Using plant growth regulators and Vesicular Arbuscular Mycorrhiza to improve growth of the slow growing indigenous *Mimusops zeyheri* seedlings and accumulation of essential nutrient elements

By

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MINI-DISSERTATION submitted in partial fulfilment of the requirements for the degree of

Master of Science in agriculture (Horticulture)

FACULTY OF SCIENCE AND AGRICULTURE

(School of agricultural and environmental sciences)

At the

UNIVERSITY OF LIMPOPO

SUPERVISOR: PROFESSOR P.W. MASHELA

2017

TABLE OF CONTENTS

DECLARATION

I declare that the mini-dissertation hereby submitted to the University of Limpopo, for the degree of Master of Science in Agriculture (Horticulture) has not previously been submitted by me for a degree at this or any other University; that it is my work in design and in execution, and that all materials contained herein had been duly acknowledged.

Radzuma M.G (Ms) Date

DEDICATION

To my son Tshegofatso Radzuma and mother Mokgadi Jane Radzuma.

ACKNOWLEDGEMENTS

I would like to give thanks to the God of possibilities, for making it possible to for me to carry out this project successfully. Indeed, nothing is impossible when the Lord God almighty is in charge. Thank you for giving me good health, strength and ability to work hard and consistently throughout this project. Truly you deserve the honour and glory. To my beloved parents, Mrs Mokgadi Jane Radzuma and Mr. Nkoshilo Phineas Radzuma, words will never be enough to express my gratitude. You have been my pillar of strength and supported me from the day I was brought into this world till now – for that I am endlessly grateful. I would also like to appreciate my brothers Simon, Alfred and Thomas for their support and patience throughout my study life, may the God Almighty continue to bless you in all areas of your lives. To my son Tshegofatso, may all my achievements be a motivation for you to be a better person in life. Kea leboga, Ditlou. I would also like to appreciate the mutual support received from everyone who assisted me to complete this study, for their hardworking, training and assistance in various areas of the project and for also giving me strength to keep on moving forward. To my supervisor, Professor P.W. Mashela, thank you for your patience, advices, guidance, support and for being my academic parent. Thank you for steering this ship to the right direction. The scientific, statistical knowledge and the life lessons I have received through your mentorship will forever be treasured and used effectively and efficiently. I also appreciate the assistance received from Dr Z.P. Dube and Dr Y.M. Maila all the assistance, suggestions and taking interest in my research field. I would also like to thank all my colleagues at the Green Technologies Research Centre (GTRC); it was an honour to work with you side by side. The assistance I received from the GTRC employees, Ms

S.M. Seabela, Ms M.A. Mawasha, Ms R.S. Mawasha, Mr M.K. Ralefatana, Mr L.T. Letswalo and Mr E.M. Letswalo is highly appreciated. I would like to appreciate the financial support I have received from the National Research Foundation of South Africa and the Agricultural Research Council-Universities Collaboration Centre' for scholarship and research, respectively. I would also like to appreciate my fellow postgraduate students in the School of Agricultural and Environmental Sciences for all the assistance I had received, one way or the other, during the execution of this study.

LIST OF LEGENDS

LIST OF TABLES

LIST OF FIGURES

LIST OF APPENDICES

8

ABSTRACT

The evergreen Transvaal Red Milkwood (*Mimusops zeyheri*) had been targeted as an urban- and rural-greening tree in Limpopo Province due to its excellent nutritional, medicinal and aesthetic attributes. However, in various surveys, the slow growth characteristics of this plant species were ranked as the highest demotivator for adoption. Slow growth in *M. zeyheri* limits the potential for domestication and commercialisation of the tree and also restricts the marginal farmers to adopt the tree as a source of income. Various sustainable products are being used for various purposes in plant production including promotion of plant growth. The objective of this study was to determine the effect of indole-3-acetic acid (IAA), gibberellic acid (GA3), 6-benzylaminopurine (6-BAP) and vesicular arbuscular mycorrhiza (VAM) each on growth and accumulation of nutrient elements, respectively, in *M. zeyheri* seedlings. Six-month old seedlings were transplanted into 30-cm diameter plastic pots containing steam-pasteurised loam soil and river sand at 3:1 (v/v) ratio. Stock solutions of IAA, GA3 and 6-BAP were prepared in 100 mL plastic containers, with 102.2 mg of each being first dissolved in 2 mL methanol and then diluted in 98 mL distilled water. Treatments, 0.0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 and 51.2 $mg.mL^{-1}$ for each plant growth regulator, were arranged in a randomised complete block design, with five replications. At 84 days after application of treatments, plant height, stem diameter, leaf number, leaf area, petiole length, dry root mass and dry shoot mass were collected. The IAA concentrations had significant effects on stem diameter and leaf number. Stem diameter and leaf number each over increasing concentration of IAA exhibited positive curvilinear quadratic relations, with the relationships explaining the model by 98 and 94% in respective variables. Leaf

number and plant height over increasing GA3 concentrations exhibited positive curvilinear quadratic relations, with the relationships being explained by 94 and 92% of the model, respectively. Leaf number, dry root mass, stem diameter and dry shoot mass over increasing 6-BAP concentrations exhibited positive curvilinear quadratic relations, with the relationships being explained by 97, 98, 94 and 87%, respectively, of the model. Vesicular arbuscular mycorrhiza had no significant effects on all plant variables and accumulation of selected essential nutrient elements in *M. zeyheri* seedlings. In conclusion, the use of IAA, GA3 and 6-BAP as foliar sprays on *M. zeyheri* demonstrated for the first time the existence of density-dependent growth (DDG) patterns in various organs on *M. zeyheri*, with the possibility of using the optimum concentrations to promote growth of this plant species, *M. zeyheri*. Plant growth regulators and VAM had no effect on nutrient content of *M. zeyheri* seedlings. Also, VAM had no significant effect on plant growth variables. Therefore, plant growth regulators could be used to increase the growth of the slow growing *M. zeyheri* seedlings.

CHAPTER 1
RESEARCH PROBLEM

1.1 Background

Slow growth in indigenous trees is a challenge as it results in prolonged waiting period prior to reproduction during which time various production inputs are required. Slow growing plants occupy the land for an extended period of time without reproduction, while the occupied land could have been used for other agricultural practices to generate income. Slow growth of indigenous plants particularly, the Transvaal red milkwood (*Mimusops zeyheri),* is a disadvantage to rural entrepreneurs who would like to make a living from the tree (Mashela *et al*., 2013a). Fruit of *M. zeyheri* provide the highest vitamin C among most edible fruits, ranging from 50-80 $mg.g^{-1}$ fresh fruit, which is much more than that in guava fruit, which produce vitamin C content of approximately 20 $mg.g^{-1}$ fresh fruit (Venter and Venter, 1996). Fruit of this tree are harvested from September through November, when exotic fresh fruits are scarce. A team of researchers in Limpopo Province, South Africa, had been researching and developing *M. zeyheri* trees for commercialisation. However, the slow growth of *M. zeyheri* had been limiting its economic potential adoption in marginal communities. Generally, the intensive exploitation of this fruit tree had since led to its extinction in some parts of South Africa.

Exposure of plants to unfavourable environmental and, extreme climatic conditions in particular, could produce stress on plants, resulting in stunted plant growth. South African soils are also low in phosphorus (P), and due to widespread high soil pH; P could exist in unavailable forms (Mangesha, 2008). Plant growth regulators (PGRs) have been an important component of best agricultural practices, but most of their

uses had been restricted to high value horticultural crops, with a few exceptional uses in indigenous plant species (Abdelgadir and Johnson, 2009). Hence, the research intended to investigate the potential effects of using synthetic plant growth regulators and mycorrhiza in improving growth of *M. zeyheri* trees.

1.2 Problem statement

Slow growth of *M. zeyheri* starts from seed germination through the seedling phases to maturity. Mkhabela (2003) demonstrated that seed germination of *M. zeyheri* took as long as eight weeks, with the optimum occurring at six weeks. *Mimusops zeyheri,* like other fruit trees, has clearly defined shoot flushes, which alternate with root flushes. Generally, when shoot flushes occur, root growth is dormant, *vice versa* (Hartmann *et al*., 1988). In *M. zeyheri,* two shoot flushes were observed, one in winter (May-July) and the other during flowering, which occurred at fruit ripening from late spring (August-September) to early summer (October-December) (Mashela *et al*., 2013a). Under natural conditions, *M. zeyheri* trees grow under marginal conditions, particularly in rocky places, where there was a diversity of limiting factors in terms of moisture and nutrient elements availability. The fruit tree is believed to be highly drought tolerant (Mashela *et al*., 2013a).

1.3 Rationale of the study

Mimusops zeyheri trees have high nutritional and medicinal values, but had been overlooked because they were previously viewed as weeds (Mashela and Mollel, 2002). The tree appears to be predisposed for flourishing under marginal conditions (Mashela *et al*., 2013a). The use of bio-stimulants such as plant growth regulators and vesicular arbuscular mycorrhiza (VAM) improve the availability of phosphorus

thereby promoting plant growth. Information on improved growth of *M. zeyheri* seedlings would promote the use of *M. zeyheri* as source of vitamin C supplements, particularly during the period when fresh fruits are in scarce supply. This tree is classified as an evergreen tree and therefore, could serve invasions aesthetic roles. Additionally, the domestication of *M. zeyheri* would avoid extinction and over utilisation of this heritage plant in South Africa as articulated in the greening policies.

1.4 Purpose of the study

1.4.1 Aim

The aim of the study was to develop technologies that could improve the slow growth rates in *M. zeyheri* seedlings.

1.4.2 Objectives

1. To determine the optimum level at which plant growth regulators would enhance growth and accumulation of essential nutrient elements in *M. zeyheri* seedlings.
2. To investigate the potential effects of vesicular arbuscular mycorrhiza on growth and accumulation of essential nutrient elements in *M. zeyheri* trees.

