49, 65, 60 and 61% in TTV of the respective variables. Plant height, leaf number and chlorophyll content each against increasing VAM levels exhibited negative quadratic relations, with 97, 83 and 80% associates, respectively. In conclusion, the use of soil type to promote growth and accumulation of essential nutrient elements on *M. zeyheri* seedlings demonstrated that clay soil could be suitable for cultivation of *M. zeyheri.* Also chloride salt concentrations included all three phases of density-dependent growth (DDG) patterns, whereas VAM levels used in the study were already in the last two phases of DDG patterns. Vesicular arbuscular mycorrhiza treatment did not have significant effects on Ca, Fe, K, Na and Zn in leaf tissues of *M. zeyheri* seedlings. In conclusion, soil with high clay content could be ideal when raising *M. zeyheri* seedlings. However, salt concentration of 8.11 mM/m^3 exhibited the stimulation of *M. zeyheri* seedlings growth, Therefore, lower concentration less than 8.11 mM/m^3 shown to be toxic by reducing the plant growth. In contrast, 5.554 g per plant of VAM will supreme for growth and development of *M. zeyheri* seedlings.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

1.1.1 Description of the research problem

Red Milkwood (*Mimusops zeyheri*) is an indigenous fruit tree to the former Transvaal Province in South Africa. The plant has appropriate attributes as an alternative fruit crop due to its high vitamin C in rural areas of Limpopo Province. Generally, *M. zeyheri* trees grow on wooded, rocky hillsides or river basins (Venter and Venter, 1996). The trees have a non-aggressive lateral root system, which is adapted to rocky conditions and marginal soils (Van Wyk, 1974). Also, *M. zeyheri* trees have attributes associated with drought tolerance (Venter and Venter, 1996). In most drought-tolerant plant species, during the seedling stage more growth is partitioned towards root growth at the expense of shoot growth (Krieg, 1983). Such plants, as observed in *M. zeyheri*, tend to be slow growers during the seedling stage.

Efforts are underway to domesticate indigenous plants as alternative crops in various regions of the world (Gardner, 1991). The advantage of indigenous plant species when compared with exotic species is that they are already adapted to indigenous conditions. Incidentally, when indigenous plants are used as alternatives to exotic crops, they have a better chance of creating more suitable agro-systems that combat land degradation and conserve the natural plant heritage. Domestication and commercialisation of genetically sustainable plant species with environmental, nutritive and economic values in rural areas is a serious challenge to stakeholders involved in integrated rural development (Mashela and Mollel, 2001).

High quality irrigation water is increasingly becoming scarce in all production systems of the world (Aylward *et al.,* 2010), including South Africa, which is a water-scarce country (DWAF, 2002). However, irrigation is becoming increasingly indispensable due to persistent droughts in the context of global warming (Aylward *et al.,* 2010). Most sources of irrigation water are increasingly becoming contaminated by industry, household and agricultural wastes - with salt levels in most water sources rising above tolerance level for most crops. Due to the scarcity of irrigation water in Limpopo Province, it is becoming unproductive to grow most exotic fruit crops, which are inherently sensitive to water and salt stress (Mashela and Mollel, 2001). Exotic fruit crop systems in rural communities had since collapsed due to their inadaptability to marginal soils and water stress (Mashela and Mollel, 2001). South Africa is a water scarce country (Steyn *et al.,* 2014), with most soils being deficient in phosphorus (DWAF, 2002). The challenges of poor quality water and low P dictate the need for using crops that are tolerant to salinity and drought, but with the capacity to co-exist with mycorrhiza (Volkmar *et al.,* 1998)**.**

1.1.2 Impact of the research problem

Various predictions had suggested that the inland South Africa faces extremes of ecological patterns due to climate change (Steyn *et al.,* 2014). Due to scarcity of water, exotic fruits are increasingly becoming unaffordable, pricewise, to communities in marginal areas of Limpopo. The uses of water include household, livestock and industrial uses such as mining. Also, most exotic crops used in agriculture are highly sensitive to salinity, whereas damage to crops by nematodes is closely associated with salinity challenges (Mashela *et al.,* 1992). *Mimusops zeyheri*

seedlings had been to be highly resistant to root-knot (*Meloidogyne* species.) nematodes (Mashela *et al.,* 2013).

1.1.3 Possible causes of the research problem

The major causes of extremes weather conditions that render most exotic fruit trees vulnerable is climate change, that result in global warming (Steyn *et al*., 2014). The causes of salinity in irrigation water are industrial, agricultural, mining and household wastes (Muchuweti *et al.*, 2006). Increased leakage to the groundwater system causes the water table to rise, thereby causing salts to accumulate in top soil layers. Generally, when the salty water table rises to within two metres of the soil surface, the process of evaporation concentrates salt ions at the surface (Muchuweti *et al.*, 2006). Salinity is exacerbated when the water that is being applied to the irrigated is derived from salty rivers or contaminated boreholes, which fields had been the case in most inland South Africa (Muchuweti *et al.*, 2006).

1.1.4 Proposed solutions

In some extreme cases, wastewater is being used in agriculture. However, recent studies (Kgopa and Mashela*,* 2017; Phadu *et al.,* 2017) Demonstrated that treated wastewater could have detrimental effects on soil structure and eventually plant growth. The use of salt-tolerant indigenous plants and those that can co-exist with mycorrhiza would provide some of the possible solutions to the research problem. Also, improved knowledge on how indigenous plants partition growth in roots and shoots would also increase the potential cultivation of these plants as alternative crops in context of climate-smart agriculture. Currently, it is not clear whether *M. zeyheri* trees could benefit from the use of mycorrhiza. Mycorrhiza fungi are

cosmopolitan microorganisms that inhabit the rhizosphere of most perennial trees (Giri *et al.,* 2003). The symbiotic association of a plant with mycorrhiza fungi allows access to mobile nutrients in nutrient poor soils (Marschner and Dell, 1994). *Mimusops zeyheri* is adapted to rocky conditions, where establishment of mycorrhiza could encounter some challenges. Mycorrhiza fungi establishment is an integral component of the natural ecosystem, and are known to exist in saline environments where they improve early plant growth and tolerance to salinity (Aliasgharzadeh *et al.,* 2001).

1.1.5 General focus of the study

The focus of the current study would be to assess *M. zeyheri* seedling growth using different soil types, and root growth versus shoot growth, followed by salinity effects in seedlings and the influence of vesicular arbuscular mycorrhiza fungi.

1.2 Problem statement

Different plants show different levels of osmotic gene expression in reaction to salt stress (Agaoglu *et al.,* 2004). Previous studies on *M. zeyheri* suggested that these trees had some potential for salt tolerance. Also, mycorrhiza fungi have the potential to ameliorate the effects of salinity in plants (Aliasgharzadeh *et al*., 2001). Mycorrhiza fungi are present among a wide range of soil microorganisms including nematodes, inhabiting the rhizosphere (Giri *et al.,* 2003). The symbiotic association of a plant with mycorrhiza fungi allows access to mobile nutrients in nutrient poor soils (Marschner and Dell, 1994). Mycorrhiza fungi establish an integral component of the natural ecosystem, and are known to exist in saline environments where they improve early plant growth and tolerance to salinity (Aliasgharzadeh *et al.,* 2001).

However, the effect of mycorrhiza and salinity levels on growth of *M. zeyheri* seedlings for optimum production is not yet known. Use of mycorrhiza fungi in protection of *M. zeyheri* seedlings from salinity could be ideal since mycorrhiza proliferate most soil types (Aliasgharzadeh *et al*., 2001).

1.3 Rationale for the study

Mimusops zeyheri is a slow growing indigenous plant, with suitable attributes for use in marginal communities of Limpopo Province (Mashela *et al*., 2013). Salinity in irrigation water and low P in soils could contribute to the slow growth of *M. zeyheri*. However, *M. zeyheri* appears to be a naturally slow grower, particularly during the seedling stage. Evidence that plants respond to limiting factors in density-dependent growth (DDG) patterns, with stimulation, neutral and inhibition phases, exists (Mashela and Kgabo, 2014). Best agricultural practices involve the use of cultural practices within the stimulation phases of their DDG patterns. Separate studies, one establishing the optimum responses of *M. zeyheri* to saline water, and the other to increasing level of mycorrhiza, would be necessary to enhance the development of good agricultural practices for *M. zeyheri,* especially during the seedling and juvenile stages.

1.4 Purpose of the study

1.4.1 Aim

The study aimed at the development of salinity and mycorrhiza conditions that would promote growth and development of *M. zeyheri* seedlings.

1.4.2 Objectives

1. To investigate the influence of soil type on root growth and foliar nutrient elements in *M. zeyheri* seedlings.
2. To determine the degree of salt tolerance in *M. zeyheri* seedlings.
3. To investigate the influence of vesicular arbuscular mycorrhiza fungi on growth of *M.* zeyheri.

1.5 Reliability, validity and objectivity

In this study, reliability of data were based on statistical analysis of data at the probability level of 5%, validity was achieved through repeating the experiments in time, while objectivity will be achieved by ensuring that the findings are discussed on the basis of empirical evidence, in order to eliminate all forms of subjectivity (Leedy and Ormrod, 2005).

1.6 Bias

Bias was minimised by ensuring that the experimental error in each experiment was reduced through replications and by assigning treatments randomly within the selected research designs (Leedy and Ormrod, 2005).

1.7 Scientific contribution

The study intends to establish whether *M. zeyheri* plant could perform better in any of the salinity levels and mycorrhiza mixed with salinity. The findings will provide information on resistance to salinity, thereby providing information on whether the plant can be planted in areas of high salinity.

1.8 Structure of the dissertation

Following the description and detailed outlining of the research problem (Chapter 1), the work done and not yet done on the research problem was reviewed (Chapter 2). Then, each of the three subsequent chapters (Chapter 3, 4 and 5) addressed each of the three objectives, sequentially. In the final chapter (Chapter 6), findings in all chapters were summarised and integrated to provide the significance of the findings and recommendations with respect to future research, culminating in a conclusion which tied together the entire study. In the citations and references the Harvard style, approved by the Senate of the University of Limpopo, was used.

CHAPTER 2
LITERATURE REVIEW

2.1 Work done on the research problem

Red milkwood (*Mimusops zeyheri*) fruit trees are indigenous to the northern parts of South Africa and have high potential to serve in economic and nutritional projects in arid and semi-arid regions (Mashela and Pofu, 2012; Venter and Venter, 1996). Trees of *M. zeyheri* are evergreen and grow up to 15 m high (Venter and Venter, 1996), with non-aggressive lateral root systems. The trees are both frost-hardy and drought-tolerant (Mashela and Mollel, 2001). Most marginal communities in Limpopo Province were historically settled in areas with soils of low agricultural potential, where clay and sandy soils are predominant (Mashela and Mollel, 2001). Incidentally, commercial farming and urban communities are situated in high potential agricultural soils such as loam. Soil type has a strong influence on the productivity of crops (Hartmann *et al.,* 2010), which may be direct through physical abrasion of soil particles on the root system and/or indirect through the influence of the soil on the availability of water and/or nutrient elements (Hartmann *et al*., 2010).

Various indigenous plants were evaluated as alternative crops in Limpopo Province, with fruit trees being ranked using edible qualities in fresh and/or beverage form (Mashela and Mollel, 2001). *Mimusops zeyheri* was top-ranked for its edible fresh fruit quality and high vitamin C. The first shoot flush in this fruit tree occurs in late winter (May-July), whereas the second, along with flowers, starts during late spring (August-October) through early summer (November-January), when fruit are ready for harvest (Mashela *et al.*, 2013).

Due to a large number of flowers and the high retention of most flowers and fruit, alternate fruit bearing is common phenomenon in *M. zeyheri* trees (Mashela *et al.*, 2013). Fruit are laterally borne (Mashela *et al.*, 2013), which implies that pruning is necessary for enhancing fruit production (Hartmann *et al.,* 2010). Fruit of *M. zeyheri* have the highest vitamin C per unit among both endemic and exotic locally available fruits (Venter and Venter, 1996). Micro-propagation and cultivation protocols for the tree had been completed for rural greening (Maila, 2001).

Trials were conducted to assess vegetative (asexual) propagation through air-layering and sexual propagation through seed scarification using sulphuric acid, hot water. The results demonstrated that asexual propagation under all treatments occurred between five to six weeks, whereas hot water treatment had moderate influence on emergence and acid scarification reduced seedling emergence (Mkhabela, 2003). Among all treatments, mechanical scarification enhanced seedling emergence, but none of the treatments improved seed germination rate (Mkhabela, 2003).

Mashela (2017) examined the effect of different salts (KCl, NaCl, Na_2CO_3 and $CaCl_2$) on growth of *M. zeyheri* seedlings for two seasons, namely, summer and winter, during shoot flushes. Sodium carbonate increased soil pH and reduced chlorophyll content during both seasons. Other salts increased electrical conductivity. Generally, Na_2CO_3 had negative impact on growth of *M. zeyheri* seedlings, apparently through binding with calcium to form calcium carbonate, which rendered Ca unavailable to seedlings. In contrast, *M. zeyheri* seedlings excelled in all chloride salts.

2.1.1 Effects of salinity on plant growth

Soil salinity is a widespread problem that restricts plant growth and biomass production, especially in arid, semi-arid and tropical areas (Apse *et al.,* 1999). Salinity affects plants through nonspecific and specific mechanisms. The nonspecific mechanism is related to decreasing osmotic potential of the soil solution that impedes transpiration and photosynthesis (Shannon and Grieve, 1999). Specific mechanisms relate to ion uptake and altered physiological processes resulting from toxicity, deficiency or changes in mineral balance (Hasegawa *et al.*, 2000; Shannon and Grieve, 1999). In citriculture, salt tolerance is the ability of root to exclude the transport of Na and Cl ions from being transported (Volkmar *et al.,* 1998). Plants generally vary in response to soil salinity (Downton, 1977). However, different rootstocks show different levels of osmotic gene expression in reaction to salt stress (Agaoglu *et al.,* 2004).

2.1.2 Effects of vesicular arbuscular mycorrhiza on plant growth

Arbuscular mycorrhiza (AM) fungi are among the most common soil fungi and the majority of plant species have associations with AM fungal species (Selvaraj and Chellappan, 2006). About 80% of vascular plants have associations with AM fungi (Hodge, 2000). Mycorrhiza fungi occur in most ecosystems (Read, 1991). Plant-mycorrhiza associations could be traced back to over 400 million years (Read, 1991). However, each plant species has a different degree of dependency on mycorrhiza, for example; faba bean (*Vicia faba*) has strong affiliation to mycorrhiza association and is depended upon its fungal association for growth and establishment (Talaat and Abdallah, 2008). Mycorrhiza fungi can also help plants to survive and grow under different environmental conditions, thereby increasing their

reproductive output (Bolandnazar *et al.,* 2007). The basic elements of the symbiosis are that the plant provides the mycorrhiza with carbohydrates, whereas the fungi provide the plant with certain nutrients needed for growth (Selvaraj and Chellappan, 2006). Estimations suggested that in compensation for the additional nutrients and water provided by mycorrhiza, a plant could provide 20% of its fixed carbon to roots for mycorrhiza establishment and maintenance of the association (Selvaraj and Chellappan, 2006).