1.5 Hypotheses

1. The optimum level at which plant growth regulators would enhance growth and accumulation of nutrient elements in *M. zeyheri* trees can be established.
2. Vesicular arbuscular mycorrhiza would play a role on growth and accumulation of essential nutrient elements in *M. zeyheri* seedlings.

1.6 Scientific contribution

Findings of the study would expand knowledge and provide the opportunity to propagate this indigenous plant as a future crop. Improved growth of *M. zeyheri* seedlings would encourage commercialisation of this plant species for its nutritional and aesthetic attributes.

1.7 Reliability, validity and objectivity

Reliability of data would be based on statistical analysis of data at the probability level of 5%. Validity would be achieved by testing treatments at various levels. Objectivity would be achieved by ensuring that the results were discussed on the basis of empirical evidence, thereby, eliminating all attributes of subjectivity (Leedy and Ormrod, 2005).

1.8 Bias

Bias was minimised by ensuring that the experimental error in each experiment was contained through sufficient replications. Also, treatments were assigned at random within the selected research designs (Leedy and Ormrod, 2005).

1.9 Structure of mini-dissertation

Chapter 1 would focus on description of the research problem, followed by Chapter 2 that addressed work done on the research problem. Chapters 3 and 4 would focus on addressing Objectives 1 and 2, respectively. In the final Chapter (Chapter 5), findings in all Chapters would be summarised and integrated to provide the significance of the findings and recommendations with respect to future research,

followed by conclusions. The citation in text and listing of references adopted the Harvard style as approved by the Senate of the University of Limpopo.

CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Indigenous plants, adapted to semi-arid and arid areas, are usually slow growers (Venter and Venter, 1996). *Mimusops zeyheri* is indigenous to the northern parts of the former Transvaal Province of South Africa (Mashela *et al*., 2013a), which is currently most of the Limpopo Province. The region is characterised by semi-arid climate with low rainfall, which occurs mainly during hot summers (October–December). Generally, slow growing plants invest their growth strategies in the development of the deep root systems (Krieg, 1983). Slow growth in fruit trees limits the commercialisation and domestication for local and international markets, which could affect the competitiveness of indigenous fruits on fresh produce markets. Indigenous fruit crops are gaining popularity within the agricultural industry and efforts to improve their growth rates are underway (Garner, 1991). Plant growth regulators (PGRs) and vesicular arbuscular mycorrhiza (VAM) had been used successfully in agriculture to manipulate plant growth and development. However, the effects of PRG and VAM on growth of *M. zeyheri* seedlings had not been documented.

2.2 Work done on problem statement

2.2.1 Propagation of *Mimusops zeyheri*

Mimusops zeyheri is propagated mainly sexually through seeds (Venter and Venter, 1996). However, sexual propagation has inherent challenges of producing seedlings that are not true-to-type (Hartmann *et al*., 1988). In addition, the seedlings emerge after an extended period, while the seeds have a short-lifespan (Venter and Venter,

1996). Attempts were made to enhance seed germination through scarification using sulphuric acid, hot water and mechanical techniques (Mkhabela, 2003). The results demonstrated that seed germination under all treatments occurred mainly from five to six weeks. Hot water treatment had moderate effects on seedling emergence, whereas and acid scarification reduced seedling emergence (Mkhabela, 2003).

Using shoot tips, Maila (2005) developed tissue culture protocols for asexually propagating *M. zeyheri* trees. The technology resulted in uniform and true-to-type plantlets that could be used in the commercialisation of the plant. The study eliminated any genetic variation by producing clonal rootstocks. However, the process of shoot proliferation, initiation of roots and the subsequent weaning in the greenhouse occurred after an extended period for at least 31 weeks (Maila, 2005). The low multiplication rates confirmed the slow growth status in this plant species. The protocols were improved using bio-char to shorten the extended period by half (Maila, 2005).

2.2.2 Characteristics of *Mimusops zeyheri*

Mimusops zeyheri trees are evergreen and could grow to the height of 15 m under favourable conditions (DAFF, 2012; Mashela *et al*., 2013a; Venter and Venter, 1996). The tree has non-aggressive root systems which enable growth under marginal conditions (Van Wyk, 1974). However, the non-aggressive root systems could limit the potential of *M. zeyheri* trees to absorb sufficient moisture and nutrients, which promote growth. Venter and Venter (1996) noted that *M. zeyheri* trees had attributes which had been associated with drought-tolerance, such as heavily waxed leaves. Generally, drought-tolerant plants are slow growers, since

plant growth is partitioned in favour to root growth as opposed to shoot growth (Krieg, 1983). *Mimusops zeyheri* leaves are glossy, dark green and are borne on a spreading crown (DAFF, 2012). *Mimusops zeyheri* organs contain high latex content and due to hairy shoots and young leaves, they appear to be leathery (Venter and Venter, 1996). Young leaves and twigs are covered in rusty-brown, velvety hairs, whereas mature leaves are dark and pale green on the dorsal and ventral sides, respectively. The creamy white flowers, originally covered by brown petals, are strongly scented and borne in clusters, whereas the yellow to deep orange fleshy fruit with a glossy brittle skin, are borne on lateral ends of the previous year shoots (Mashela *et al*., 2013a). In pomology, two types of fruit bearing had been identified, namely, lateral- and tip-bearing habits. Generally, in lateral-bearing fruit trees, tree pruning is mandatory, whereas in tip-bearing trees, pruning is not essential for promoting yield.

The *M. zeyheri* trees have a strong alternate fruit bearing habit, unless they are properly irrigated and fertilised throughout the year, but especially after the winter shoot flushes (Mashela *et al*., 2013a). *Mimusops zeyheri* fruit contain shiny brown to black pigmented seeds, whereby each fruit could carry one to two seed (Motlhanka *et al.,* 2008), with other fruit containing three seeds (Mashela *et al*., 2013a), depending on the area of origin of the trees. Historically, the fruit carpus was dried and ground for use as food during wars and long journeys (Mashela *et al.*, 2013a).

The tree bark is grey and rough on mature trees or dark brown and smooth in small trees (Venter and Venter, 1996). Upon emergence, the epicotyls are etiolated and should be hardened-off to brown colours prior to transplanting into plastic bags. The

bark of *M. zeyheri* trees also contains latex (Janick and Paull, 2008). This tree had been considered to be a multipurpose tree, since it could be used for general purpose timber, as food supplements and aesthetic purposes (Mashela *et al*., 2013a). Apparently, as the tree gains popularity, other uses would be realised.

2.2.3 Natural habitat

Mimusops zeyheri flourished in marginal areas in rocky hillsides, kloof and riverine vegetation (Venter and Venter, 1996). In South Africa the tree is found in the wild in Limpopo, Gauteng, North West, Mpumalanga, KwaZulu-Natal and Free State Provinces (Venter and Venter, 1996). This indigenous tree grows best at temperature ranges from 12 to 25ºC, with the average annual rainfall of 464 mm and is believed to have some potential to withstand moderate frost (Venter and Venter, 1996).

Soil type plays a major role in the productivity of *M. zeyheri* seedlings. Most environmental factors could have an effect on the morphology of the *M. zeyheri* (Ledwaba, 2008). Incidentally, sandy soils were observed to reduce productivity of *M. zeyheri* seedlings, whereas loam and clay soils had no significant effect on different parameters (Ndhukula, 2006). Similarly, in a pot study conducted to observe the performance of *M. zeyheri* seedlings in three different soil types, it was observed that relative to loam, clay soil increased leaf growth by 19 to 20% and 9 to 58% at 9 and 12 months, respectively, after transplanting (Mashela *et al*., 2013a). Relative to loam, sandy soil improved leaf and petiole growth by 10 to 88% and 21 to 49% at 9 to 12 months, respectively, after transplanting (Mashela *et al.*, 2013a). The results of that study suggested that *M. zeyheri* trees could be grown successfully in clay and

sandy soils, where the majority of marginal communities in Limpopo Province had been historically settled.

2.2.4 Influence of salinity

Nchabeleng (2004) investigated the effect of potassium chloride, sodium chloride, sodium carbonate and calcium chloride salts on growth of *M. zeyheri* seedlings for two seasons, namely, summer and winter, under greenhouse conditions. Sodium carbonate reduced chlorophyll content and increased soil pH during both growing seasons, whereas the other salts increased electrical conductivity. Generally, sodium carbonate impacted negatively on growth of *M. zeyheri* seedlings through binding with calcium to form calcium carbonate (Nchabeleng, 2004), with calcium becoming unavailable to plants. It could be concluded that growth of *M. zeyheri* trees was improved under chloride salinity, whereas growth was inhibited under carbonate salinity. It was evident that *M. zeyheri* required sodium ion, which is an essential element in C4 plants (Nchabeleng, 2004). However, it is not clear whether *M. zeyheri* is a C3 or a C4 plant.

2.2.5 Genetic variability

The genetic diversity of *M. zeyheri* in Limpopo Province was investigated using a cluster analysis technique through the unweighted pair group method with arithmetic means separating individual *M. zeyheri* bans into distinct clusters with average genetic similarity estimates ranging from 47 to 89% (Maputla, 2002). Approximately 91% generic variability occurred among *M. zeyheri* populations in Limpopo Province, with within population variation being at 9% (Maputla, 2002). The report provided

information on genetic variability and partitioning of genetic diversity, which could help in species identification and classification within the breeding programmes.

Legend 2.1 Matured *Mimusops zeyheri* tree.

2.2.6 Functional nutrients in *Mimusops zeyheri* fruit

Mimusops zeyheri fruits have high vitamin C content which could be used as a supplement to eliminate diseases associated with vitamin C deficiencies (Venter and Venter, 1996). *Mimusops zeyheri* trees could close vitamin C deficiency gap during spring since the trees produce fruits from mid-spring to mid-summer when most indigenous and exotic fruit tress produce their fruits in summer (Mashela and Mollel, 2002). According to Chivandi (2012), *M. zeyheri* seeds were observed to have a high vitamin E content, which ranged from 0.50 to 48.7 µg g^{-1}, whereas Venter and Venter (1996) reported that fruit contained 50-80 $mg.g^{-1}$ fresh fruit. Seed oil in this plant species contains glutamic acid at the highest concentration, when compared to other amino acids, which had 1.38% of the crude protein content of total fat. *Mimusops zeyheri* seed oil also contains oleic acid and the essential fatty acids, linoleic acid and α-linoleic acid (Chivandi, 2012). The fatty acid profile of seed oil in this plant species did not have squalene, which is a fat soluble anti-oxidant that is produced

naturally during cholesterol synthesis. The findings demonstrated that *M. zeyheri* seeds could further be exploited as source of oleic acid, but due to low crude protein concentration the seeds could not serve as a potential source of protein concentrate in animal feeds (Chivandi, 2012).

2.2.7 Regional effect

Accumulation of essential nutrient elements in soil and leaves of *M. zeyheri* trees after fruiting differed with location and harvest time throughout the three locations (Ledwaba, 2008). At Cheunespoort, leaf copper and phosphorus were not affected, whereas soil copper increased by 19%. Accumulation of zinc in leaves was observed in high quantities in Chuenespoort after fruiting. There was an observed decrease in leaf magnesium and leaf manganese in *M. zeyheri* trees. *Mimusops zeyheri* trees in Bochum had an increase in accumulation of leaf manganese, leaf phosphorus and soil zinc, whereas there was a decrease in soil copper, leaf zinc and leaf magnesium after fruiting.

At Sekgosese, *M. zeyheri* trees displayed an increase in leaf manganese and a decrease in leaf magnesium and copper, whereas there was no change in zinc and phosphorus. From these findings it can be concluded that soil conditions and different environmental and climatic conditions across the three regions could play a major role on availability of essential nutrient elements in soil and leaves of *M. zeyheri* seedlings. Location and time were demonstrated to play a major role on accumulation of essential nutrient elements of *M. zeyheri* seedlings. Soil pH and soil electrical conductivity were observed to have an effect on *M. zeyheri* trees across Chuenespoort, Bochum and Sekgosese, which are climatically different

(Ledwaba, 2008). Electrical conductivity values were significantly higher in Chuenespoort when compared to those in Bochum and Sekgosese, whereas soil pH was significantly higher in Bochum when compared to Sekgosese and Chuenespoort (Ledwaba, 2008).

2.2.8 Pests on *Mimusops zeyheri*

Claims abound that the aboveground organs of *M. zeyheri* trees are pest-free due to the high latex content, whereas the fruits were host to an unidentified fruit borer (DAFF, 2012). *Mimusops zeyheri* trees were observed to be resistant to the highly injurious root-knot (*Meloidogyne* species) nematodes, which is a pest to a wide range of crops (Mashela *et al*., 2013b). Generally, exposed to various levels of *M. incognita,* it was observed that the nematode was not able to feed and reproduce on roots of *M. zeyheri* seedlings. However, plant resistance to pathogens could be lost when the tree is stressed either through environmental conditions or attack by certain foliar pests. Under greenhouse conditions, *M. zeyheri* seedlings attracted aphids and mealybugs, which was a concern since aphids carry viruses which could result in reduced yields, while both pests release copious quantities of honeydew, which could result in failure of nematode resistance (Pofu *et al*., 2011).

Mimusops zeyheri fruit were recently shown to be a host to the Mediterranean fruit fly (*Ceratitis capitata*). A study was conducted after observing symptoms of the Mediterranean fruit fly on *M. zeyheri fruits*, whereby *M. zeyheri* fruit were collected and kept in plastic pots containing steam pasteurised growing media covered with mesh sheath for 16 to 21 days (Dube *et al*., 2016). The findings of the study led to the conclusion that *M. zeyheri* fruits were hosts to *C. capitata*. *Mimusops zeyheri*

trees, with their fruit occurring in spring to mid-summer could serve as a reservoir of *C. capitata* for the tropical and subtropical fruit industries in South Africa (Dube *et al*., 2016).

2.3 Improving growth in other plant species

All best agricultural practices are intended to improve plant growth and productivity. In this literature review, only plant growth regulators and VAM were reviewed.

2.3.1 Plant growth regulators and selective uses in horticulture

Plant growth regulators (PGRs) are chemical compounds that modify plant physiological processes (Harms and Oplinger, 1988). The PGRs had been used over the years for their beneficial qualities of either stimulating or inhibiting plant growth through cell division, cell enlargement and cell differentiation. Synthetic PGRs are widely used commercially to facilitate physiological processes such as flowering, fruit development and ripening. Post bloom application of benzyl adenine had been documented in the successful thinning of young fruitlets of mature Empire apple trees (Elfving and Cline, 1993). The product, when used for thinning of apple fruitlets, it produced better results individually than when mixed with naphthalene acetic acid or carbaryl (Elfving and Cline, 1993).

A combined use of auxins, gibberellins and cytokinins was observed to improve fruit production in kiwifruit when applied after flower development (Lorenzo *et al*., 2007). Generally, when plant growth regulators were applied together, combining two or all three, there could be positive synergistic effects that increased fruit size, growth, fruit diameter and length (Lorenzo *et al*., 2007). Gibberellic acid was successfully used in

improving height of tamarind (*Tamarindus indica*), whereas there were no significant treatment effects in variables such as stem diameter, root length, shoot and root dry mass and the dry root mass to dry shoot mass ratio (Dantas *et al*., 2012).

An increase in the number of branches was observed in both field and shade house conditions when *Jatropha curcas* plants were treated with foliar application of BA at 3.0, 6.0, 9.0 and 12.0 mM (Abdelgadir and Johnson, 2009). A significant increase in branch number and leaf number was observed at the concentration of 15 Mm and 12 mM in the greenhouse and field conditions, respectively (Abdelgadir and Johnson, 2009). However, plant height was reduced under greenhouse conditions in all tested concentrations. Similar results were observed in baby rubber plant (*Peperomia obtusifolia)* plants treated with BA where there was a significant increase in branch number, but a decrease in plant height was observed, with a rosette of leaves (Henny, 1986). The latter suggested that BA application in *P. obtusifolia* induced zinc deficiency.

Plant growth regulators are used mainly in plant tissue culture for root and shoot initiation in explants. Nishiwaki *et al*. (2000) observed the formation of somatic embryos directly from the epidermal cells of carrot (*Daucus carota*) seedlings in the medium containing ABA produced somatic embryos in sunflower (*Helianthus annuus*) plants (Charriere and Hahne, 1998). Maila and Mashela (2009) observed a highly significant effect on shoot regeneration and increased root number in *Cucumis africanus* plants treated with BAP and IBA with treatment levels 0.0, 0.2, 0.4, 0.6, 0.8 and 1.0 $mg.L^{-1}$ under *in vitro* conditions. The optimum levels at which stimulated shoot number were observed occurred at 0.80 $mg.L^{-1}$, whereas that which was

required for regenerating the optimum number of roots was at 0.50 mg.L^{-1} IBA in full strength Murashige and Skoog (MS) medium.

2.3.2 Use of vesicular arbuscular mycorrhiza fungi in agriculture

Vesicular arbuscular mycorrhiza (VAM) fungi had been documented to form a symbiotic relationship with plant roots aiding in environmentally stressed soil conditions such as drought, salinity and water logging, which has direct impact on plant growth parameters (Sharma and Yadav, 2013). Plant-VAM symbiosis also improves nutrient absorption, especially in phosphorus deficient soils. According to Berea and Jeffries (1995), VAM fungi obtain carbon compounds and essential nutrient elements from the symbiotic plant roots and supply the plants with immobile nutrient elements from the soil solution.

Improved growth of semi-wood olive trees was observed when the plants were treated with VAM fungi (Sidhoum and Fortas, 2013). Powell and Nakrishnan (1986) observed an increase in plant height on hardwood cuttings of kiwifruit treated with VAM and phosphorus. A significant difference in shoot length was observed at 28 days, but at 39 days there were no significant differences in shoot length. Improved growth on VAM-infested plants had often been related with the more efficient uptake of nutrients, especially phosphorus from soil (Bolan, 1991). Jayne and Quigley (2013) observed an improved growth of both annuals and perennials through symbiosis, with perennials responding more favourably to colonisation than annuals. Plants inoculated with VAM had better growth and reproductive response than control plants.

The VAM colonised roots of cucumber (*Cucumis sativus*), resulting in better plant growth and yield (Ortas, 2010). The efficacy of VAM varies with plant species since the application depends on plant size. Generally, bigger plants require higher levels of VAM when compared to small horticultural crops (Dames, 2016: Personal com.). The effect of VAM could be observed after an extended period of time, mainly because the plant at early exposure periods had failed to form symbiosis with the VAM. According to Borkowska (2008), VAM symbiosis could take a long period since the fungi can utilise the soil nutrients during the first year which were supposed to be supplied to the tree and as a result the tree does not benefit from the symbiotic relationship.

However, the VAM could improve plant growth in nutrient deficient soils. The VAM has the ability to acquire nutrients and moisture from long distances where plant roots are unable to reach through the developed fungal structures. Incidentally, VAM in a dose of 1000 units per plant and a half dose of fertilisers without phosphorus had a significant influence on vegetative growth of plum trees expressed by the trunk cross-sectional area, whereas there was no significant effect on vigour of growth of sour cherry trees treated with VAM (Świerczyński and Stachowiak, 2010). Therefore, it appears that the relationship could benefit organs in plants.