Arbuscular mycorrhiza is important for plant biodiversity and the health of ecosystems (Malcova *et al.,* 2003) and could help plants withstand different forms of environmental stress. The fungi enable plants to grow and reproduce in heavy metal-contaminated sites (Malcova *et al*., 2003; Sudova and Vosatka, 2008) as well as increasing the uptake of soil moisture by the host plant, thereby allowing them to withstand the effects of drought better (Auge, 2001). Furthermore, mycorrhiza could help plants to resist extreme temperatures (Zak *et al.,* 2000) as well as reduce the stress of herbivore attacks (Gange and West 1994; Gehring *et al*., 2006; Wamberg *et al.,* 2003) and to also protect plants from diseases and pathogens (Newsham *et al*., 1995; Sharma *et al.,* 1992). Arbuscular mycorrhiza fungi had been classified as belonging to the phylum *Glomeromycota* (Gerdemann, 1975). Unfortunately, there had not been accurate estimates of species richness in mycorrhiza fungi, but some estimates had been above 150 species (Morton and Benny, 1990). Arbuscular mycorrhiza fungal associations are composed of three main structures. First, hyphae work as external filamentous arms searching for nutrients around the root zone (Hodge, 2000). Second, there are specialised vesicles within the root, which are

thought to be storage organs, especially for lipids (Hirsch and Kapulnik, 1998). Arbuscular are the third important part of the AM association.

2.1.3 Effects of salinity-mycorrhiza relations on plant growth

Arbuscular mycorrhiza fungi are ubiquitous among a wide array of soil microorganisms inhabiting the rhizosphere (Giri *et al.,* 2003). The symbiotic association of a plant with AM fungi allows access to mobile nutrients in nutrient poor soils (Marschner and Dell, 1994). Arbuscular mycorrhiza fungi constitute an integral component of the natural ecosystem and are known to exist in saline environments where they improve early plant growth and tolerance to salinity (Aliasgharzadeh *et al.,* 2001). Many researchers have reported that AM fungi could enhance the ability of plants to cope with salt stress (Rabie, 2005; Yano-Melo *et al.,* 2003) by improving plant nutrient uptake (Asghari *et al.,* 2005) and ion balance (Giri *et al.,* 2007), protecting enzyme activity (Giri and Mukerji, 2004) and facilitating water uptake (Ruiz-Lozano and Azcon, 1995). In salt-affected soil, AM fungi are thought to improve the supply of mineral nutrients to plants, especially the supply of P, as it tends to be precipitated by ions like Ca, Mg and Zn (Al-Karaki *et al.,* 2001). Giri *et al.* (2003) reported that AM fungi counter-balanced the adverse effects of salinity stress and thereby increased plant growth. Rabie (2005) suggested that AM fungi protected the host plants against the detrimental effects of salt. An increasing occurrence of soil salinity, drought and declining productivity of grape varieties in India had since made use of a suitable rootstocks imperative (Rabie, 2005).

According to Ebrahim (2014), the results he obtained from the trials indicated that mycorrhiza did not actually help the plant overcome salinity at higher stress. Also,

different levels of salinity and different salt types influenced mycorrhiza species interaction with plants in different ways. In the first field experiment with higher salinity levels and mixed commercial mycorrhiza, the results were impressive regarding the plant offspring quality. In the second experiment with reduced levels of salinity and the addition of individual mycorrhiza species, the results were not conclusive. Thus, it was recommended that the second field experiment be repeated under controlled conditions for comparison of results.

2.1.4 Salt tolerance in plants

According to Munns and Tester (2008), there are three distinct mechanisms that enable plants to tolerate salinity stress. The first mechanism involves enhancing the tolerance of the plant to osmotic stress under salinity, thus potentiating leaf growth and stomata conductance. The second mechanism is based on Na exclusion, which in turn restricts it from accumulating to toxic levels within the leaves. Tissue tolerance to accumulated Na is the third mechanism that helps plants overcome salt stress. This process entails the sequestration and accumulation of the ions in the cytoplasm so that salt particles would not interfere with physiological and biochemical processes during plant development (Munns and Tester, 2008). However, with the exception of halophytes plants that survives highly saline habitats (Flowers and Colmer, 2008). The majority of plants and crops still fail to resist soil salinity concentrations above 4 dS/m (USDA–ARS, 2008). To address the above-mentioned impacts on plant growth and agricultural losses, novel methods for overcoming salinity had been investigated and introduced.

There had been interest in growing new plants that can cope with salinity, and at the same time be suitable as new crop plants for human and animal consumption (Gallagher, 1985). Another approach was to breed existing crop plants specifically to develop strains that can withstand salt stress (Cuartero and Fernandez-Munoz, 1999). Genetic engineering had also taken on an important role in overcoming salinity by developing plants with genes that enable adaptation to high salinity conditions (Wu *et al.,* 2010). Apart from these biological methods to combat soil salinity, mechanical methods could also be used. These include using chemicals to leach excessive salts from soil and the use of desalination machines to remove salts from irrigation water (Muralev *et al.,* 1997). However, the problem remains that conventional methods for fighting soil salinity are expensive and most farmers in developing countries cannot afford the associated financial burden (Cantrell and Linderman, 2001).

2.2 Work not yet done on the research problem

The influence of mycorrhiza and salinity levels on *M. zeyheri* indigenous plants had not been documented. Information on the two aspects would enhance the potential uses of *M. zeyheri* trees in rural areas of South Africa.

2.3 Addressing the identified gaps

Mimusops zeyheri is an indigenous fruit tree, which has appropriate attributes for use as a fruit crop and source of vitamin-C in rural areas of Limpopo Province. The identified gaps in the current review were the shortage of information on the influence of mycorrhiza to improve the growth and production of indigenous *M.*

zeyheri fruit tree under soil with salinity. Empirical studies were conducted to overcome all this gaps.

CHAPTER 3
INFLUENCE OF SOIL TYPE ON ROOT GROWTH AND FOLIAR NUTRIENTS IN *MIMUSOPS ZEYHERI* SEEDLINGS

3.1 Introduction

Red milkwood *(Mimusops zeyheri* Sond.*)* is a slow-growing fruit tree, with the highest vitamin C per unit among both endemic and exotic fruits that are locally available in South Africa (Venter and Venter, 1996). Soil type and environmental factors play a major role in productivity of *M. zeyheri* and have an effect on morphology and phenology of the trees (Ledwaba, 2008). Soil type determines nutrient and soil moisture availability in plants (Mayer and Poljakoff, 1989), with *M. zeyheri* trees being able to grow on rocky conditions (Venter and Venter, 1996). Generally, the key restrictions in most soil types include deficiencies and toxicities of certain macro and micro-nutrient elements, which result in overall nutrient imbalances in different plant organs.

In sandy soil, most of the essential nutrient elements are not available due to their being leached out of the effective root-zones (Mayer and Poljakoff, 1989). In heavy clay soil, although the nutrient elements and waterholding capacity might be high, these growth factors could be out of reach for roots due to existing strong adsorption forces on surfaces of soil particles (Mayer and Poljakoff, 1989). In contrast, due to high pH in calcareous soil, most essential nutrient elements might be in unavailable form, whereas others might be in toxic concentrations. In a recent study (Mashela, 2017), it was shown that different soil types had different effects on growth of *M. zeyheri* seedlings, with limited information on root growth. The objective of this study

was to investigate the influence of soil type on root growth and foliar nutrient elements in *M. zeyheri* seedlings.

3.2 Materials and methods

3.2.1 Location of the study

The study was conducted at the Green Biotechnologies Research Centre of Excellence, University of Limpopo, in Limpopo Province, South Africa (23°53'10"S, 29°44'15"E). Summer (November-January) minimum/maximum temperatures average 10/38ºC, whereas average annual rainfall is less than 500 mm. Seventeen-cm diameter, one-metre long polystyrene pipes were held upward by a steel structure and filled with different soil types (Legend 3.1). The study was conducted during summer in 2015 and repeated in 2016.

Legend 3.1 *Mimusops zeyheri* seedlings in polystyrene pipes with loam, clay, calcareous and sandy soils.

3.2.2 Treatments and experimental design

The four treatments, namely, clay, calcareous, loam and sandy soils, were laid out in a randomised complete block design, with ten replications, and repeated in 2016 with nine replications.

3.2.3 Procedures

Seedlings were raised in 160-hole-seedling trays filled with Hygromix-T (Hygrotech, Pretoria North) under greenhouse conditions. At emergence of true leaves, seedlings were hardened-off outside of the greenhouse through intermittent withdrawal of irrigation water. Two weeks after hardening-off, uniform seedlings were transplanted into polystyrene pipes filled with steam-pasteurised (300°C for 1 h) soil types. Soil pH, soil texture and N, P and K for each soil type were quantified prior to transplanting seedlings. The seedlings were fertilised after transplanting using 5 g NPK 2:3:2 (26) + 0.5% Zn + 5% S + 5% Ca and 1 g NPK 2:1:2 (43) Multifeed to provide 0.47 N, 0.43 K, 0.43 P, 121 Mg,1 Fe, 0.10 Cu, 0.47 Zn, 1.34 B, 4.02 Mn and 0.09 mg Mo per mL water (Mashela, 2002).

3.2.4 Data collection

At 60 days after initiating the treatments, hypocotyl length was measured from the soil level to the petiole of dicotyledonous leaves; epicotyl length was measured from petiole of dicotyledonous leaves to the end of the meristematic tip. Chlorophyll content of dicotyledonous leaves; secondary leaves were measured from three mature leaves per plant using a chlorophyll meter (MINOLTA, SPAD-502) and shoot severed. Root systems were removed from the polystyrene pipes and immersed in water to remove soil particles Roots branches were counted and total root length

measured on each plant. Roots and shoots were oven-dried in air-forced ovens for 72 h at 60°C for dry matter. Dried leaves were separated from the stem and ground into powder using a pestle and mortar. The powdered samples were each digested in 5% nitric acid, which was then mixed with a vortex meter. Samples were then incubated in a warm water-bath at 95°C for an hour, left to cool down to room temperature, filtered and the container covered with a foil (SW-846 EPA Method 3050B) to minimise direct contact with light. Samples were then subjected to Atomic Absorption Spectrometry (AAS) at Limpopo Agro-food Station (LATS) to quantify Na, Ca, K, Fe and Zn in leaf tissues.

3.2.5 Data analysis

Data for plant variables and essential nutrient elements were subjected to analysis of variance through the SAS software (SAS Institute, 2008). Prior to ANOVA, leaf numbers were transformed through $\log_{10}(x + 1)$ to normalise the variances (Gomez and Gomez, 1984), but untransformed means were reported. Mean separation for significant ($P \leq 0.05$) treatment effects was achieved through the Fisher's least significance difference test at the probability level of 5%. Unless otherwise stated, treatments were discussed at the probability level of 5%.

3.3 Results

Seasonal interaction for plant variables was not significant. Data for the two seasons were therefore pooled (n = 76) and re-analysed using SAS software.

3.3.1 Plant growth variables

Soil type had highly significant ($P \leq 0.01$) effects on root length, root branch number and dry root mass, contributing 74, 80 and 40% in total treatment variation (TTV) of the respective variables (Table 3.1). Soil type had significant effects on plant height, leaf number, chlorophyll of dicotyledonous leaves and chlorophyll of secondary leaves, contributing 60, 72, 84 and 85% in TTV of the respective variables (Table 3.3). Treatments had no significant effect on hypocotyl length and dry shoot mass.

In root variables, clay soil significantly increased root length, but the effects were not different to those in sandy soil, which were also not different to those of loam and calcareous soils (Table 3.2). The effects of loam, calcareous and sandy soils on root branching were not different from one another, but were each significantly higher than those of clay soil, where the variable was reduced by 100%. Dry root mass of seedlings on loam and sandy soils were not different from each other, but were significantly higher than those in clay and calcareous soils by 30% in each soil type (Table 3.2). Relative to loam, clay soil increased epicotyl length, secondary leaf number, dicot leaf chlorophyll and secondary leaf chlorophyll by 59, 42, 8 and 57%, respectively (Table 3.4). However, the effects of loam, calcareous and sand soils on growth of shoot variables were not different.

Table 3.1 Partitioning mean sum of squares of root growth variables in various organs of *Mimusops zeyheri* seedlings under different soil types at 60 days after the treatments (n = 76).

		Root length		Root branch no.		Dry root mass	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	18	20.17	12	1.76	12	1.47	9
Treatment	3	128.7	74***	11.82	80***	6.83	40***
Error	54	25.76	14	1.22	8	8.75	51
Total	75	174.6	100	14.8	100	17.05	100

***Highly significant at P ≤ 0.01.

Table 3.2 Influence of clay, calcareous and sandy soils to loam soil on root growth variables in *Mimusops zeyheri* seedlings at 60 days after the treatments (n = 76).

	Root length		Root branch no.		Dry root mass	
Treatment	Variable (cm)[z]	R.I. (%)	Variable	R.I. (%)	Variable (g)	R.I. (%)
Loam	13.07[b]	_	1.84[a]	_	0.10[a]	_
Clay	18.54[a]	42	0.00[b]	− 100	0.07[b]	− 30
Calcareous	13.95[b]	7	1.16[a]	− 37	0.07[b]	− 30
Sandy	16.21[ab]	24	1.42[a]	− 22	0.10[a]	0

[z]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's least significant difference test.

Table 3.3 Partitioning mean sum of squares of shoot growth variables in various organs of *Mimusops zeyheri* seedlings under different soil types at 60 days after the treatments (n = 76).

		Shoot growth variables				Chlorophyll content			
		Height		Leaf no.		Dicot leaf		Secondary leaf	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	18	0.77	24	0.68	14	72.62	9	194.1	7
Treatment	3	1.95	60**	3.52	72***	649.9	84***	2370.2	85***
Error	54	0.51	16	0.71	14	49.20	7	230.75	8
Total	75	3.23	100	4.91	100	771.7	100	2795	100

***Highly significant at P ≤ 0.01, **Significant at P ≤ 0.05. (DF) Degree of freedom. (MSS) Mean sum of squares.

Table 3.4 Influence of clay, calcareous and sandy soils to loam soil on shoot growth variables in *Mimusops zeyheri* seedlings at 60 days after the treatments (n = 76).