Application of VAM could effectively improve growth of stressed plants. When applied on drought stressed Orchard grass (*Dacrylis glomerata*) plants, VAM showed a significant increase in shoot dry weight, tiller weight and the number of leaves relative to the untreated control (Kyriazopoulos *et al*., 2014). Salt stress in plants could be a major challenge and new salt tolerant crops are being bred in order to

minimise crop losses. Aliasgharzadeh *et al*. (2001) observed that the VAM spores were not significantly reduced by soil salinity, but an increase in spore numbers was observed (mean of 100 in 10 g soil). The use of VAM therefore could enable the survival of trees under salt stress conditions, which would allow farmers an opportunity to grow trees of their choice.

2.4 Work not yet done on problem statement

Work on improving growth in *M. zeyheri* seedlings under greenhouse conditions using PGRs and VAM had not been documented. Indigenous fruits are greatly consumed in rural areas, but research carried out on these crops is scanty, mainly due to lack of interest in *M. zeyheri* for commercialisation due to its slow growing traits. Based on the fact that no research had been conducted on about improving the inherent slow growth of *M. zeyheri* using bio-stimulants, it was imperative that an assessment be made using PGRs and VAM.

CHAPTER 3
RESPONSE OF *MIMUSOPS ZEYHERI* SEEDLINGS TO PLANT GROWTH REGULATORS

3.1 Introduction

Slow tree growth of agricultural crops is an undesirable trait since trees occupy the land for an extended period of time without producing fruits, while costs are incurred for maintenance of the crop (Venter and Venter, 1996). Commercialisation and domestication of indigenous trees is limited by slow growth which results in extinction of the trees since they are viewed as weeds. Indigenous crops are documented to contain high nutritional benefits and medicinal value but slow tree growth limits the use of such crops for economic purposes by local farmers. Slow tree growth results in decreased number of consumable crops, over exploitation of natural resources and extinction of known food sources.

Slow tree growth can be as a result of changes in hormonal balance in response to environmental stresses since the tree responds by frequently producing more abscisic acid and less cytokines (Chapin *et al*., 1988). According to Chapin (1991), hormonal changes triggers reduced growth in response to environmental stress and low availability of resource supply which activates the stress response system. Plant growth regulators are the most important component of agricultural production but their use is limited to high value horticultural crops as opposed to field crops (Abdelgadir and Johnson, 2009). The objective of this study was to determine the effect of indole-3-acetic acid (IAA), gibberellic acid (GA3) and 6-benzylaminopurine (BAP) each on growth and accumulation of essential nutrient elements of *M. zeyheri* seedlings.

3.2 Materials and methods

3.2.1 Growth conditions and preparation of materials

The study was conducted under greenhouse conditions at the Green Technologies Research Centre (GTRC), University of Limpopo, South Africa (23°53'10"S, 29°44'15"E). Three separate experiments were conducted with IAA, GA3 and 6-BAP as treatments (Legend 3.1-Legend 3.6). *Mimusops zeyheri* seeds were extracted from fresh fruits and shade dried. Two-month old seedlings were transplanted into 20-cm diameter plastic pots containing steam pasteurised loam soil and sand growing mixture at 3: (v/v) ratio. The experiments were laid on greenhouse benches with the inter and intra row spacing of 30 cm (Legend 3.1-Legend 3.6). Stock solutions of indole-3-acetic acid (IAA), gibberellic acid (GA3) and 6-benzylaminopurine (6BAP) were prepared in 100 mL plastic containers whereby 102.2 mg material was dissolved in 2 mL of alcohol (methanol) and 98 mL of distilled water was added into the solution. A pipette was used to draw different concentrations from the container into the spray bottles, which were topped up with 100 appropriate volumes of distilled water mixed with a sticker and wetter. The plants were treated weekly with foliar application using 20 mL test solution per seedling. The foliar sprays were applied in such a way that the test solution covered the entire plant in direct contact of leaves, stem and meristem.

3.2.2 Experimental design

Indole-3-acetic acid trials: The experiment was carried out under greenhouse conditions with ten treatments, namely, 0.0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 and 51.2 $mg.mL^{-1}$ PRGs arranged in a randomised complete block design, with five replications.

Legend 3.1: Effect of indole-3-acetic acid on growth of *Mimusops zeyheri* seedlings under greenhouse conditions.

Gibberellic acid trials: The experiment was laid in a randomised complete block design under greenhouse conditions with ten treatments, namely, 0.0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 and 51.2 $mg.mL^{-1,}$ with five replicates.

Legend 3.2: Effect of gibberellic acid on growth of *Mimusops zeyheri* seedlings under greenhouse conditions.

6-benzylaminopurine trials: The experiment was conducted under greenhouse conditions with ten treatments, namely, 0.0, 0.2, 0.4, 0.8, 1.6, 3.2, 6.4, 12.8, 25.6 and 51.2 $mg.mL^{-1}$, replicated five times.

Legend 3.3: Effect of 6-benzylainopurine on growth of *Mimusops zeyheri* seedlings under greenhouse conditions.

3.2.3 Cultural practices

NPK fertilisers were applied a day after transplanting with 5 g of 2:3:2 (26)+ 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 fertilisers was applied twice a month 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). Plants were irrigated every other day with 250 mL tapwater. Pest management was achieved through monitoring. Aphids were observed and managed with Malasol and weeding of plants that attracts aphids.

3.2.4 Data collection

Plant variables: At 84 days after initiation and application of treatments, chlorophyll content was measured from three mature leaves per plant using chlorophyll meter (Minolta Spad-502). Plant height was measured from the soil surface to the tip of the flag leaf and numbers of leaves per plant were counted. Stem diameter was measured 2 cm from the distal end of stem before oven drying for 70 ^{o}C for 72 h and weighed. Root system were removed from pots, immersed in water, blotted dry and weighed. Area of the leaf was obtained through measuring the leaf length and leaf width (AM350 Portable Leaf Area Meter). Petiole length was also measured.

Nutrient analysis: At 84 days after initiation and application of treatments, plants were severed 2 cm from the soil surface and oven dried at 70 ^{o}C for 72 h. Dried leaves were separated from the stem and ground into powder form using pestle and mortar. The powdered sample was digested in 5% nitric acid, which was then mixed with a vortex meter. The samples were then incubated in a warm water bath for an hour at 95 ^{o}C, cooled at room temperature and filtered, with the container covered with a foil (SW-846 EPA Method 3050B). Samples were then submitted to Limpopo Agro-food Technology Station (LATS), where they were quantified using the Inductively coupled plasma atomic emission spectrometry (ICP) for mineral elements.

3.2.5 Data analysis

Data for plant variables and mineral nutrient elements were subjected to analysis of variance using SAS (SAS Institude 2008). Discrete data for leaf number was 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 test was used to separate means which were significant at 5% level of probability. Significant plant variables were subjected to lines of the best fit, with quadratic equations generated used to compute optimum concentration values. Unless stated otherwise, treatment effects were discussed at 5% level of probability.

3.3 Results

3.3.1 Effects of indole-3-acetic acid

Effects of IAA concentrations were highly significant ($P \leq 0.01$) on leaf number and stem diameter, but had no effect on plant height, dry shoot mass, dry root mass, chlorophyll content, leaf length, leaf width and petiole length. Treatments contributed 55 and 40% in TTV of leaf number and stem diameter, respectively (Table 3.1). Relative to untreated control, concentrations of IAA decreased leaf number from 18 to 1% and stem diameter was increased by 7 to 17% (Table 3.2). The optimum values for the stem diameter and leaf number were attained at 16.6 and 12.0 $mg.mL^{-1}$, respectively. (Table 3.3)

The density-dependent growth patterns (DDG) had stimulation effect at low concentrations, whereas at high concentrations there was inhibition effect (Table 3.2). Using the relation $x = -b_1/2b_2$, the optimum level at which growth was enhanced 14.0 $mg.mL^{-1}$ distilled water for stem diameter (Table 3.3). Plant variables over increasing IAA exhibited negative quadratic relations for leaf number with 98% of the model being explained by the equation (Figure 3.1), whereas there was a positive quadratic relation for stem diameter with the model being explained by 98% (Figure 3.2). There was no optimum value for leaf number due to reduced growth (Figure 3.1). Foliar application of

IAA had no significant difference on leaf nutrient content of *M. zeyheri* seedlings. Treatments contributed 48, 22, 27, 24, 34 and 20% in total treatment variation of magnesium, phosphorus, calcium and zinc, respectively (Table 3.4).

Legend 3.4: Effect of indole-3-acetic acid on growth of *Mimusops zeyheri* seedlings under greenhouse conditions.

Table 3.1 Partitioning of mean sum of squares for leaf number and stem diameter affected of *Mimusops zeyheri* as affected by indole-3-acetic acid.

		Leaf number		Stem diameter	
Source	DF	MS	TTV (%)	MS	TTV (%)
Rep	4	0.00617	27	0.22881	42
Treatment	9	0.01263	55***	0.21473	40**
Error	36	0.00422	18	0.09839	18
Total	49	0.02302	100	0.54193	100

**Significant at P ≤ 0.05, ***Significant at P ≤ 0.01.

Table 3.2 Effect of indole-3-acetic acid on leaf number (LN) and stem diameter (STD) of *Mimusops zeyheri* seedlings.

Treatment (%)	Leaf number[y]	Impact (%)	STD (mm)	Impact (%)[z]
0.0	78.49[a]	−	78.00[a]	−
1.6	76.13[a]	−3	83.20[b]	7
3.2	77.15[b]	−2	85.80[b]	10
6.4	78.07[c]	−1	88.80[b]	14
12.8	78.68[a]	0	91.00[c]	17
25.6	75.23[e]	−4	86.20[b]	11
51.2	64.02[f]	−18	64.00[d]	−18

[y]Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

[z]Impact (%) = [(treatment/control) –1] × 100.

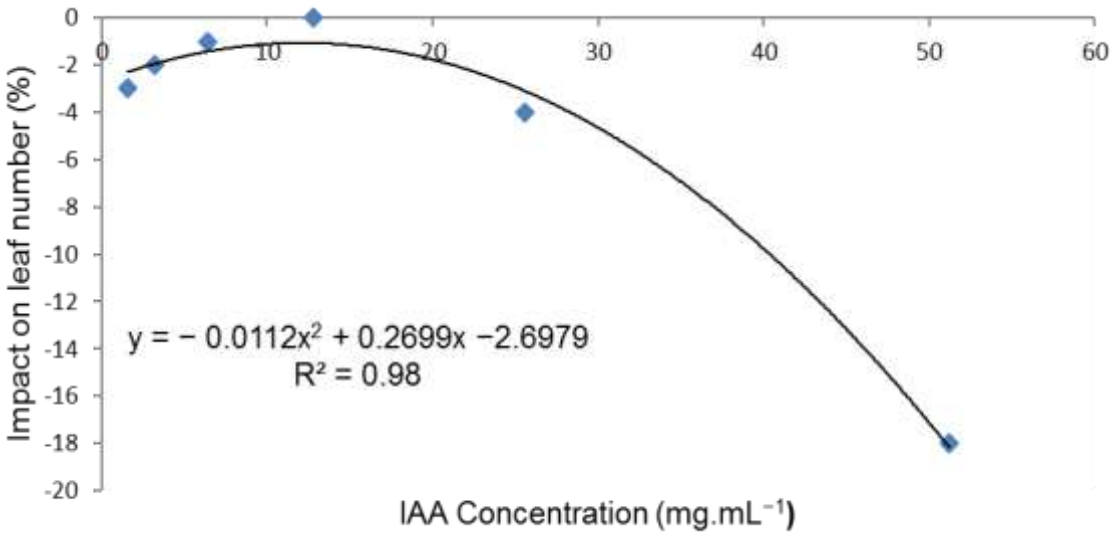

Figure 3.1 Response of leaf number of *Mimusops zeyheri* to concentrations of indole-3-acetic acid.

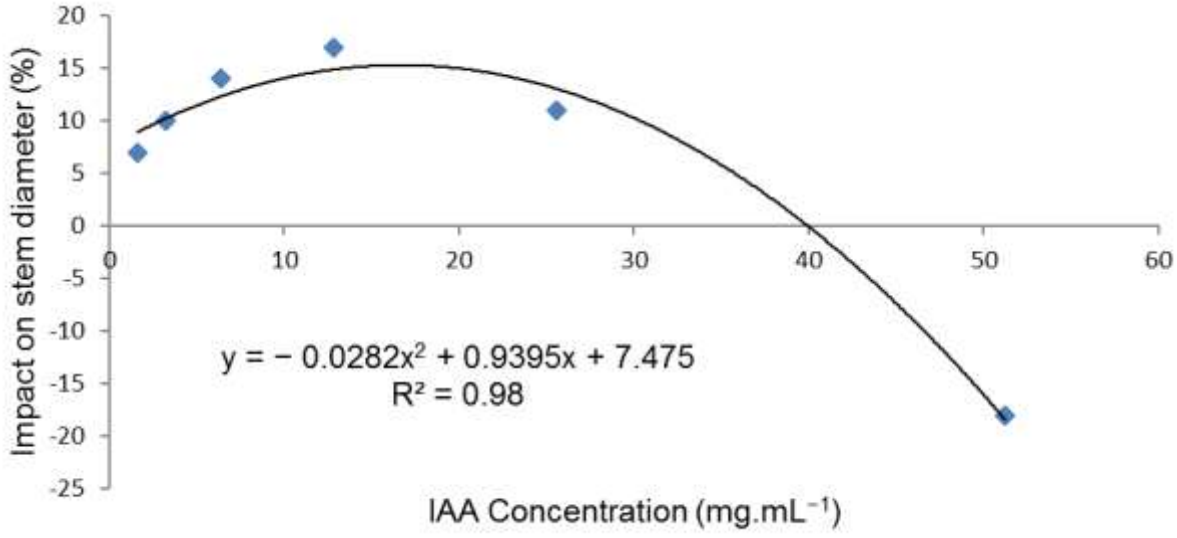

Figure 3.2 Response of stem diameter of *Mimusops zeyheri* to concentrations of indole-3-acetic acid.

Table 3.3 Quadratic relationship, coefficient of determination and computed optimum response concentration of indole-3-acetic acid for leaf number and stem diameter (SDM) of *Mimusops zeyheri.*

Variable	Quadratic relation	R^2	(x) mg/ml
Leaf number	$y = -0.0112x^2 + 0.2699x - 2.697$	0.94	12.0
Stem diameter	$y = -0.0282x^2 + 0.9395x + 7.475$	0.98	16.6
Mean optimum			14.3

$x = -b_1/2b_2$, where x is optimum concentration.

Table 3.4 Partitioning of mean sum of squares for accumulation of magnesium (Mg), calcium (Ca), phosphorus (P) and zinc (Zn) *Mimusops zeyheri* as affected by indole-3-acetic acid.

		Magnesium		Calcium		Phosphorus		Zinc	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replication	4	0.58430	14	60.9057	28	249.778	47	0.65694	50
Treatment	9	1.99647	48^{ns}	72.8943	34^{ns}	142.668	27^{ns}	0.26166	20^{ns}
Error	36	1.53597	37	82.8548	38	142.329	27	0.38863	30
Total	49	4.11674	99	216.654	100	534.775	101	1.30723	100

^{ns}Not significant at P ≥ 0.05.

3.3.2 Effects of gibberellic acid

Gibberellic acid had a significant effect ($P \leq 0.05$) on plant height and leaf number, with no effect on stem diameter, dry shoot mass, dry root mass, chlorophyll content, leaf length, and leaf width and petiole length. Treatments contributed 54 and 36% in TTV of plant height and leaf number, respectively (Table 3.5). Relative to untreated control, concentrations of GA3 increased plant height and leaf number by 19 to 25% and 46 to 66%, respectively (Table 3.6). Stimulated growth was observed at low levels and inhibition was experienced at high levels. An increase in plant height was observed with increasing concentration of gibberellic acid with the optimum concentration observed at 25.6 $mg.mL^{-1}$ and reduced at a concentration of 51.2 $mg.mL^{-1}$ (Table 3.6).

The DDG patterns had stimulation effect at low concentrations, whereas at high concentrations had inhibition effect (Table 3.7). Using the relation $x = -b_1/2b_2$, the optimum levels at which growth was enhanced was 1.6 and 3.4 $mg.ml^{-1}$ distilled water for leaf number and plant height, respectively (Table 3.7). Leaf number and stem diameter exhibited positive quadratic relations, with the relationship contributing 94 and 92%, respectively (Figure 3.3-Figure 3.4). The optimum values for the leaf number and plant height were attained at 1.6 and 3.4 $mg.mL^{-1}$, respectively (Table 3.7).

Application of GA3 had no significant effect on accumulation of essential nutrient elements in *M. zeyheri* seedlings. Treatments contributed 36, 21, 17 and 38% in total treatment variation of magnesium, phosphorus, calcium and zinc, respectively (Table 3.8).

Legend 3.5: Effect of gibberellic acid on growth of *Mimusops zeyheri* seedlings under greenhouse conditions.

Table 3.5 Partitioning of mean sum of squares for plant height and leaf number *Mimusops zeyheri* as affected by of gibberellic acid.

		Plant height		Leave number	
Source	DF	MS	TTV (%)	MS	TTV (%)
Rep	4	30.6988	35	0.03061	52
Treatment	9	47.5396	54**	0.02108	36**
Error	36	9.7670	11	0.0073	12
Total	49	88.0054	100	0.05904	100

**Significant at P ≤ 0.05.

Table 3.6 Effect of gibberellic acid on plant height and leave number of *Mimusops zeyheri* seedlings.

Treatment (%)	Plant height[y] (cm)	Impact (%)[z]	Leaf number	Impact (%)
0.0	78.49[a]	–	5.20[a]	–
3.2	93.27[b]	19	7.80[a]	50
6.4	93.58[b]	19	8.20[b]	58
12.8	97.20[c]	24	8.65[c]	66
25.6	97.99[c]	25	8.65[c]	66
51.2	93.07[b]	19	7.60[d]	46

[y]Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

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

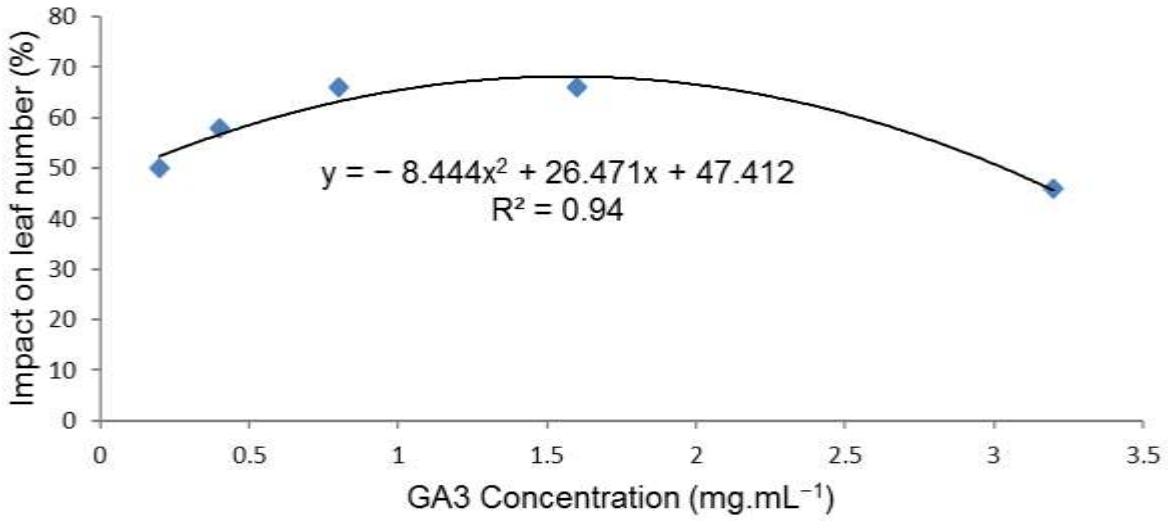

Figure 3.3 Response of leaf number of *Mimusops zeyheri* to concentrations of gibberellic acid.