	Epicotyl length		Dicot leaf Chlorophyll		Secondary leaf			
					Number		Chlorophyll	
Treatment	Variable (cm)[z]	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Loam	1.05[b]	_	59.76[b]	_	1.74[b]	_	35.47[b]	_
Clay	1.67[a]	59	64.80[a]	8	2.47[a]	42	54.08[a]	57
Calcareous	1.21[b]	15	55.50[bc]	− 7	1.74[b]	0	36.85[b]	4
Sandy	0.98[b]	− 7	51.13[c]	− 14	1.47[b]	16	27.57[b]	− 22

[z]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's least significant difference test. (R.I.) Relative impact.

3.3.2 Nutrient element variables

Soil type affected accumulation of essential nutrients elements in *M. zeyheri* leaf tissues (Table 3.5). Soil type had significant effects on Ca, K and Na in leaf tissues of *M. zeyheri* seedlings, contributing 51, 56 and 60% in TTV of the respective variables (Table 3.5). However, soil type had no significant effect on Fe and Zn content in leaf tissues of the test plant.

Seedlings on clay had the highest Ca in leaf tissues than those in calcareous and sandy soil, but the effects of clay and loam were not different (Table 3.6). Relative to loam, clay soil increased Ca in leaf tissues of *M. zeyheri* seedlings by 9%. Relative to loam, sand reduced K in leaf tissues of seedlings by 31%. In contrast, relative to loam soil, clay, calcareous and sand reduced Na in leaf tissues of seedlings by 22, 23 and 31%, respectively.

Table 3.5 Partitioning mean sum of squares of essential nutrient elements in leaf tissues of *Mimusops zeyheri* seedlings in different soil types at 60 days after the treatments (n = 76).

		Ca		Fe		K		Na		Zn	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	18	146.9	33	44.71	45	153.3	28	1.26	29	0.023	34
Treatment	3	229.6	51**	4.81	5ns	309.6	56**	2.67	60***	0.020	30ns
Error	54	74.48	16	49.62	50	92.45	16	0.49	11	0.024	36
Total	75	451	100	99	100	555	100	4.42	100	0.07	100

***Highly significant at P ≤ 0.01, **Significant at P ≤ 0.05, nsNot significant at P ≥ 0.05.

Table 3.6 Accumulation of essential nutrient elements in leaves of *Mimusops zeyheri* seedlings raised on selected soil types relative to those on loam soil at 60 days after the treatments (n = 76).

	Calcium (ppm)		Potassium (ppm)		Sodium (ppm)	
Treatment	Variable[z]	R.I. (%)	Variable	R.I. (%)	Variable	R.I. (%)
Loam	29.69[ab]	-	31.33[a]	−	2.83[a]	−
Clay	32.23[a]	9	27.43[ab]	− 13	2.21[b]	− 22
Calcareous	26.30[b]	− 11	27.92[a]	− 11	2.17[b]	− 23
Sand	24.43[b]	− 18	21.59[b]	− 31	1.95[b]	− 31

[z]Column means with the same letter were not different (P ≤ 0.05) according to Fisher's least significant difference test.

3.4 Discussion

3.4.1 Root growth variables

Soil type had highly significant effects on root length, root branch number and dry root mass. Previously it was shown that soil type affected root length in *Moringa oleifera* seedlings (Mashela, 2017). Postma *et al*. (2014) demonstrated that soil type had significant effects on lateral root branching in maize plants, which may increase fluctuations in root/shoot allocation, which complicated the relationship of lateral root branching with sustained root growth, nutrient uptake and plant growth.

In the current study, soil type had significant effects on dry root mass of *M. zeyheri* seedlings, which contradicted earlier observations (Mashela *et al.,* 2013). In an earlier study (Mashela *et al.,* 2013), clay soil improved dry root mass, whereas in the

current study clay reduced the variable, which are rather difficult to explain with the limited empirical information in the current study. Basically, the increase in dry root mass suggested that most photosynthates were being channelled towards this organ.

In root variables, clay soil significantly increased root length. Correspondingly results were observed in *M. oleifera,* root length increased the growth in clay soil and sandy soil than other soil tested (Mashela, 2017). The effects of loam, calcareous and sandy soils on root branching were not different from one another, but were each significantly higher than those of clay soil, where the variable was reduced. Contradictions were observed in maize plants Postma *et al*. (2014) why soil type had significant effects or enhancing lateral root branching, which might be due to increased fluctuations in root/shoot allocation of carbohydrates. Mobilisation of photosynthates complicates the relationship of lateral root branching with sustained root growth, nutrient uptake and plant growth (Postma *et al*., 2014).

Carbon availability for root growth plays an important role in our results, as greater sink strength of the root system must be balanced with sufficient source strength in order to have greater root growth and sub-sequent greater soil exploration. The reduction of root branching by 100% in clay soil confirms with the previous studies (Pan and Bassuk, 1985) root branches and growth was reduced due to compaction. The branching habit was altered despite impeded elongation on soil compaction on *Alianthus altissima.* The observation suggested that root branches occur in a soil with cracks, with clay soil having the advantage of compaction that would eventually affect the root branching appearance (Pan and Bassuk, 1985).

Dry root mass of seedlings on loam and sandy soils were not different from each other, but were significantly higher than those in clay and calcareous soils. The significant reduction of dry root mass in *M. zeyheri* on clay, calcareous and sandy confirmed the results of other studies where the variable was the lowest (Hegazi, 2015; Mashela, 2017; Pahla *et al*., 2013). Relative to loam, clay soil increased epicotyl length, secondary leaf number, dicot leaf chlorophyll and secondary leaf chlorophyll. The findings confirms the results of Mashela (2017), clay soil improved leaf number and outperformed the once on sandy and calcareous soils growing *M. oleifera* seedlings (Mashela, 2017). Clay soils increased chlorophyll content in the leaves of the seedlings, except calcareous soil were it consequently reduced the chlorophyll content, results confirmed that clay soil has high nutrient adsorption capabilities and high water holding capacities (Hartmann *et al*., 2010).

Commonly soil with high clay content has large organic matter and mineral elements, beside with more capacity to preserve water when related to loam, sandy and calcareous soils. Texture of the soil play an essential factor where clay soils generally can compromise the cation exchange in the soils. Due to clay soil with the benefit of their small size particles have the most surface area and therefore, the most exchange sites (Mashela *et al*., 2013). However, the effects of loam, calcareous and sand soils on growth of shoot variables were not different. The results indicate substantial decline on shoot growth variable in sand relative to other soils, outcomes evident the soil has deprived soil moisture content and high occurrence of nutrient element absence that may describe the observed poor performances as observed to other crops (Mashela *et al*., 1992). Mashela *et al*. (1991) demonstrated that sandy soil consistently suppressed growth of Alyceclover (*Alysicarpus vaginalis*), a

leguminous forage crop with a fibrous root system in Florida, USA. The root length growth of *M. zeyheri* seedlings in all soils investigated confirms plants invest in development of the root system (Krieg, 1983).

3.4.2 Shoot growth variables

Soil type had significant effects on plant height, leaf number, chlorophyll of dicotyledonous leaves and chlorophyll of secondary leaves. Mashela *et al.* (2013) observed that soil type had no effect on plant height and leaf number of *M. zeyheri,* which contradicted results of the current study, thereby suggesting that the availability of essential nutrient elements was crucial in the soil systems for promoting the growth of seedlings (Mashela *et al*., 2016). Later, Mashela (2017) showed that effects of soil type on *M. zeyheri* seedlings were registered leaves, with the effects on this variable being pronounced on dicotyledonous leaves.

3.4.3 Nutrient element variables

Accumulation of essential nutrient elements in *M. zeyheri* leaf tissues was variously affected by soil type. Soil type had significant effects on Ca, K and Na in *M. zeyheri* leaf tissues. Observations in this study confirmed findings where soil type had an effect on nutrient elements accumulation of leaf tissues in *M. oleifera* seedlings (Mashela, 2016). However, soil type had no significant effect on Fe and Zn content in *M. oleifera* leaf tissues (Mashela, 2016). Al-rawi *et al.* (2016) also observed that soil type had no effect on accumulation of Fe and Zn in peach seedlings leaf tissues, whereas an increase in Ca, K and Na accumulation in peach leaf tissues occurred.

Mimusops zeyheri seedlings on clay soil accumulated high Ca in leaf tissues. The reduction of K and Na in *M. zeyheri* leaf tissues on various soils in the current study except on clay might be due to fact that clay soil has high adsorption capabilities to K and Na ions but tended to be non-fixing to K (Arafin *et al*., 1973). The increased accumulation of Ca in *M. zeyheri* leaf tissues on clay soils suggested that Ca was required in large quantities in this plant species. Cations are absorbed by plant roots, but also held on exchange sites in soils. The positive charges of Ca are attracted to negative electrical charges on exchange sites of clay particles and organic matter. The higher amount of clay and organic matter, results in large attraction of Ca which would be absorbed by plants (Leal *et al*., 2009). Mashela (2017) suggested that certain nutrient elements such as Ca ions, could be absorbed at high level by plants even under adverse conditions, due to their indispensable roles in various structures and physiology of plants

Relative to loam, calcareous and sand soil, each reduced the accumulation of certain cations in *M. zeyheri* leaf tissues. Similarly, others noted the reduction of Ca, K and Na in *M. zeyheri* leaf tissues in calcareous soil (Mashela, 2017), which might be due to the chemistry of calcareous soil. Calcareous soil has high Ca ions, which result in high soil pH. Under high soil pH, the nutrient elements, K and Na might be available in toxic quantities, which might result in challenges to a plant to absorb excess nutrients in toxic amounts, whereas Ca might be unavailable to certain plants (Mashela, 2016).

The current findings demonstrated poor accumulation of Ca, K and Na in leaf tissues of *M. zeyheri* seedlings in sandy soils, which confirmed observations in *M. oleifera*

seedlings (Mashela, 2016). The observation could be ascribed to the characteristics features of sand which are different to those of clay or loam soils. Poor soil moisture content and high incidence of nutrient element unavailability could provide possible reasons of the observed poor nutrient in *M. zeyheri* leaf tissues (Mashela *et al*., 1992). Ledwaba (2008) suggested that nutrient element accumulation in *M. zeyheri* leaf tissues could change or differ due to fruiting time, soil type and area of plantation. Generally, nutrient uptake is faster in warmer soils than in cold soils (Mashela, 2017). Since most nutrients are taken up via soil solution, soil water is needed to dissolve them and bringing them into contact with root surfaces.

3.5 Conclusion

In conclusion, results of this study demonstrated that soil with high clay and loam content improved the root growth and essential nutrients accumulation comparable to those on other soils such as, calcareous and sandy soils. In this study it was shown that the essential nutrient elements in *M. zeyheri* could be affected by soil type, particularly the clay soil. Because the soil type influence on chemical compounds should always be taken into consideration when establishing *M. zeyheri* projects on marginal soils. Therefore, this could imply that soil with high clay content may be used when raising *M. zeyheri* for sufficient accumulation of essential nutrient elements for optimal production in smallholder farmers.

CHAPTER 4
DEGREE OF SALT TOLERANCE IN *MIMUSOPS ZEYHERI* SEEDLINGS

4.1 Introduction

Due to scarcity of water inland, supplementary irrigation for the drought-tolerant Red Milkwood (*Mimusops zeyheri* Sond.) would rely much on underground water. Generally, most underground water in the region is salty. Salinity is a cyclic phenomenon with salts accumulating in the rhizosphere during irrigation with salty water. However, salts are leached out of the rhizosphere during the rainy seasons (Mashela *et al*., 1992). In citriculture, cyclic salinity increased population densities of the citrus nematode, *Tylenchulus semipenetrans* Cobb (Mashela *et al*., 1992). Also, using observations in citriculture, infection by *T. semipenetrans* on salt-tolerant plants breaks salt tolerance (Mashela *et al*., 1992). Salt tolerance in plants is measured as the ability of roots to exclude the movement of Na or Cl ions to leaves (Hamdia *et al*., 2004). Generally, salt-tolerant citrus rootstocks are susceptible to *T. semipenetrans,* whereas all *T. semipenetrans*-resistant rootstocks have no salt tolerant genotypes (Mashela *et al*., 1992). *Mimusops zeyheri* seedlings are highly resistant to root-knot (*Melodigyne species)* nematodes (Mashela *et al.,* 2003). However, the degree of salt tolerance in *M. zeyheri* seedlings had not been documented. The objective of this study was to determine the degree of salt tolerance in *M. zeyheri* seedlings.

4.2 Materials and methods

4.2.1 Location of the study

The study was conducted under greenhouse conditions at the Green Biotechnologies Research Centre (GBRC) of Excellence, University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). Maximum day temperatures were controlled through

thermostatically-activated fans to 25ºC. The experiments of different salts levels were initiated during spring (July-September) 2016 and repeated in Autumn (January-March) 2017.

4.2.2 Experimental design and procedures

Mimusops zeyheri seeds were removed from fresh fruits and shade-dried. Eight treatments, namely, 0, 2, 4, 8, 16, 32, 64 and 128 NaCl + $CaCl_2$ mM/m^3 at 3:1 ratio, to maintain the integrity of cell membranes in roots (Mashela *et al.,* 1992) were arranged in a randomised complete block design, with six replications. Uniform three-month-old seedlings, at four leaf stage, were transplanted into 30-cm diameter pots, filled with pasteurised (300°C for 1 h) loam soils that were mixed with Hygromix-T at 3:1 ratio (v/v). Pots were placed on greenhouse benches at 0.2 m intra-row and 0.3 m inter-row spacing. The plants were treated every second day with irrigation application of the solution at 500 ml/seedling (Legend 4.1).

4.2.3 Cultural practices

Fertilisers were applied a day after transplanting at 5 g NPK 2:3:2 (26)+ 0.5% Zn + 5% S + 5% Ca fertiliser mixture per plant to provide a total of 155 mg N, 105 mg P and 130 mg K per ml water. Multifeed fertilisers was applied twice a month to provide 0.47 mg N, 0.43 mg K, 0.43 mg P, 121 mg Mg,1 mg Fe, 0.10 mg Cu, 0.47 mg Zn, 1.34 mg B, 4.02 mg Mn and 0.09 mg Mo per mL water (Mashela, 2002). Plants were irrigated every other day with 500 ml tapwater. Pest management was achieved through daily monitoring. Aphids were observed and managed with Malasol as per label instruction.

Legend 4.1 *Mimusops zeyheri* seedlings on greenhouse benches for salinity trial.

4.2.4 Data collection

At 90 days after initiating the treatments, plant height was measured from the soil level to the tip of the flag leaf using a ruler. The number of leaves was counted, with chlorophyll content being measured using a Chlorophyll meter (MINOLTA, SPAD-502). Root systems and shoots were removed from the pots and roots immersed in water to remove soil particles and total root length plant measured. Stem diameter was measured at 5 cm above the severed ends of shoots using a digital vernier caliper. Leaf areas, along with leaf length and leaf width of the first leaf below the flag leaf were measured using a leaf area meter (AM350 Portable Leaf Area Meter). Petiole length was also measured. Both roots and shoots were oven-dried at 60ºC for 72 h for dry matter determination. Mature dried leaves were separated from the branches and ground into powder using pestle and mortar. The powdered samples were each digested in 5% nitric acid, which was then mixed on a vortex meter.

Samples were then incubated in a warm water bath for an hour at 95°C, left to cool down at room temperature, filtered and the container covered with a foil (SW-846 EPA Method 3050B). Samples were then subjected to Atomic Absorption Spectrometry (AAS) to quantify Na, Ca, K, Fe, and Zn in leaf tissues at Limpopo Agro-food Technology Station.

4.2.5 Data analysis

Data for plant variables were subjected to analysis of variance (ANOVA) using SAS software (SAS Institute 2008). The degree of freedom and their mean sum of squares were partitioned to provide the total treatment variation (TTV) for different sources of variation. The Waller-Duncan multiple range tests were used to separate means which were different at the probability level of 5% level of probability. Significant variables were subjected to lines of the best fit with the independent (x-axis) variable transformed using $\log_2 2$ (Tseke and Mashela, 2017). Quadratic equations generated used to compute optimum concentration values. Unless stated otherwise, treatment effects were discussed at the probability level of 5%.

4.3 Results

The seasonal interaction on plant and nutrient element variables measured were not significant therefore, data were pooled (n = 96) and re-subjected to ANOVA using SAS software.

4.3.1 Plant growth variables

Responses of growth to salts treatments had significant effects on leaf number, dry shoot mass, root length and leaf length, contributing 73, 60, 50 and 64% in TTV of

the respective variables (Table 4.1). Treatments had no significant effects on plant height, dry root mass and chlorophyll content.

In plant growth variables, salinity significantly increased the leaf number of *M. zeyheri* at high concentrations, with the variable decreasing at low concentrations after application of salts (Table 4.2). The effects of increasing salt concentration increased dry shoot mass from 25 to125%. Relative to untreated control, salt increased root length by 10%. Leaf length of *M. zeyheri seedlings* was reduced by salinity from 8 to 21% (Table 4.2).

Leaf number (Figure 4.1), dry shoot mass (Figure 4.2), root length (Figure 4.3) and leaf length (Figure 4.4) over increasing salt concentrations, each exhibited positive quadratic relations. The models for each relation were explained by 73, 90, 95 and 66% associations, respectively. Using $x = -b_1/2b_2$ relation for each model, leaf number, dry shoot mass, root length and leaf length were each optimised at 1.819, 0.607, 26.254 and 3.779 NaCl + $CaCl_2$ mM/m^3 at 3:1 ratio, with mean optimisation being at 8.115 NaCl + $CaCl_2$ mM/m^3 (Table 4.3).

Table 4.1 Partitioning mean sum of squares of growth in various organs of *Mimusops zeyheri* seedlings under salinity levels at 90 days after the treatments (n = 96).

		Leaf no.		Plant height		Dry shoot mass		Dry root mass		Root length		Chlorophyll		Leaf length	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	11	0.83	5	8.54	18	0.57	13	0.28	36	110.5	33	85.9	48	672.3	23
Treatment	7	11.4	73***	23.5	49ns	2.54	60**	0.30	40ns	168.6	50***	53.0	29ns	1819	64***
Error	77	3.33	21	16.2	33	1.12	27	0.18	24	55.91	17	41.6	23	365.7	13
Total	95	15.6	100	48.2	100	4.23	100	0.76	100	335.01	100	180.5	100	2857	100

***Highly significant at P ≤ 0.01, **Significant at P ≤ 0.05, nsNot significant at P ≤ 0.05.

Table 4.2 Relative impact of salinity levels to control on growth variables in *Mimusops zeyheri* seedlings at 90 days after the treatment initiations (n = 96).

Treatment	Leaf number		Dry shoot mass		Root length		Leaf length	
	Variable[z] (cm)	R.I. (%)	Variable (g)	R.I.[y] (%)	Variable (cm)	R.I. (%)	Variable (mm)	R.I. (%)
0	8.2[bc]	–	0.8[bc]	–	29.5[b]	–	95.7[a]	–
2	7.3[c]	− 11	0.7[bc]	− 13	29.6[b]	0.34	84.5[c]	− 12
4	7.0[bc]	− 15	0.7[c]	− 13	30.6[b]	4	75.6[c]	− 21
8	7.8[bc]	− 5	1.1[ab]	38	30.7[b]	4	80.5[bc]	− 16
16	8.2[bc]	0	1.2[ab]	50	30.8[b]	4	80.6[bc]	− 16
32	8.4[b]	4	1.1[ab]	38	31.4[b]	6	85.1[bc]	− 11
64	8.4[b]	4	1.3[bc]	63	32.3[a]	10	80.7[bc]	− 16
128	9.3[a]	13	1.8[a]	125	32.3[a]	10	87.8[bc]	− 8
Cv %	22.33		108.9		23.36		21.93	

z Column means with the same letter were not different (P ≤ 0.05) according to Waller-Duncan multiple range test.

y Relative impact (%) = [(treatment/control) −1] × 100.

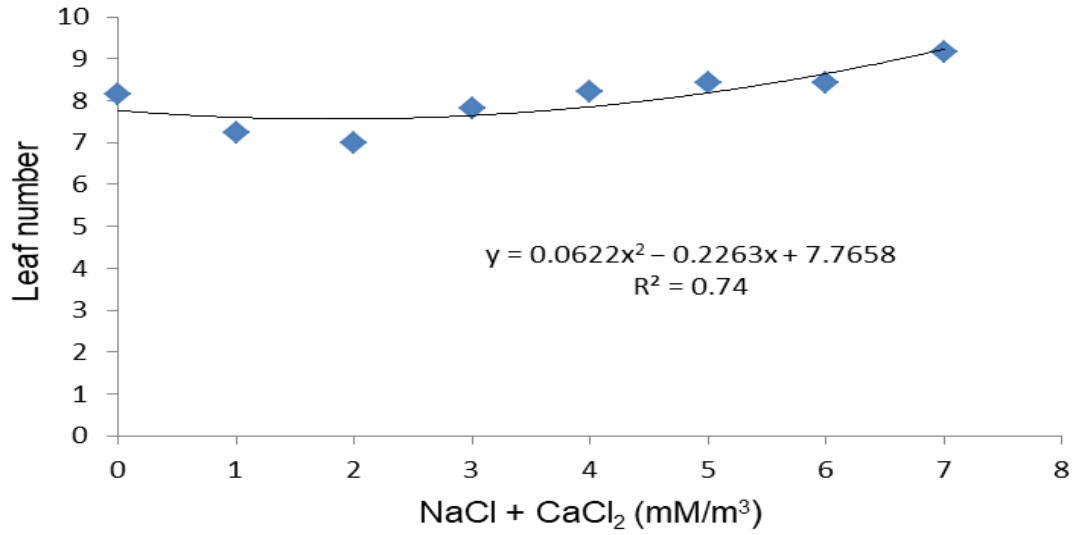

Figure 4.1 Response of leaf number in *Mimusops zeyheri* to salinity at 90 days after the treatments.

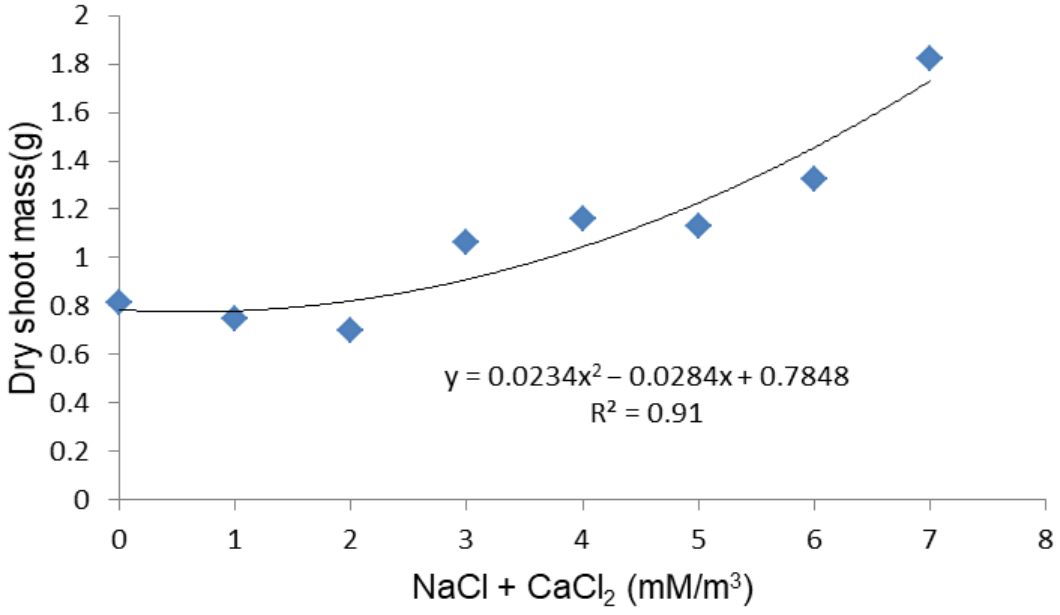

Figure 4.2 Response of dry shoots in *Mimusops zeyheri* to salinity at 90 days after the treatments.

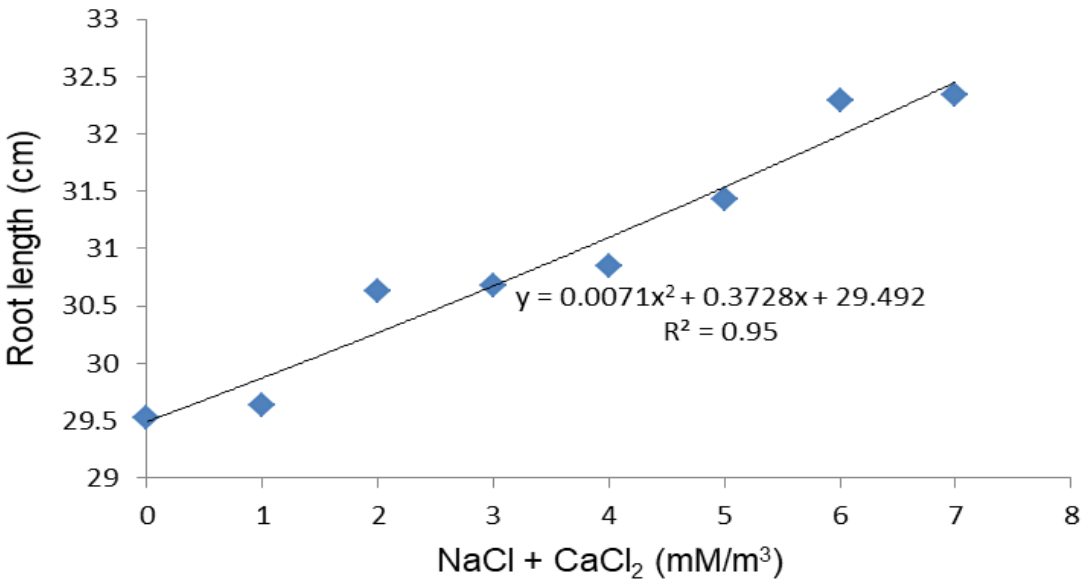

Figure 4.3 Response of root length in *Mimusops zeyheri* to salinity at 90 days after the treatments.

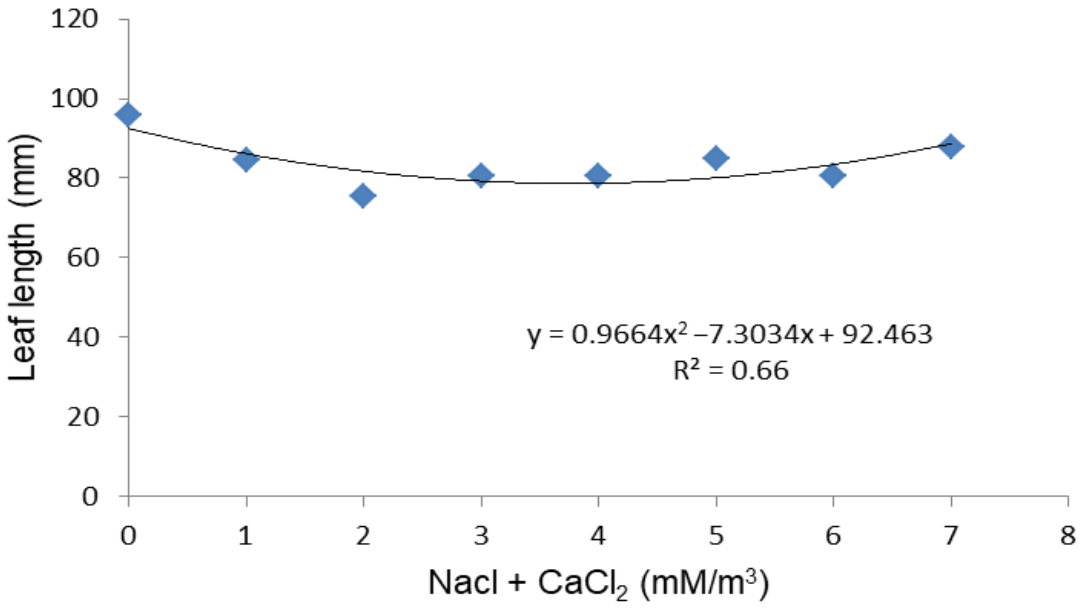

Figure 4.4 Response of leaf length in *Mimusops zeyheri* to salinity at 90 days after the treatments.

Table 4.3 Quadratic relationship, coefficient of determination and computed minimum response concentration of different salinity levels for leaf number, dry shoot mass, root length and leaf length of *Mimusops zeyheri* at 90 days after the treatments.

Organ	Quadratic relation	R^2	(x) mM/m^3
Leaf number	$y = 0.0622x^2 - 0.2263x + 7.7658$	0.74	1.819
Leaf length	$y = 0.9664x^2 - 7.3034x + 92.463$	0.66	3.779
Dry shoot mass	$y = 0.0234x^2 - 0.0284x + 0.7848$	0.91	0.607
Root length	$y = 0.0071x^2 + 0.3728x + 29.492$	0.95	26.254
Mean optimum value			8.115

$x = -b_1/2b_2$.