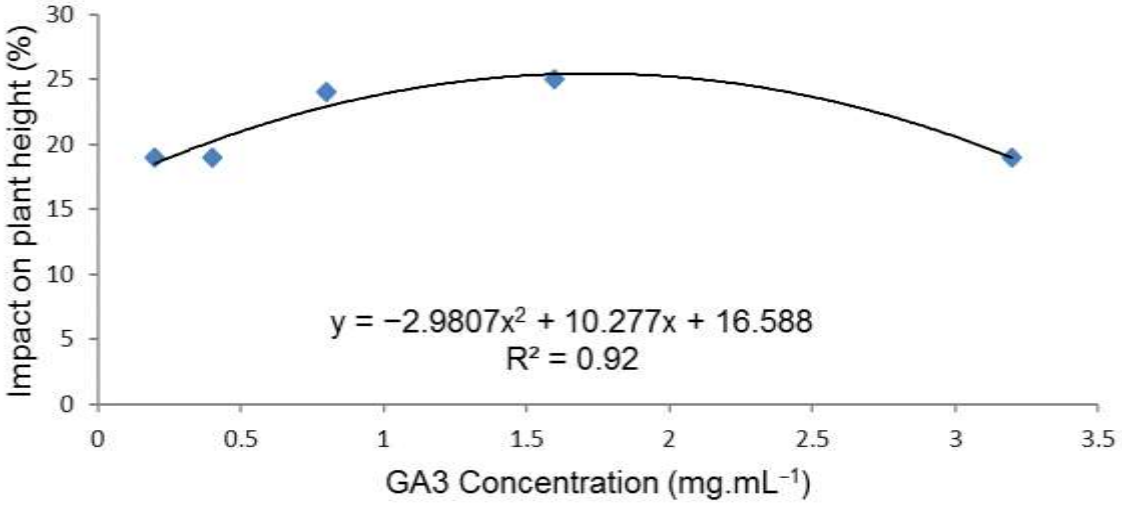

Figure 3.4 Response of plant height of *Mimusops zeyheri* to concentrations gibberellic acid.

Table 3.7 Quadratic relationship, coefficient of determination and computed optimum response concentration of gibberellic acid (GA3) for leaf number (NOL) and plant height (PTH) of *Mimusops zeyheri*.

Variable	Quadratic relation	R^2	(x) mg/ml
Leaf number	$y = -8.444x^2 + 26.471x + 47.412$	0.94	1.6
Plant height	$y = -2.9807x^2 + 10.277x + 16.58$	0.92	3.4
Mean optimum value			2.5

$x = -b_1/2b_2$, where x is optimum concentration.

Table 3.8 Partitioning of mean sum of squares for accumulation of magnesium (Mg), phosphorus (P), calcium (Ca) and zinc (Zn) *Mimusops zeyheri* as affected by gibberellic acid.

		Magnesium		Calcium		Phosphorus		Zinc	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replication	4	0.4243	35	57.851	63	299.84	50	0.048	18
Treatment	9	0.4311	36^{ns}	15.949	17^{ns}	127.37	21^{ns}	0.100	38^{ns}
Error	36	0.3424	29	18.692	20	132.61	24	0.112	43
Total	49	1.1978	100	92.4936	100	559.826	95	0.26	100

^{ns}Not significant P ≥ 0.05.

3.3.3 Effects of 6-benzylaminopurine

Application of 6-benzylaminopurine had a significant effect ($P \leq 0.05$) on leaf number, dry root mass, stem diameter and dry shoot mass, but had no effect on plant height, chlorophyll content, leaf length, leaf width and petiole length. Treatments contributed 55, 50, 53 and 25% in TTV of leaf number, dry root mass, stem diameter and dry shoot mass, respectively (Table 3.9). Relative to untreated control, concentration of 6-BAP decreased leaf number by 3 to 7% with slight increase by 1% (Table 3.10). Stem diameter, dry root mass and dry shoot mass were increased by 14 to 24, 3 to 23 and 12 to 17%, respectively (Table 3.10-Table 3.11).

Increasing levels of 6-BAP stimulated dry shoot mass with the optimum level reached at 1.6 $mg.mL^{-1}$, whereas at higher level of 3.2 $mg.mL^{-1}$ growth was inhibited. Significant effect on stimulated leaf number and stem diameter was observed with increasing concentrations of 6-BAP with an optimum level reached at the concentration of 25.6 $mg.mL^{-1}$, whereas inhibition was observed at a higher concentration of 51.2 $mg.mL^{-1}$ of the solution. The DDG patterns had stimulation effect at low concentrations, whereas at high concentrations had inhibition effect (Table 3.10-3.11).

Using the relation $x = -b_1/2b_2$, the optimum levels (x) at which growth was enhanced were 25.9, 1.6, 29.6 and 1.7 $mg.mL^{-1}$ distilled water for leaf number, dry root mass, stem diameter and dry shoot mass, respectively (Table 3.12). Plant variables over increasing 6-BAP exhibited negative quadratic relations with 97, 98, 94 and 87% of the model for leaf number, dry root mass, stem diameter and dry shoot mass being explained by the equation (Figure 3.5-Figure 3.8). Foliar application of 6-benzyaminopurine had no significant effect on accumulation of essential nutrient elements *of M. zeyheri* seedlings.

Treatments contributed 21, 29, 52, 57, 30 and 39% in total treatment variation for magnesium, phosphorus, calcium, and zinc, respectively (Table 3.13).

Legend 3.6: Effect of 6-benzylaminopurine on growth of *Mimusops zeyheri* seedlings under greenhouse conditions.

Table 3.9 Partitioning of mean sum of squares for leaf number, dry shoot mass, stem diameter and dry root mass of *Mimusops zeyheri* as affected by 6-benzylaminapurine.

		Leaf number		Dry root mass		Stem diameter		Dry shoot mass	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Rep	4	0.21600	23	0.14650	37	0.11887	25	1.34780	64
Treatment	9	0.52106	55**	0.19744	50**	0.25344	53**	0.52480	25**
Error	36	0.206	22	0.05283	13	0.10954	23	0.23758	11
Total	49	0.94306	100	0.39677	100	0.48185	100	2.1102	100

** Significant at $P \leq 0.05$.

Table 3.10 Effect of 6-Benzylaminopurine on leaf number and stem diameter of *Mimusops zeyheri* seedlings.

Treatment (%)	Leaf number[y]	Impact (%)[z]	Stem diameter (cm)	Impact (%)[z]
0.0	84.33[a]	–	46.60[a]	–
3.2	79.15[b]	−6	53.00[b]	14
6.4	81.59[a]	−3	54.80[b]	18
12.8	84.17[a]	0	56.60[b]	21
25.6	85.59[a]	1	57.60[b]	24
51.2	78.73[b]	−7	55.40[b]	19

[y]Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

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

Table 3.11 Effect of 6-Benzylaminopurine on dry root mass and dry shoot mass of *Mimusops zeyheri* seedlings.

Treatment (%)	Dry root mass[y] (g)	Impact (%)[z]	Dry shoot mass (g)	Impact (%)
0	1.40[a]	–	1.80[b]	–
0.2	1.52[a]	9	1.50[a]	–17
0.4	1.58[a]	13	1.80[b]	0
0.8	1.62[a]	16	1.88[b]	4
1.6	1.72[a]	23	1.98[c]	10
3.2	1.44[ab]	3	1.58[a]	–12

[y]Column means followed by the same letter were not different (P ≤ 0.05) according to Waller-Duncan Multiple Range test.

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

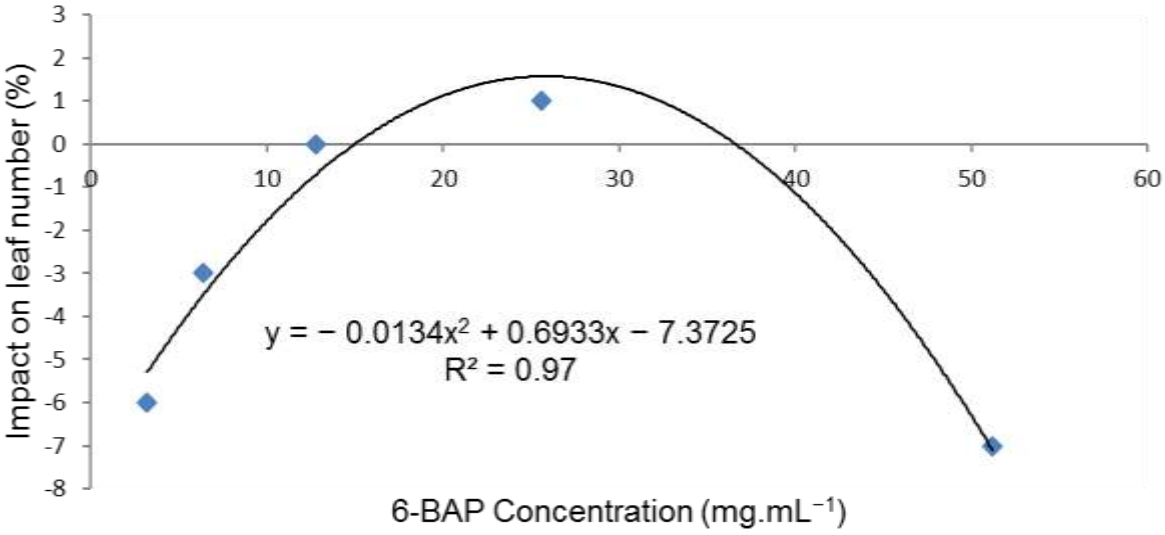

Figure 3.5 Response of leaf number of *Mimusops zeyheri* to concentrations of 6-benzylaminopurine.