4.3.2 Nutrient element variables

Responses of essential nutrient accumulation in leaf tissues of *M. zeyheri* to salt treatments had significant effects on K and Na, contributing 28 and 19% in TTV of the respective variables (Table 4.4). However treatments had no significant effects on Ca, Fe and Zn. In essential nutrient elements accumulation, salinity increased leaf K of *M. zeyheri* seedlings from 5−137%, respectively, but significantly reduced the leaf Na accumulation by 4−32%, respectively (Table 4.5).

Potassium (Figure 4.5) over increasing salt concentrations exhibited positive quadratic relations and Na (Figure 4.) over increasing salt concentrations, exhibited negative quadratic relations. The models for each relation were explained by 95 and 86%, respectively. Using $x = -b_1/2b_2$ for each model, K and Na in leaf tissues were each optimised at 1.586 and 0.828 mM/m^3 NaCl with $CaCl_2$ at 3:1 ratio, with the average optimisation being at 1.206 mM/m^3 concentration (Table 4.6).

Table 4.4 Partitioning mean sum of squares of essential nutrient elements in leaf tissues of *Mimusops zeyheri* seedlings in salinity at 90 days after the treatments (n = 96).

		Ca		Fe		K		Na		Zn	
Source	DF	MSS	TTV (%)[z]	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	11	23134	66	6284	93	12018	65	4935	73	2182	88
Treatment	7	6062	17^{ns}	167	3^{ns}	5009	28^{***}	1252	19^{**}	173	7^{ns}
Error	77	6148	17	273	4	1346	7	533	8	125	5
Total	95	35344	100	6724	100	18373	100	6720	100	2480	100

***Highly significant at P ≤ 0.01; **Significant at P ≤ 0.05.

Table 4.5 Response of potassium and sodium in *Mimusops zeyheri* leaf tissues to concentrations of NaCl with $CaCl_2$ (n = 96).

	Potassium		Sodium	
	Variable (ppm)	R.I. (%)	Variable (ppm)	R.I. (%)
Salt (mM/m^3)				
0	45.18^{bz}	−	25.45^{a}	−
2	47.51^{b}	5	23.11^{b}	− 9
4	51.39^{b}	14	23.93^{b}	− 6
8	53.31^{b}	18	24.48^{b}	− 4
16	50.09^{b}	11	24.09^{b}	− 5
32	64.40^{b}	43	20.58^{b}	− 19
64	79.40^{b}	76	17.39^{b}	− 32
128	107.14^{a}	137	17.45^{b}	− 31
Cv %	29.8		18.8	

[z]Column means with the same letter were not different (P ≤ 0.05) according to Waller-Duncan multiple range test.

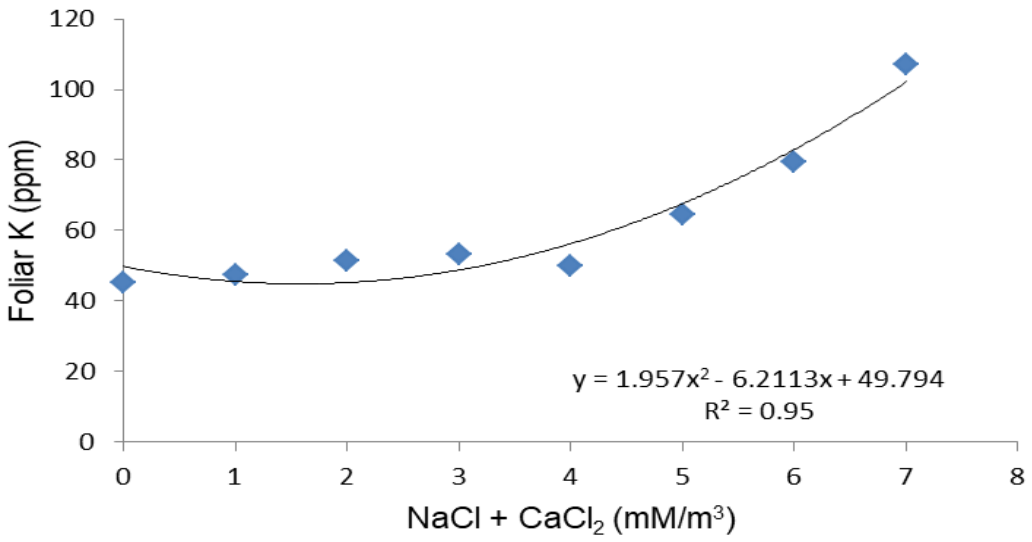

Figure 4.5 Response of potassium in *Mimusops zeyheri* leaf tissues at 90 days after the treatments.

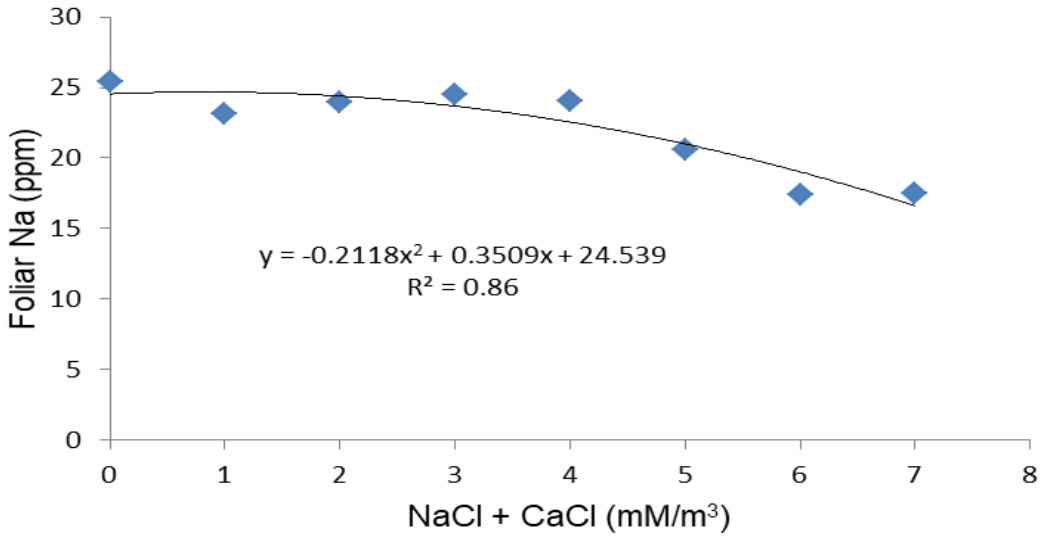

Figure 4.6 Response of sodium in *Mimusops zeyheri* to salinity at 90 days after the treatments.

Table 4.6 Quadratic relationship, coefficient of determination and computed minimum response concentration of NaCl with $CaCl_2$ on potassium and sodium in *Mimusops zeyheri* leaf tissues at 90 days after the treatments.

Foliar cation	Quadratic relation	R^2	(x) mM/m3
K	$y = 1.957x^2 - 6.2113x + 49.794$	0.95	1.586
Na	$y = -0.2118x^2 + 0.3509x + 24.539$	0.86	0.828
Mean optimum value			1.206

$x = -b_1/2b_2$.

4.4 Discussion

4.4.1 Plant growth variables

Growth response of *M. zeyheri* seedlings to salinity treatments had an effect on leaf numbers, dry shoots, root length and leaf length. Mashela (2017) confirmed the recent results with the previous observations that salinity treatment had an effect on leaf numbers and dry shoot mass of *M. zeyheri* seedlings. Ebrahim (2014) also agreed that salt had a significant effect on plant organs of ribwort plantain (*Plantago lanceolata* L.). Contradictions existed when the root length of *M. zeyheri* was not affected by the salt type during summer (Mashela, 2017), which suggested that sodium influenced photosynthesis by altering water balance in roots. Also, Belew *et al.* (2010) demonstrated that there was a substantial effect of salinity in both shoot length and internode length on growth responses of grape rootstocks.

The absence of treatment effects on plant height, dry root mass and chlorophyll content, agreed with findings on *M. zeyheri* in an earlier study (Mashela, 2017). However, salt type did not have an effect on plant height and dry root mass of *M. zeyheri* seedlings in both seasons. Previous studies disagreed with the current

findings that salinity concentrations might have an effect on leaf chlorophyll (Ali *et al.,* 2004). The toxicity of Na and Cl ions in plant cells may even affect the photosynthesis process, which would eventually affect the constituents of the chloroplast. Also, Na and Cl ions in chloroplasts of *M. zeyheri* leaves could have been affected by salinity due to interference with other essential nutrient elements and photosynthates (Ali *et al.,* 2004).

At low salt concentrations, salinity increased leaf number of *M. zeyheri* seedlings in agreement with findings by Ebrahim (2014), who observed increased leaf number in ribwort plantain treated with NaCl at low concentrations. Dry shoots mass of *M. zeyheri* was significantly increased, which confirmed other observations on dry shoot mass of *M. zeyheri* seedlings under the saline conditions (Mashela, 2017). In the study, Mashela (2017) suggested that it was probable that Na, which is an essential nutrient element for C4 plants, was required for plant growth in *M. zeyheri*. However, in tomato plants, which is a C3 plant, Na ions reduced plant growth, although the ions are required for improving tomato fruit quality (Hajer *et al.,* 2006).

Root length of *M. zeyheri* seedlings was significantly increased by salt treatment, which did not support observations in tomato plants, where the variable was reduced by salinity under field conditions (Snapp and Shennan, 1992). , and these findings demonstrate that Na in soil plays a major role inhibiting root growth. Therefore, high levels of Na disturb Ca nourishment when accumulated in cytoplasm and inhibits many enzymes (Hamdia *et al*., 2004). Current findings were confirmed by Mashela (2017), when the root length of *M. zeyheri* improved by the salinity conditions. Reduction of *M. zeyheri* leaf length under salinity conditions agree with the former

studies (Mashela, 2017), various salt types significantly declined the variable relative to the control in both winter and summer seasons.

Increasing levels of salinity applied on *M. zeyheri* trees had a significant effect on tree growth, which suggests that it was operating at the stimulation phase as we observed increasing of plant growth. Salts treatments such as NaCl in plants resulted in enhanced moisture and nutrient uptake which can be observed through increased leaf number, dry shoots and leaf length. Further similar studies indicated that increasing NaCl alone showed rapid increase in growth of Canola (*Brassica napus*) at 20ºC, but contradictory the severely reduction of growth due to the increment of the NaCl persuaded salinity exceeded levels of an EC (Line *et al*., 2008). The less decrement severity of growth was observed when NaCl combined with $CaCl_2$, which supported the findings by Line *et al*. (2008) that Ca helps to protect the integrity of cell membranes, reducing the permeability of membranes while inhibiting ion leakage due to environmental stress. Also the adverse osmotic gradients combinations cause these effects, and the inhibitory effects of salts and ions on cell metabolism, as well as nutrient disproportion. Calcium had an ameliorative effect on the growth of NaCl stressed plants, by moderating whole metabolism (Jaleel *et al*., 2008). The results of the current study clearly support the findings of Ashraf and Fatima (1994) that $CaCl_2$ clearly reduces the toxic effect of NaCl with regard to the growth of the wheat and canola plant.

Extensive experimental trials demonstrated salinity irrigation increased nematodes egg production by two in all citrus rootstocks without changing any relative levels of nematodes resistance among rootstocks (Mashela *et al.,* 1992). The range at which

plant organs respond to treatment depends on concentration applied (Maila 2001) and as a result some levels can stimulate one process, while inhibiting other processes within the same plant organ (Hartmann *et al*., 2010). Consequently increased, concentrations of the applied salts still stimulate the plant growth without reaching the optimal level of *M. zeyheri* seedlings growth.

The relationship observed between leaf number, dry shoot weight, root length, leaf length and NaCl suggests that one or both ions were essential in growth of *M. zeyheri* seedlings. The density-dependent growth (DDG) patterns observed in all trials afforded an opportunity to compute the concentrations that would confer optimum growth (Salisbury and Ross, 2005). The DDG patterns are categorised by three phases, namely, stimulation, neutral and inhibition (Liu and An, 2005).

Using concentrations at the optimum level as observed in this study would result in concentrations accumulating towards the stimulation phase, which would increase plant growth instead of inhibiting it (Mashela *et al*., 2015). In generating the DDG patterns, salts levels behaved as allelochemical at low concentrations (Liu and An, 2005). Suggesting that above the observed optimum values the products might be stimulating the plants growth by giving the plants an essential nutrient element to stimulate the growth of *M. zeyheri* seedlings. Lack of significant effects on other tested plant growth variables of *M. zeyheri* seedling such as plant height, dry root mass and chlorophyll content has led to a conclusion that application of lower levels of salinity had no effect on optimal growth in peach trees (Borkowska *et al*., 2008).

Findings of this study prove that there is an association between salinity and growth of *M. zeyheri* seedlings, which could be explained by great significant effect on plant growth variables. The total dry shoot mass of *M. zeyheri* seedling were improved and the inhibition was observed on the leaf length on application of salinity levels. Under normal circumstances *M. zeyheri* trees have a non-aggressive root system, which enable growth on rocky areas. However, the root system could limit the ability of the trees to absorb enough moisture and nutrients to enhance tree growth under marginal conditions.

4.4.2 Nutrient element variables

Essential nutrient elements to salt treatments had significant effects on potassium and sodium, the current study is confirmed with the earlier trials which demonstrated salinity had significant effects on leaf tissues of *M. zeyheri* seedlings on K and Na (Mashela, 2017). Salinity treatment did not have an effect on Ca, Fe and Zn. Findings of this study disagree with the results observed by Mashela (2017), when Ca accumulation on leaf tissues was significantly affected by salinity, which suggested that at high soil pH levels $CaCl_2$ was converted into Ca that was readily available in plants. Therefore, supplement calcium had been reported to improve growth of various plant species that have been exposed to salt stress by excluding Na from roots and shoots (Marschener and Dell, 1994). Mashela (2016) also confirmed the accumulation of Ca on leaf tissues of *M. oleifera* under different soil types, which could explain that, current results on the absence of Ca in leaf tissues might be the pH of the soil was at low level (Marschener and Dell, 1994).