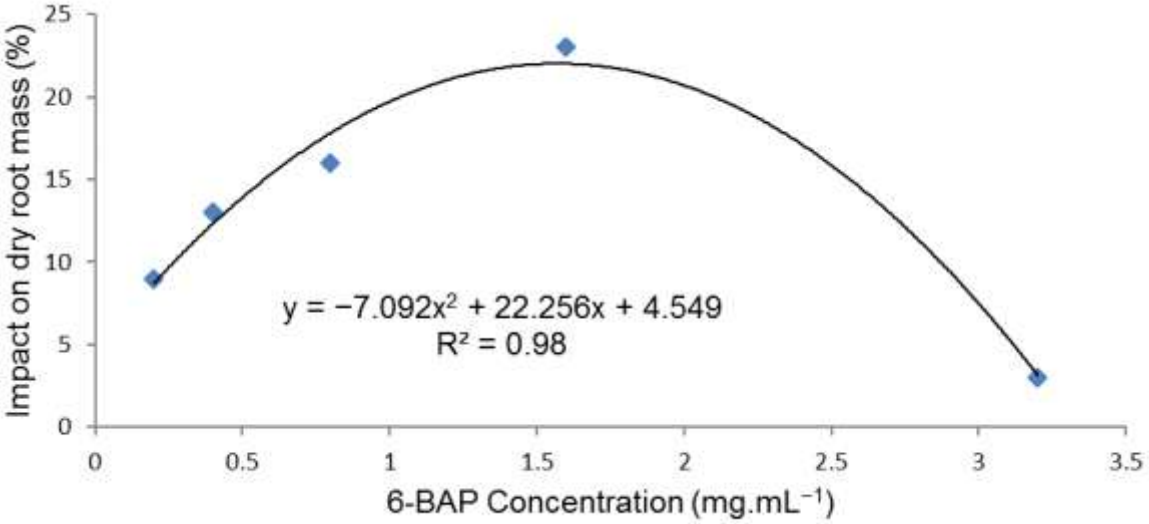

Figure 3.6 Response of dry root mass of *Mimusops zeyheri* to concentrations of 6-benzylaminopurine.

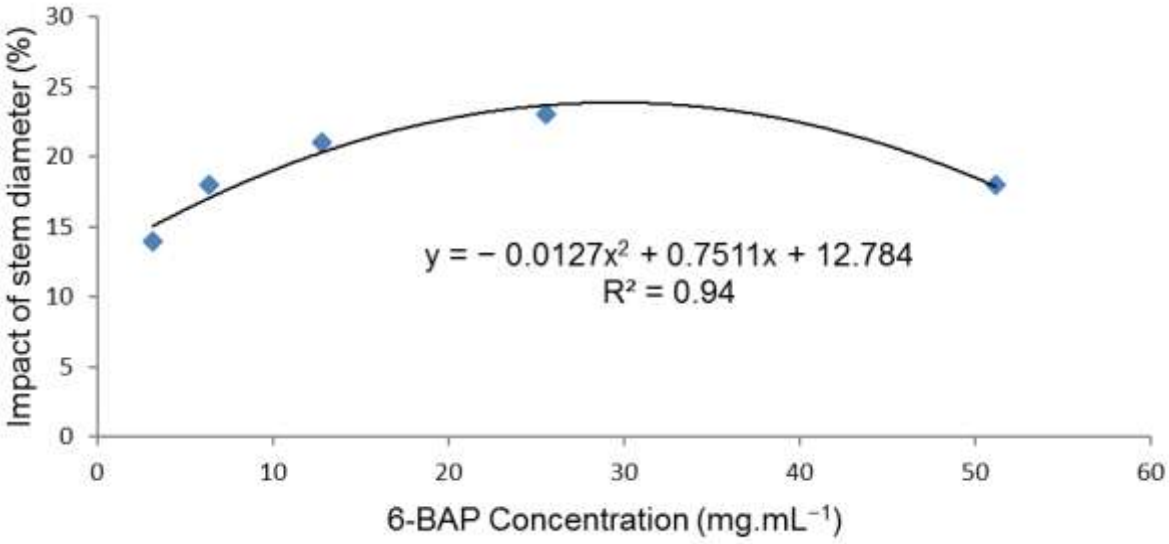

Figure 3.7 Response of stem diameter of *Mimusops zeyhe*ri to concentrations of 6-benzylaminopurine.

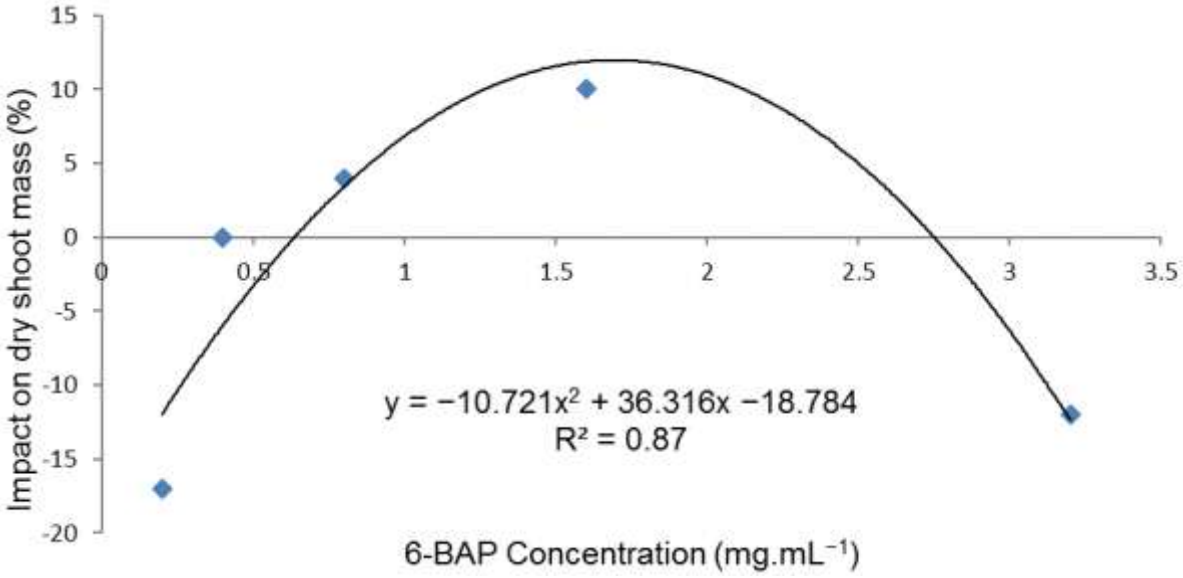

Figure 3.8 Response of dry shoot mass of *Mimusops zeyhe*ri to concentrations of 6-benzylaminopurine.

Table 3.12 Quadratic relationship, coefficient of determination and computed minimum response concentration of 6-benzylaminopurine for leaf number, dry root mass, stem diameter and dry shoot mass of *Mimusops zeyheri.*

Variable	Quadratic relation	R^2	(x) mg/ml
Leaf number	$y = -0.0134x^2 + 0.6933x - 7.3725$	0.97	25.9
Dry root mass	$y = -7.092x^2 + 22.256x + 4.549$	0.98	1.6
Stem diameter	$y = -0.0127x^2 + 0.7511x + 12.784$	0.94	29.6
Dry shoot mass	$y = -10.721x^2 + 36.316 - 18.784$	0.87	1.7
Mean optimum value			14.63

$x = -b_1/2b_{2,}$ where x is optimum concentration.

Table 3.13 Partitioning of mean sum of squares for accumulation of magnesium (Mg), phosphorus (P), calcium (Ca) and zinc (Zn) on *Mimusops zeyheri* as affected by 6-benzylaminopurine.

		Magnesium		Calcium		Phosphorus		Zinc	
Source	DF	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)	MS	TTV (%)
Replication	4	9.46070	46	46.9098	23	43.379	18	0.5969	29
Treatment	9	4.39398	21^{ns}	62.0475	30^{ns}	124.964	52^{ns}	0.7960	39^{ns}
Error	36	6.58381	32	99.3398	48	73.620	30	0.6695	32
Total	49	20.43849	99	208.2971	101	241.963	100	2.0625	100

^{ns}Not significant at P ≥ 0.05.

3.4 Discussion

3.4.1 Effects of indole-3-acetic acid

Increasing concentrations of IAA had significant effect on leaf number and stem diameter of *M. zeyheri* seedlings. Incidentally, IAA is the main auxin in plants, regulating growth and developmental processes such as cell division and elongation, tissue differentiation, apical dominance, and responses to light, gravity, and pathogens (Aloni *et al*., 2006). Application of IAA did not increase total root biomass of *M. zeyheri* seedlings. Lack of significant effect on dry root mass could be as a result of trees being insensitive to very low IAA levels. Ahmed *et al*. (2012) observed gradual increase in shoot height with increasing concentrations of IAA on Valencia oranges but increased shoot diameter at low concentrations.