The absence of significant effects of salinity on Fe in the leaf tissues of *M. zeyheri* appears that no previous study conducted on *M. zeyheri* accumulating Fe in leaf tissues. The previous study shows a significant effect of salinity treatment in Fe accumulation on sunflower leaf tissues (Achakzai *et al.,* 2010). The findings were also in line with results obtained in sorghum and maize seedlings subjected to various levels of irrigation salinity stress conditions (Achakzai, 2008), as well as (Achakzai *et al*., 2010) uptake and accumulation of macronutrients by sunflower varieties. However, other findings contradicted with the abovementioned results, with most studies suggesting that in saline and saline sodic soils, the solubility of micronutrients including Fe was particularly low, with plants grown in such soils often facing deficiencies of micronutrients (Page *et al*., 1990).

Results of the current study indicated that there was no significant effect on Zn in *M. zeyheri* leaf tissues. However, earlier results showed that salinity had an effect on Zn in *M. zeyheri* leaf tissues (Mashela, 2017). The results contradicted with the findings of the current study which might be difficult to explain, because the uptake of microelements was usually inhibited at extreme values of pH (Shuman and McCracker, 1999). $CaCl_2$ salts are known for their capabilities to reduce pH of the soil, which could explain the Zn effect in leaf tissues of *M. zeyheri* seedlings.

Salinity increased leaf K of *M. zeyheri*, similarly results confirm the previous study when leaf K of *M. zeyheri* increased in accumulation and was highest before and after fruiting in Bochum and Sekgosese (Ledwaba, 2008). Also, Henry and Shongwe (1995) observed that exchangeable potassium tended to be equally available in all leaves of sugarcane (*Saccharum officinarum*), significant differences in distributions

of non-exchangeable and exchangeable K percentage saturation levels between soils were found when sorted according to clay categories and clay mineralogy.

Sodium accumulation in *M. zeyheri* leaf tissue was reduced after the salinity treatment application, results agree with the previous trials when *M. oleifera* leaves accumulated lower Na content on the plant (Mashela, 2017). According to Loneva (1988), confirms with the present findings that decrease in Na contents in the leaf tissues lead to increase in K contents. Potassium and Na ions ratios in plant leaves can be attributed to the effect of competition between Na and K ions on the absorptive sites of the plant roots.

Salinity treatment had a significant effect on K when applied on *M. zeyheri* seedlings, which confirms that the treatment was functioning at the stimulation phase as we witnessed the improved accumulation of K on *M. zeyheri* leaf tissues. Salts treatments such as NaCl in *M. zeyheri* leaf tissues indicated the declining accumulation of Na when the salt concentration increases, which may be that the increment of K adversely reduces the Na accumulation in the leaves (Loneva, 1988).

The density-dependent growth patterns (DDG) are categorised in three different phases, namely, stimulation, neutral and inhibition (Liu and An, 2005). Using concentrations at the optimum level observed in the findings result that K accumulation was working towards the stimulation phase instead of inhibition phase (Mashela *et al.,* 2015). Formulating the DDG patterns, salt levels stimulated the accumulation of K elements in leaf tissues of *M. zeyheri* seedlings. In contrast, salts inhibited the accumulation of Na, suggesting that *M. zeyheri* seedlings were tolerant

to Na ions. In plant production, salt tolerance had been described as exclusion of transport of Na ions to shoots by roots (Mashela *et al.,* 1991).

4.5 Conclusion

Growth of *M. zeyheri* seedlings and increasing concentrations of NaCl with $CaCl_2$ salt exhibited positive quadratic relations, this suggested that at low and high concentrations salt stimulated and inhibited growth of *M. zeyheri* seedlings, respectively. In contrast, K and Na versus increasing salt concentrations exhibited positive and negative quadratic relations, respectively. The latter was an indication that *M. zeyheri* seedlings were tolerant to NaCl salinity. In conclusion, chloride salinity could be used at low concentrations to stimulate growth of nursery *M. zeyheri* seedlings.

CHAPTER 5
INFLUENCE OF VESICULAR ARBUSCULAR MYCORRHIZA FUNGI ON GROWTH OF *MIMUSOPS ZEYHERI*

5.1 Introduction

Red Milkwood (*Mimusops zeyheri* Sond.) trees have hardly root systems, which enable the tree to grow in rocky mountainous areas (Venter and Venter, 1996). Generally, in such marginal habitats, the tree gives an impression that it could be suited for cultivation in low-input agricultural systems. In such systems, essential nutrient elements could be limiting to the successful husbandry of fruit trees. Vesicular arbuscular mycorrhiza (VAM) is suitable for use in low-input agricultural systems, with respect to improving the availability of nutrients to plants (Gavito *et al*., 2002). The potential use of VAM in improving growth and nutrient elements in *M. zeyheri* had not been documented. The objective of this study was to investigate the influence of vesicular arbuscular mycorrhiza fungi on growth of *M. zeyheri*.

5.2 Materials and methods

5.2.1 Growth conditions and preparations

The study was conducted under greenhouse conditions at the Green Biotechnologies Research Centre (GBRC) of Excellence, University of Limpopo, South Africa (23°53'10"S, 29°44'15"E) during spring (July-September) through summer (October-December) 2015 and validated in time during the same seasons 2016. Day/night temperatures averaged 5/15^{o}C, with maximum temperatures being controlled using thermostatically-activated fans. Relative humidity was maintained at a high level through wet walls. *Mimusops zeyheri* seedlings were raised and hardened-off as described previously (Chapter 3). Uniform seedlings were

transplanted into 30-cm-diameter plastic pots, containing steam-pasteurised (300ºC for 1 h) loam soil and river sand at 3:1 ratio. Pots were placed on greenhouse benches with inter-row and intra-row spacing of 0.45 m each. Strains of VAM, namely, Mycoroot (Mycoroot, Pietermaritzburg), were procured for each season, with the left overs being discarded.

5.2.2 Experimental design and inoculation

Eight treatments, namely, 0, 10, 20, 30, 40, 50, 60 and 70 g per plant Mycoroot, were arranged in a randomised complete block design, with six replications (Legend 5.1).

Legend 5.1 *Mimusops zeyheri* seedlings on greenhouse benches in a vesicular arbuscular mycorrhiza trial.

5.2.3 Cultural practices

Fertilisers were applied a day after transplanting with 2:3:2 (26) NPK 5 g + 0.5% Zn + 5% S + 5% Ca fertiliser mixture per plant, which provided a total of 155 mg N, 105 mg P and 130 mg K per ml water. Multifeed fertiliser was applied twice a month at 5 g per 4 L water to provide 0.47 mg N, 0.43 mg K, 0.43 mg P, 121 mg Mg, 1 mg Fe, 0.10 mg Cu, 0.47 mg Zn, 1.34 mg B, 4.02 mg Mn and 0.09 mg Mo per ml water (Mashela, 2002). Plants were irrigated with 500 ml chlorine-free water every other day. Pests were scouted, with aphids controlled once using Malasol as per label instruction.

5.2.4 Data collection

At 90 days after the treatment, plant height was measured from the soil level to the tip of the flag leaf. Chlorophyll content was measured using a chlorophyll meter (MINOLTA, SPAD-502) and leaves were counted. Shoots were severed at the soil surface. Roots were removed from pots, immersed in water to remove soil particles and root length measured. Stem diameter was measured at 5 cm above the severed ends using a digital vernier caliper. Leaf area was measured, along with the leaf length and leaf width of three leaves below the flag leaf (AM350 Portable Leaf Area Meter). Petiole length was measured using a ruler. Both shoots and roots were oven-dried at 60º C for 72 h for dry shoot mass and dry root mass determination.

Dried mature leaves were separated from the shoot and ground into powder using pestle and mortar. The powdered samples were each digested in 5% nitric acid, which was then mixed using a vortex meter. Samples were incubated in a warm bath with water at 95°C for 1 h, left to cool down to room temperature and filtered with the

container covered with a foil (SW-846 EPA Method 3050B). Samples were then subjected to Atomic Absorption Spectrometry (AAS) to quantify Na, Ca, K, Fe and Zn in leaf tissues at Limpopo Agro-food Technology Station.

5.2.5 Data analysis

Data for plant variables were subjected to analysis of variance (ANOVA) using SAS software (SAS Institute, 2008). Discrete data for leaf number were transformed through $\log_{10}(x + 1)$ to homogenise the variances (Gomez and Gomez, 1984), but untransformed means were reported. The degree of freedom and their mean sum of squares were partitioned to provide the total treatment variation (TTV) for different sources of variation. The Waller-Duncan multiple range tests were used to separate treatment means which were significant at probability level of 5%. Significant plant variables were subjected to lines of the best fit, with quadratic equations used to compute optimum or minimum concentration values using $x = -b_1/2b_2$ relation (Gomez and Gomez, 1984). Unless stated otherwise, treatment effects were discussed at probability level of 5%.

5.3 Results

Seasonal interaction did not have significant effects on the variables. Data were pooled (n = 96) and subjected to ANOVA using SAS software.

5.3.1 Plant growth variables

Growth response to VAM treatment had highly significant ($P \leq 0.01$) effects on plant height, leaf number, stem diameter and chlorophyll content, contributing 49, 65, 60 and 61% in TTV of the respective variables (Table 5.1). Treatments had no

significance effects on dry shoot mass, dry root mass and leaf length. Relative to untreated control, VAM reduced plant height, leaf number and chlorophyll content on *M. zeyheri* seedlings by 2-8, 15-27 and 11-24%, respectively (Table 5.2). In contrast, the treatment increased stem diameter of *M. zeyheri* seedlings from 0 to 11% (Table 5.2).

Stem diameter over increasing VAM level, exhibited positive quadratic relations with the model explained by 97% (Figure 5.3). Plant height (Figure 5.1), leaf number (Figure 5.2) and chlorophyll content (Figure 5.4) over increasing VAM level, each exhibited positive quadratic relations. The models for each relation were explained by 97, 83 and 80%, respectively. Using $x = -b_1/2b_2$ relation for each model, plant height, leaf number, stem diameter and chlorophyll content were optimised at 5.418, 5.327, 3.274 and 8.198 g VAM, respectively, with overall mean optimisation of 5.554 g (Table 5.3).

Table 5.1 Partitioning mean sum of squares of growth in various organs of *Mimusops zeyheri* seedlings under different mycorrhiza levels at 90 days after the treatments (n = 96).

		Plant height		Leaf no.		Dry shoot		Dry root		Stem diam.		Leaf length		Chlorophyll	
Source	DF	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)	MSS	TTV (%)
Replication	11	24.9	38	2.7	18	2.3	61	0.6	60	0.4	27	692.6	56	83.0	21
Treatment	7	29.6	49***	9.9	65***	0.5	13ns	0.1	10ns	0.9	60***	216.3	18ns	250.7	61***
Error	77	8.5	13	2.6	17	1.0	26	0.3	30	0.2	13	330.6	26	74.9	18
Total	95	63	100	15.2	100	3.8	100	1	100	1.5	100	1239.4	100	408.6	100

***Highly significant at $P \leq 0.01$, ns not significant at $P \leq 0.05$.

Table 5.2 Relative impact of different mycorrhiza levels to control on growth variables in *Mimusops zeyheri* seedlings at 90 days after the treatments (n = 96).

	Plant height		Leaf number		Stem diameter		Chlorophyll content	
Treatment	Variable[z] (cm)	R.I. (%)	Variable	R.I.[y] (%)	Variable (mm)	R.I. (%)	Variable	R.I. (%)
0	18.5[a]	−	9.8[a]	−	2.7[b]	−	65.1[a]	−
10	18.1[b]	− 2	8.1[b]	− 17	2.9[b]	7	58.0[b]	− 11
20	17.5[b]	− 5	8.3[b]	− 15	2.9[b]	7	56.6[b]	− 13
30	17.2[b]	− 7	8.3[b]	− 15	3.0[a]	11	55.3[b]	− 15
40	17.2[b]	− 7	7.2[b]	− 27	3.0[a]	11	54.2[b]	− 17
50	16.8[b]	− 9	7.4[b]	− 25	2.9[b]	7	55.6[b]	− 15
60	16.9[b]	− 9	7.4[b]	− 25	2.8[b]	4	54.3[b]	− 17
70	17.1[b]	− 8	7.5[b]	− 24	2.7[b]	0	49.2[b]	− 24

[z]Column means with the same letter were not different (P ≤ 0.05) according to Waller-Duncan multiple range test.

[y]Impact (%) = [(treatment/control) −1] × 100.

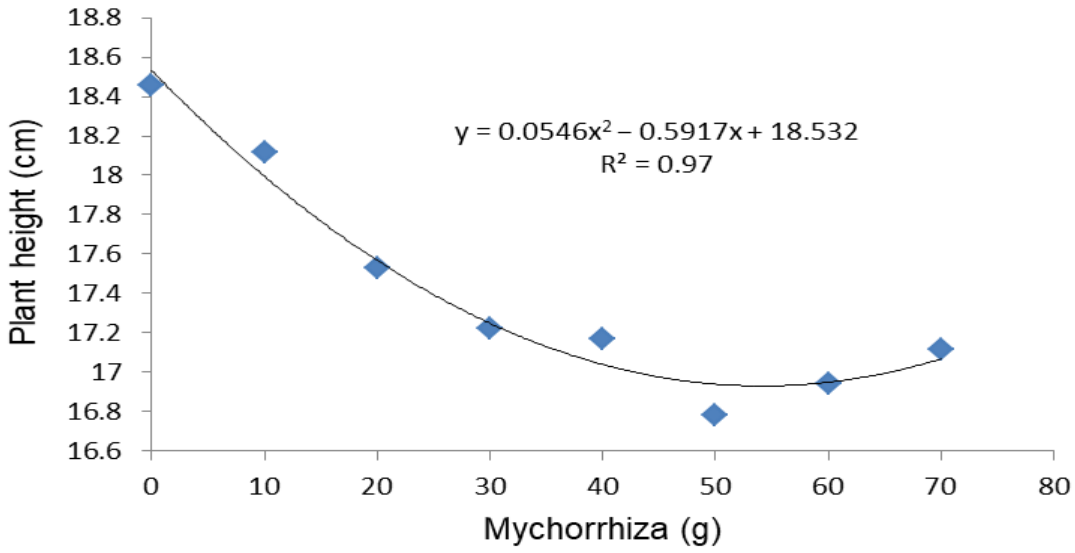

Figure 5.1 Response of plant height in *Mimusops zeyheri* to vesicular arbuscular mycorrhiza at 90 days after the treatments.

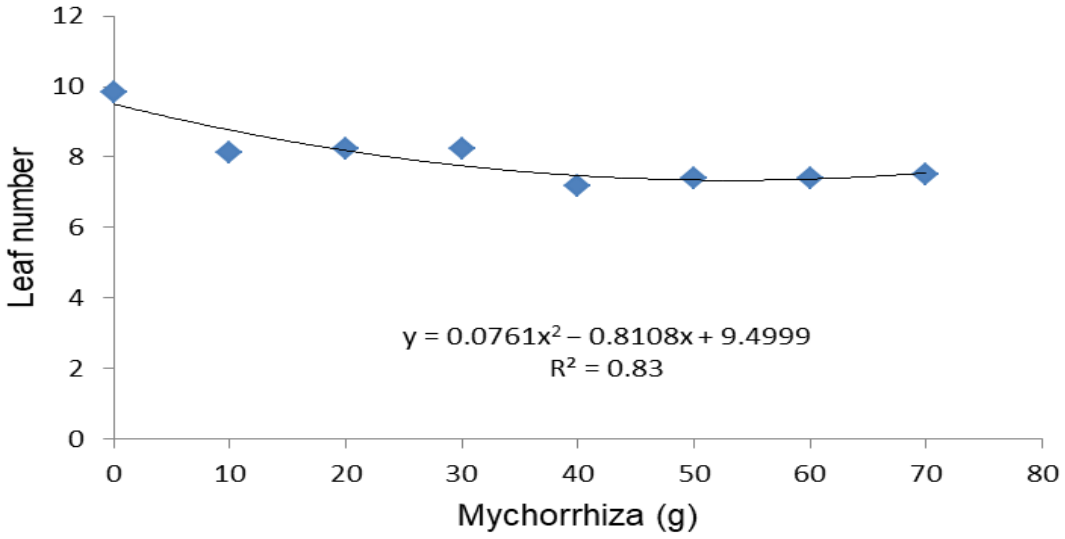

Figure 5.2 Response of leaf number in *Mimusops zeyheri* to vesicular arbuscular mycorrhiza at 90 days after the treatments.

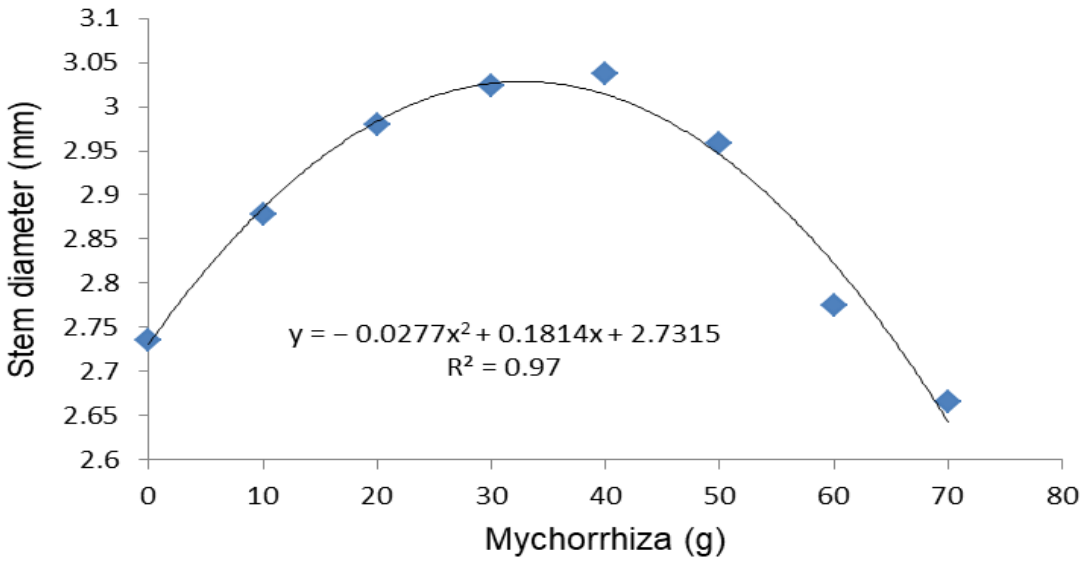

Figure 5.3 Response of stem diameter in *Mimusops zeyheri* to vesicular arbuscular mycorrhiza at 90 days after the treatments.

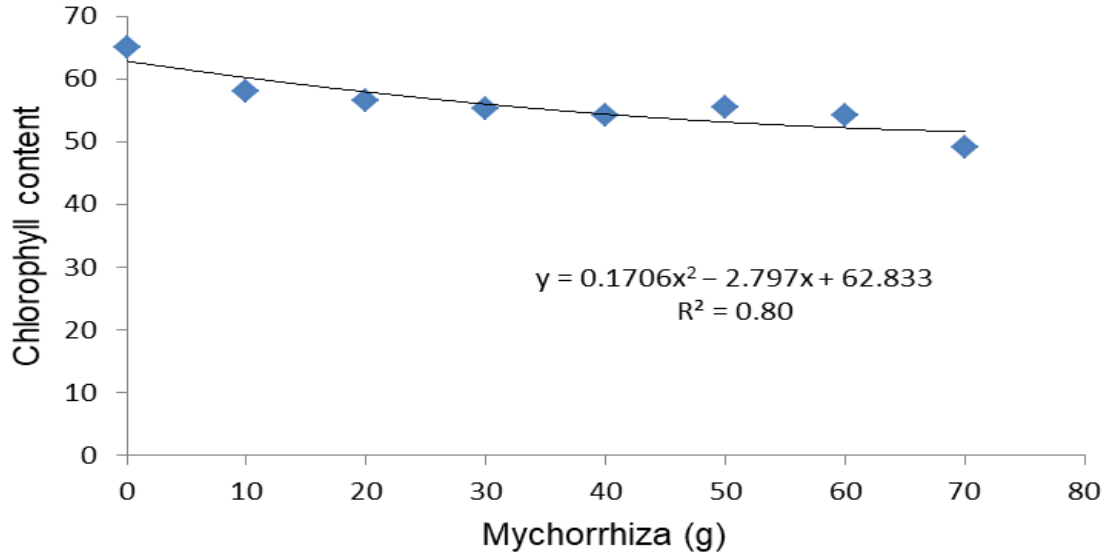

Figure 5.4 Response of chlorophyll content in *Mimusops zeyheri* to vesicular arbuscular mycorrhiza at 90 days after the treatments.

Table 5.3 Quadratic relationship, coefficient of determination and computed minimum response concentration of different mycorrhiza levels for plant height, leaf number, stem diameter and chlorophyll of *Mimusops zeyheri* at 90 days after the treatments.

Organs	Quadratic relation	R^2	(x)
Plant height	$y = 0.0546x^2 – 0.5917x + 18.532$	0.97	5.418
Leaf number	$y = 0.0761x^2 – 0.8108x + 9.4999$	0.83	5.327
Stem diameter	$y = -0.0277x^2 + 0.1814x + 2.7315$	0.97	3.274
Chlorophyll	$y = 0.1706x^2 – 2.797x + 62.833$	0.80	8.198
Mean optimum value			5.554 g

$x = -b_1/2b_2$.

5.3.2 Nutrient element variables

Vesicular arbuscular mycorrhiza treatment did not have significant effects on Ca, Fe, K, Na and Zn in leaf tissues of *M .zeyheri* seedlings (Table 5.4). Treatment means for the nutrient elements and their coefficient of variation were summarised (Table 5.5).

Table 5.4 Partitioning mean sum of squares of essential nutrient elements in leaf tissues of *Mimusops zeyheri* seedlings under different mycorrhiza levels at 90 days after the treatments (n = 96).

		Ca		Fe		K		Na		Zn	
Source	DF	MSS	TTV (%)[z]	MSS	TTV (%)	MSS	TTV(%)	MSS	TTV (%)	MSS	TTV (%)
Replication	11	102.4	32	38.97	38	136.7	36	0.67	34	0.019	31
Treatment	7	111.3	34[ns]	15.89	15[ns]	123.3	32[ns]	0.59	31[ns]	0.016	26[ns]
Error	77	110.5	34	48.77	47	121.1	32	0.69	35	0.026	43
Total	95	324.2	100	103.63	100	381.1	100	1.95	100	0.061	100

[ns] not significant at P ≤ 0.05.

Table 5.5 Influence of vesicular arbuscular mycorrhiza on essential nutrient elements accumulation in *Mimusops zeyheri* seedlings at 90 days after the treatments (n = 96).

Treatment	Ca	Fe	K	Na	Zn
0	29.286	7.3248	29.638	2.507	0.2507
10	24.117	4.6237	27.317	2.0800	0.2675
20	30.175	6.9792	30.308	2.2925	0.2850
30	24.875	7.8920	24.375	1.9600	0.2771
40	23.350	8.3283	25.300	1.8600	0.2260
50	23.808	7.3602	24.950	2.1000	0.2600
60	30.758	7.0737	32.860	2.3008	0.3338
70	23.993	8.0800	24.390	1.9257	0.2139
Cv (%)	39.41	96.89	40.18	39.12	62.08

5.4 Discussion

5.4.1 Plant growth variables

Vesicular arbuscular mycorrhiza treatments had highly significant effects on plant height, leaf number, stem diameter and chlorophyll content. The current results contradicted those where VAM did not have significant effects on *M. zeyheri* seedlings that were much older (Radzuma, 2017). In the current study seedlings were 90 days old, whereas in the other study (Radzuma, 2017) they were older. Also Jifon *et al*. (2002) observed that application of mycorrhiza could depress growth of sour orange (*Citrus aurantium* Rosid.) seedlings under high P supply. Similarly, results suggested that when a significant effect in leaf area, plant height, stem

diameter, chlorophyll content and plant biomass was observed in orange (*Citrus sinensis* L.) seedlings during screening of five *Glomus* species of mycorrhiza, namely, *Glomus, mosseae (UK), G. mosseae (USA), G. clarium, G. caledonium and G. etunicatum* (Ortas, 2012). The observed reduction effects could be in terms of competition for carbohydrates and other resources, particularly at colonization stage by VAM.

Vesicular arbuscular mycorrhiza treatments had no significant effects on dry shoot mass, dry root mass and leaf length, which confirmed similar studies on older *M. zeyheri* trees (Radzuma, 2017). In addition to being organ-specific, it appeared that the influence of VAM on *M. zeyheri* seedlings was also plant age-specific. However, since growth of other variables was initially stimulated and eventually reduced, the latter would eventually have indirect effects on other variables such as biomass

Vesicular arbuscular mycorrhiza significantly stimulated and then inhibited plant height, leaf number and chlorophyll content, which might imply that the symbiotic relationship between VAM and *M. zeyheri* seedlings had density-dependent growth (DDG) patterns, as depicted by various quadratic relationships (Mashela *et al.,* 2015; Salisbury and Ross, 2015). On the basis of DDG patterns, there are three phases, namely, stimulation, neutral and inhibition phases (Mashela *et al.,* 2015). When the independent (x-axis) factor is within the neutral, the factor did not have have significant effects on the variables, as observed in this current and other VAM studies (Świerczyński and Stachowiak, 2010; Tong *et al*., 2006).

The observed DDG patterns on stem diameter of *M. zeyheri* seedlings and VAM provided some light in how external factors influence the variable. Generally, the root-knot (*Meloidogyne species*) (Mashela *et al.,* 2003), drought (Mafeo, 2006), root-pruning (Mashela and Nthangeni, 2002), salinity (Mashela, 2017) and VAM (Bagyaraj and Revanna, 2017) decreased stem diameter in various plants. In contrast, Wu and Xia (2010) demonstrated that VAM increased stem diameter in *Poncirus trifoliata* (bonsai) seedling rootstock when water content in the soil was at 12-20%. The DDG patterns, for the first time in the current study, provided an explanation where VAM concentrations stimulated, had no effect and reduced stem diameter in plants. The effects of VAM on stem diameter were therefore, density dependent, as explained in interactions of plants with phytonematicides (Mashela *et al.,* 2015).

Using the concept DDG patterns, regardless of whether ANOVA had significant effects or not, VAM and *M. zeyheri* seedlings had symbiotic relationships. Generally, when organs are stimulated or inhibited, ANOVA outputs invariably suggest significant effects (Mashela *et al.,* 2015), which would occur at one level of an external factor. Mashela *et al.* (2015) outlined the conceptual fact that when a few treatment levels within the stimulation phase were used, the relation with responding variables would be positive linear, whereas within the inhibition phase the relation would be negative linear. In contrast, within the neutral phase, the relation between dependent and independent variables would not be significant (Dube *et al.,* 2016). However, when the level of the independent factor spans all the three phases of DDG patterns, ANOVA would be significant, with the relations characterised by either negative or positive quadratic relationships (Mashela *et al.,* 2015). Generally,

the quadratic responses are dependent upon whether the starting concentration is within the stimulation, neutral or inhibition point. In the assessment of mean concentration stimulation point (MCSP) using the curve-fitting allelochemical response dosage (CARD) computer model, the relation included all three phases from stimulation to inhibition (Mashela *et al.,* 2015). The relations had almost always, positive quadratic relations. However, in the assessment of bioactivities of cucurbitacin containing phytonematicides, the relations started from neutral, through inhibition to stimulation, characterised by negative quadratic relations (Dube, 2016).

In the current study, certain variables such as plant height, leaf number, stem diameter and chlorophyll content with VAM level, had DDG patterns, characterised by quadratic relations. The four variables were optimised at different VAM levels, with the overall mean being at 5.554 g VAM. In other words, for *M. zeyheri* seedlings, VAM should not be applied beyond 5.554 g since it would then inhibit the listed variable and then eventually affect the overall growth of the seedlings.

5.4.2 Nutrient element variables

Application VAM levels on *M. zeyheri* seedlings had no significant effect on nutrient accumulation. Increasing concentrations of VAM had no effect on accumulation of Ca, Fe, K, Na and Zn. There was no observed pattern in all treatment level in regard to accumulation of essential nutrient elements in *M. zeyheri* seedlings. Similar results were observed when nutrient element variables of *M. zeyheri* leaf tissues, such as Mg, P, K, Ca, and Fe were not affected by VAM (Radzuma, 2017). This results contradicts with findings in Orange (*Citrus sinensis*) trees treated with *Glomus clarium* species increased Ca, K, Zn and Na contents relative to the untreated

control (Ortas, 2012).similar results were observed when VAM fungi was applied on citrus, there were no significant effect in uptake of essential nutrient elements such as phosphorus, calcium, zinc, copper and iron was (Sriavastava *et al*., 2002). These findings concur with the observation that the presence of certain mineral elements in trees can be limiting to the availability of other nutrient elements (Ledwaba 2008).

Results of this study might indicate that VAM was working at a neutral phase, since there was no mycorrhizal symbiosis between the fungus and the roots of *M. zeyheri* seedlings subsequently nutrient uptake of the tree was not enhanced of affected. This could be as a result that the fungus was not exposed to the trees for a sufficient period of time which could have subsequently resulted in symbiosis and enhanced tree growth. Mycorrhizal symbiosis can take a long period of time since the fungi can utilise the nutrients during the first year which was supposed to be supplied to the tree and as a result the tree does not benefit from the symbiosis (Borkowska, 2008).

Extensive studies had proven that mycorrhizal application on trees enhance nutrient uptake especially of scarce and immobile nutrients such as phosphorus and nitrogen (Bolan, 1991; Sidhoum and Fortas, 2013). However, lack of significant effect on nutrient content of *M. zeyheri* seedling has led to a conclusion that application of VAM had no effect on nutrient elements accumulation in *M. zeyheri* since it was operating at a neutral phase.

5.5 Conclusion

The other plant variables, including the nutrient elements, which were not significantly affected by VAM, suggested that the VAM level was within the neutral

phase for the variable. Vesicular arbuscular mycorrhiza levels used in the study were amenable to all phases of DDG patterns for plant height, leaf number, stem diameter and chlorophyll content as characterised by the quadratic relations. However, the VAM levels for other variables were within the neutral phase of DDG patterns as signified by ANOVA outputs that were not significant at the probability level of 5%. In conclusion, observations in the current study suggested that VAM was capable of colonizing *M. zeyheri* seedlings to establish a symbiotic relationship.

CHAPTER 6
SUMMARY OF FINDINGS, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

6.1 Summary of findings

The study focused on the (1) influence of soil type on root growth and foliar nutrient elements in Red milkwood (*Mimusops zeyheri* Sond.*)* seedlings, (2) degree of salt tolerance in *M. zeyheri* seedlings and (3) influence of vesicular arbuscular mycorrhiza (VAM) fungi on growth of *M. zeyheri* seedlings. The results of the study demonstrated that soil with high clay and loam content improved root growth and accumulation of essential nutrients when compared to those on calcareous and sandy soils. In this study, it was shown that the essential nutrient elements in *M. zeyheri* could be affected by soil type, particularly the clay soil. Influence of soil type on chemical compounds should always be taken into consideration when establishing *M. zeyheri* projects on marginal soils. Findings suggested that soil with high clay content might be used when raising *M. zeyheri* seedlings in order to improve root growth with subsequent increased accumulation of essential nutrient elements in leaf tissues. The results suggested that *M. zeyheri* seedlings were tolerant to chloride salts, with results suggesting that Na and Cl ions were potential essential nutrient elements for growth of *M. zeyheri* seedlings.

The VAM levels used in the study were amenable to all phases of density-dependent growth (DDG) patterns for plant height, leaf number, stem diameter and chlorophyll content as characterised by the quadratic relations. However, the VAM levels for essential nutrient element variables were within the neutral phase of DDG patterns as signified by ANOVA outputs that were not significant at the probability level of 5%.

Therefore, the observations in the current study suggested that VAM was capable of colonizing *M. zeyheri* seedlings to establish a symbiotic relationship.

6.2 Significance of findings

Mimusops zeyheri seedlings grow rapidly and accumulate high levels of nutrients in clay soil, suggesting that the growing mixture for raising seedlings in the nursery should be high in clay content. Also, *M. zeyheri* seedlings performed better under chloride salinity following the DDG patterns, suggesting that the two salts used in the study improved growth and accumulation of certain nutrient elements in leaf tissues of *M. zeyheri* seedlings. However, VAM levels used in the current study were not suitable for *M. zeyheri* seedlings as illustrated by reduced plant growth and lack of significant effects on essential nutrient elements in leaf tissues.

6.3 Conclusions

In conclusion, soil with high clay content and chloride salts improved growth of roots and accumulation of selected essential nutrient elements in *M. zeyheri* seedlings. In contrast, VAM levels used in the current study were already in the inhibition phase for growth in *M. zeyheri* seedlings whereas other nutrient element variables were operating at neutral phase of DDG patterns for the plant species. Validation of optimal for chloride salt and VAM levels on clay soils could provide evidence on whether the products and the soil type could not serve as a suitable growing mixture for *M. zeyheri* seedlings in nursery systems.

6.4 Recommendations

Growing mixtures for raising *M. zeyheri* seedlings should be high in clay content. Also, seedlings could benefit from irrigation with water containing some chloride salts. Generally, it had been shown that C4 plants require Na as an essential nutrient element (Shommer-Ilan and Waisel, 1973) and it could be beneficial to establish the photosynthetic pathway of *M. zeyheri*. Also, since VAM levels used in the current study were already in the inhibition phase of DDG patterns (Mashela *et al*., 2015), it would be necessary to reduce VAM levels so that DDG patterns, could be generated. Also, the optimum chloride salt and VAM levels should be validated under clay to establish whether they could improve growth of *M. zeyheri* seedlings under nursery conditions prior to rolling-out the findings to nurseries.

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Appendix 3.1 Analysis of variance for root length of *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	18	363.2	20.2		
Treatment	3	386.0	128.7	4.99	0.01
Error	54	1391.2	25.8		
Total	75	2140.4			

Appendix 3.2 Analysis of variance for root branch number of *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	18	31.7	1.8		
Treatment	3	35.5	11.8	9.7	0.01
Error	54	66.0	1.2		
Total	75	133.2			

Appendix 3.3 Analysis of variance for dry root mass of *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	18	0.02	1.5		
Treatment	3	0.03	6.8	7.8	0.01
Error	54	0.05	8.6		
Total	75	0.1			

Appendix 3.4 Analysis of variance for hypocotyl length of *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P
Replication	18	56.34	3.13		
Treatment	3	0.42	0.14	0.23	0.87
Error	54	32.23	0.59		
Total	75	88.99			

Appendix 3.5 Analysis of variance for epicotyl length of *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	18	13.83	0.77		
Treatment	3	5.85	1.95	3.80	0.05
Error	54	27.76	0.51		
Total	75	47.44			

Appendix 3.6 Analysis of variance for number of new leaves of *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	18	12.16	0.68		
Treatment	3	10.57	3.52	4.92	0.01
Error	54	38.68	0.72		
Total	75	61.4079			

Appendix 3.7 Analysis of variance for chlorophyll content on dicotyledonous leaf of *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	18	1307.2	72.62		
Treatment	3	1949.7	649.89	13.21	0.01
Error	54	2656.6	49.19		
Total	75	5913.5			

Appendix 3.8 Analysis of variance for chlorophyll content on secondary leaf of *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	18	3493.1	194.06		
Treatment	3	7110.5	2370.18	10.27	0.01
Error	54	12460.7	230.75		
Total	75	23064.4			

Appendix 3.9 Analysis of variance for dry shoot mass of *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P
Replication	18	0.12	6.46		
Treatment	3	0.03	9.67	2.06	0.12
Error	54	0.25	4.69		
Total	75	0.39			

Appendix 3.10 Analysis of variance for calcium accumulation in *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	18	2644.8	146.9		
Treatment	3	688.70	229.6	3.08	0.05
Error	54	4021.8	74.5		
Total	75	7355.3			

Appendix 3.11 Analysis of variance for iron accumulation in *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P
Replication	18	804.82	44.71		
Treatment	3	14.44	4.81	0.10	0.96
Error	54	2679.48	49.62		
Total	75	3498.74			

Appendix 3.12 Analysis of variance for potassium accumulation in *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	18	2760.00	153.33		
Treatment	3	928.70	309.57	3.36	0.05
Error	54	4981.61	92.25		
Total	75	8670.31			

Appendix 3.13 Analysis of variance for sodium accumulation in *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	18	22.68	1.26		
Treatment	3	8.02	2.67	5.36	0.01
Error	54	26.92	0.49		
Total	75	57.66			

Appendix 3.14 Analysis of variance for zinc accumulation in *Mimusops zeyheri* in response to soil type under shade house conditions at 60 days after the treatments.

Source	DF	SS	MS	F	P
Replication	18	0.42	0.02		
Treatment	3	0.06	0.02	0.86	0.47
Error	54	1.27	0.02		
Total	75	1.75			

Appendix 4.1 Analysis of variance for leaf number of *Mimusops zeyheri* in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	11	9.12	0.83		
Treatment	7	80.24	11.46	3.44	0.01
Error	77	256.64	3.33		
Total	95	345.99			

Appendix 4.2 Analysis of variance for plant height of *Mimusops zeyheri* in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	93.91	8.54		
Treatment	7	164.22	23.46	1.45	0.19
Error	77	1244.46	16.16		
Total	95	1502.59			

Appendix 4.3 Analysis of variance for dry shoot mass of *Mimusops zeyheri* in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	11	6.23	0.57		
Treatment	7	17.76	2.54	2.26	0.05
Error	77	86.38	1.12		
Total	95	110.37			

Appendix 4.4 Analysis of variance for dry root mass of *Mimusops zeyheri* in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	3.10	0.28		
Treatment	7	2.11	0.30	1.68	0.13
Error	77	13.81	0.18		
Total	95	19.02			

Appendix 4.5 Analysis of variance for root length of *Mimusops zeyheri* in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	11	1215.95	110.541		
Treatment	7	1179.85	168.51	3.01	0.01
Error	77	4305.41	55.91		
Total	95	6701.21			

Appendix 4.6 Analysis of variance for stem diameter of *Mimusops zeyheri* in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	5.37	0.49		
Treatment	7	1.02	0.15	0.65	0.71
Error	77	17.22	0.22		
Total	95	23.61			

Appendix 4.7 Analysis of variance for petiole length of *Mimusops zeyheri* in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	0.11	0.01		
Treatment	7	0.12	0.02	1.59	0.15
Error	77	0.82	0.01		
Total	95	1.05			

Appendix 4.8 Analysis of variance for chlorophyll content of *Mimusops zeyheri* in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	944.89	85.89		
Treatment	7	371.26	53.04	1.28	0.27
Error	77	3200.22	41.56		
Total	95	4516.37			

Appendix 4.9 Analysis of variance for leaf length of *Mimusops zeyheri* in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	11	7395.5	672.32		
Treatment	7	12734.7	1819.24	4.97	0.01
Error	77	28161.6	365.73		
Total	95	48291.8			

Appendix 4.10 Analysis of variance for leaf width of *Mimusops zeyheri* in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	16436	1494.22		
Treatment	7	7418	1059.65	0.70	0.67
Error	77	116608	1514.39		
Total	95	140462			

Appendix 4.11 Analysis of variance for calcium accumulation in *Mimusops zeyheri* leaves in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	254471	23133.7		
Treatment	7	42436	6062.3	0.99	0.45
Error	77	473411	6148.2		
Total	95	770318			

Appendix 4.12 Analysis of variance for iron accumulation in *Mimusops zeyheri* leaves in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	69128.1	6284.38		
Treatment	7	1174.8	167.82	0.61	0.74
Error	77	21039.4	273.24		
Total	95	91342.3			

Appendix 4.13 Analysis of variance for potassium accumulation in *Mimusops zeyheri* leaves in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	11	132203	12018.5		
Treatment	7	35067	5009.5	3.72	0.01
Error	77	103668	1346.3		
Total	95	270938			

Appendix 4.14 Analysis of variance for sodium accumulation in *Mimusops zeyheri* leaves in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	11	54290	4935.47		
Treatment	7	8769	1252.77	2.35	0.05
Error	77	41052	533.14		
Total	95	104111			

Appendix 4.15 Analysis of variance for zinc in *Mimusops zeyheri* leaves in response to salinity under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	24006.0	2182.36		
Treatment	7	1211.1	173.01	1.38	0.23
Error	77	9640.1	125.20		
Total	95	34857.2			

Appendix 5.1 Analysis of variance for plant height of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	11	274.35	24.94		
Treatment	7	207.68	29.67	3.48	0.01
Error	77	656.19	8.52		
Total	95	1138.22			

Appendix 5.2 Analysis of variance for leaf number of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	11	29.87	2.71		
Treatment	7	69.66	9.95	3.84	0.01
Error	77	199.72	2.59		
Total	95	299.24			

Appendix 5.3 Analysis of variance for dry shoot mass of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	2571	2.34		
Treatment	7	3.71	0.53	0.52	0.82
Error	77	7881	1.02		
Total	95	108.23			

Appendix 5.4 Analysis of variance for dry root mass of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	6.50	0.59		
Treatment	7	0.48	0.11	0.22	0.98
Error	77	23.96	0.31		
Total	95	30.93			

Appendix 5.5 Analysis of variance for petiole length of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	0.23	0.02		
Treatment	7	0.04	0.01	0.44	0.87
Error	77	0.97	0.01		
Total	95	1.24			

Appendix 5.6 Analysis of variance for root length of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	272.64	24.79		
Treatment	7	376.46	53.78	1.30	0.26
Error	77	3195.58	41.50		
Total	95	3844.68			

Appendix 5.7 Analysis of variance for stem diameter of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	11	4.40	0.40		
Treatment	7	6.47	0.92	6.16	0.01
Error	77	1155	0.15		
Total	95	22.42			

Appendix 5.8 Analysis of variance for leaf length of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	7618.1	692.56		
Treatment	7	1513.8	216.26	0.65	0.70
Error	77	25457.6	330.62		
Total	95	34589.6			

Appendix 5.9 Analysis of variance for leaf width of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	821.07	74.6428		
Treatment	7	601.30	85.9001	1.62	0.14
Error	77	4092.82	53.1534		
Total	95	5515.19			

Appendix 5.10 Analysis of variance for chlorophyll content of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P ≤
Replication	11	913.09	83.01		
Treatment	7	1754.55	250.65	3.35	0.01
Error	77	5769.57	74.93		
Total	95	8437.21			

Appendix 5.11 Analysis of variance for calcium accumulation in *Mimusops zeyheri* leaves in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	1126.2	102.386		
Treatment	7	778.8	111.256	1.01	0.43
Error	77	8504.6	110.450		
Total	95	10409.7			

Appendix 5.12 Analysis of variance for iron accumulation in *Mimusops zeyheri* leaves in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	428.69	38.9717		
Treatment	7	111.23	15.8904	0.33	0.94
Error	77	3755.30	48.7701		
Total	95	4295.22			

Appendix 5.13 Analysis of variance for potassium accumulation in *Mimusops zeyheri* leaves in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	1503.2	136.650		
Treatment	7	862.9	123.274	1.02	0.43
Error	77	9327.8	121.140		
Total	95	11693.9			

Appendix 5.14 Analysis of variance for sodium accumulation in *Mimusops zeyheri* leaves in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	7.2386	0.65806		
Treatment	7	4.1403	0.59147	0.85	0.54
Error	77	53.3896	0.69337		
Total	95	64.7686			

Appendix 5.15 Analysis of variance for zinc accumulation in *Mimusops zeyheri* leaves in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after the treatments.

Source	DF	SS	MS	F	P
Replication	11	0.21101	0.01918		
Treatment	7	0.11566	0.01652	0.61	0.74
Error	77	2.07202	0.02691		
Total	95	2.39869			