Indole-3-acetic acid had no significant effect on plant height which resulted in short and bushier trees, which could be as a result of apical dominance caused by auxins being localised at the terminal buds. Similarly, IAA reduced shoot height in eucalyptus trees by causing a reduction in both cell numbers and cell length (Bachelard, 1968). Findings of this study displayed no significant effect on leaf area, plant height, chlorophyll content, petiole length, dry root and shoot mass of *M. zeyheri* trees treated with IAA. Contradictory results were observed by Khudhur and Omer (2015), who observed a significant effect on stem cuttings, number of main branches, diameter of main branches, root length, number of main roots, leaf area, fresh and dry weight of shoot, fresh and dry weight of root, dry matter of shoot and root, biomass of shoot and root on *Dalbergia sissoo* trees treated with 500 ppm IAA.

Increasing concentration of IAA had inhibited dry shoot and root mass which are said to be poor indicators of growth (Maila, 2005). Incidentally, plant height, stem diameter are considered to be good indicators of *M. zeyheri* tree growth in variety of soil types. The significant effect in stem diameter in trees treated with IAA could be due to cell division in the cambium and xylem development which proceed in an intact stem (Little and Savidge, 1987). Variations in cambial growth patterns along the stem have been explained by the postulated existence of longitudinal concentration gradients of IAA (Aloni and Zimmermann, 1983).

Plant growth regulators operate at a cellular level through cell enlargement, cell elongation and cell division (Harms and Oplinger, 1988). Plant growth regulators were observed to have no significant effect on the nutrient content of *M. zeyheri* seedlings. According to Mashela *et al*. (2013b), accumulation of a certain nutrient in *M. zeyheri* trees during and after fruiting resulted in an increase or decrease of the other essential element. Leaf accumulation of magnesium, potassium, phosphorus and calcium resulted in limiting the content of zinc and iron in *M. zeyheri* trees.

Soil factors are said to greatly influence availability of essential nutrient elements in the soil systems (Mashela *et al*., 2013b). Extremely low iron levels were observed in all trees treated with plant growth regulators with level which were below detection in other treatments. Zinc was also observed to be available at low levels in *M. zeyheri* seedlings subjected to foliar applications of plant growth regulators. However, findings of this research demonstrated increased levels of magnesium, phosphorus, potassium and calcium.

Increasing concentrations of foliar application of IAA had no significant effect of accumulation of zinc in *M. zeyheri* seedlings. Zinc was available in extremely content in *M. zeyheri* seedlings. Contradictory results were observed when foliar application of IAA in Valencia orange trees increased zinc concentration in trees (Ahmed *et* al., 2012). Incidentally, zinc is required for the synthesis of IAA. Also, low levels of iron were observed in *M. zeyheri* trees treated with increasing concentrations of IAA. Similarly, low iron content were recorded in leaves of Valencia orange trees sprays with 100, 200 and 300 ppm of IAA under zinc deficiency relative to the untreated control (Ahmed *et* al., 2012).

3.4.2 Effects of gibberellic acid

Gibberellic acid had a significant effect on plant height and leaf number of *M. zeyheri* seedlings. At lower levels which were below the optimum level, increase in leaf number and plant height was observed. Similarly, application of gibberellins was observed to promote shoot elongation mainly through internode extension and inhibited terminal bud development in '*Manzanillo*' but not in '*Ascolano*' olive cultivars (Badr and Hartmann, 1972). The highest average of branches length of 26.26 and 28.19 cm for both seasons, respectively, were observed on 3 year old peach trees treated with 100 $mg.L^{-1}$ GA3 (Al-Rawi *et al*., 2016). This could be due to the counter effect of gibberellic acid which when applied in high concentrations inhibits growth of terminal buds. Generally, gibberellic acid controls cell elongation and division in plant shoots (Harms and Oplinger, 1988).

Similarly, an increase in shoot length was also observed in eucalyptus trees sown from seeds treated with 50 mg/L gibberellic acid solution. However, seedling growth was accompanied by smaller dry weight (Bachelard, 1968). Increasing concentration of GA3

had no significant effect on root growth and development of *M. zeyheri* seedlings which resulted in a small root to shoot ratio. Similarly in eucalyptus trees treated with GA roots were observed to be shorter and lighter than the untreated seeds. Gibberellic acid had no effect on stem diameter of *M. zeyheri* seedlings. Contradictory results were observed where an increase in stem diameter of 8.63 and 18.68 mm for two consecutive seasons were observed on 3 year old peach trees treated with 100 $mg.L^{-1}$ GA3 (Al-Rawi *et al*., 2016).

Application of increasing concentrations of GA3 resulted in a lack of significant effect on leaf area of *M. zeyheri* seedlings, which contradicts with the findings that demonstrated a significant increase in leaf area of 2168 and 2897 cm^2 of 3 year old peach trees treated with GA3 sprays at 100 $mg.L^{-1}$ (Al-Rawi *et al*., 2016). *Mimusops zeyheri* trees treated with GA had long leaves which were thinner when compared to tree leaves treated with IAA and 6-BAP. Leaf length was greater than leaf width in *M. zeyheri* trees treated with foliar application of GA3. Similar observations were recorded in eucalyptus leaves where application of GA3 caused alterations in leaf shape and leaf thickness whereby the leaves appeared to be thinner than the control (Bachelard, 1968). The alterations were attributed to changes in cell division rather than cell elongation.

The response of a plant organ to a hormone is dependent on the concentration of the hormone. Incidentally, one hormone can stimulate one process, while inhibiting other processes within one organ (Hartmann *et al*., 1988). This research demonstrated an increase in height of *M. zeyheri* trees treated with GA3, whereas trees treated with 6-BAP and IAA were short and had more branches. Treatment of seeds with GA3 prior to planting could modify hypocotyl growth. The increased shoot length following seed

treatment of seeds with GA3 was due primarily to increased hypocotyl extension and to a lesser extent, to extension of the first internode. This can be due to the counter effect of gibberellic acid which when applied in high concentrations inhibits growth of terminal buds. Generally, gibberellic acid controls cell elongation and division in plant shoots (Harms and Oplinger, 1988). The most consistent and obvious effect of GA on plant growth is increased internode extension (Brian, 1959).

Application of increasing concentration of GA3 had no effect on accumulation of potassium and phosphorus in *M. zeyheri* seedlings. The leaf content of phosphorus and potassium was higher than that of zinc and iron in tree leaves. An increase in phosphorus and potassium was observed in peach trees treated with foliar sprays of GA3, however, a lack in the significant effect on leaf zinc, manganese and iron was observed (Al-rawi *et al*., 2016). The result agrees with the findings of this study where there were no significant effect on leaf zinc and iron.

3.4.3 Effects of 6-benzylaminopurine

Application of 6-BAP had a significant effect on leaf number, stem diameter, dry root mass and plant dry shoot mass of *M. zeyheri* seedlings. Stimulated growth was observed at low levels and inhibition was experienced at high levels. An increase in dry shoot mass was observed with increasing concentration of 6-BAP with the optimum concentration observed at 1.6 $mg.mL^{-1}$ and reduced at a concentration of 3.2 $mg.mL^{-1}$. Maila and Mashela (2009), demonstrated an optimum level of 0.80 and 0.35 µM BAP for shoot regeneration of *C. myriocarpus* and *C. africanus,* respectively, under *in vitro* propagation conditions which contradicted with the relatively high optimum levels obtained in this

study. This could be explained in terms of the resistance induced by physical structures such as suberins and waxes in the current study.

Increasing 6-BAP concentrations inhibited leaf area of *M. zeyheri* seedlings. Contradictory results were observed when the cytokine BA had a significant effect on leaf area of olive tree at 3.91 and 3.90 cm^2 for two consecutive seasons with the control being lowest at 3.59 and 3.62 cm^2 also for two consecutive seasons (Abou-Aziz *et al*., 2011). Lack of significant effect on leaf area of *M. zeyheri* trees treated with 6-BAP could be as a result of a documented high content of latex on leaves of *M. zeyheri* leaves (Mashela *et al*., 2013a) which could inhibit leaf expansion. However, an increase in leaf width and petiole length of *M. zeyheri* trees by 19% was observed when trees were grown in clay soil within a period of 9 to 12 months (Mashela *et al*., 2013a).

Application of 6-BAP had a stimulating effect on leaf number of *M. zeyheri* seedlings and similarly it was observed that foliar application of the cytokinin BA at a concentration of 15 $mmol.L^{-1}$ had a significant increase in number of leaves of five month old *Jatropha curcas* trees relative to the control under shade house conditions (Abdelgadir and Johnson, 2009). However, application of BA was reported to have no significant effect on plant height, crown and stem diameter under field conditions. At high treatment levels which were above the optimum, 6-BAP had an inhibitive effect on plant growth. Incidentally, application of 6-BAP had a significant effect on stem diameter and plant height of *M. zeyheri* seedlings. *Mimusops zeyheri* trees treated with 6-BAP were short and highly branched with a significant effect on increase in number of leaves and stem diameter relative to untreated control. Consequently, results of this study exhibited an

increase in both dry root and shoot biomass. Incidentally, Cytokinins, including BAP, promote cell division, bud formation and stem branching in trees.

Ledwaba C.R (2008) observed increased potassium leaf content and decreased phosphorus and magnesium contents, which contradicts with findings of this study. This could imply that the nutrients supplied to the plant during the cause of the experiment were not sufficient and further studies should increase fertiliser application taking into consideration the law of the minimum quantities. When applied to morula (*Sclerocarya birrea*) trees, 150 mg/L BA significantly increased morula tree mineral uptake of leaf phosphorus, sodium, calcium, magnesium, nitrogen and potassium when compared to the control. However, at lower levels of 50 and 100mg/L BA there were no significant differences with respect to leaf phosphorus, sodium, nitrogen and potassium contents whereas at 100 mg/L BA there was an observed significant increase in leaf calcium and magnesium content relative to the control (Moashe *et al*., 2011).

3.4.4 Density-dependent growth patterns in *Mimusops zeyheri* trials

The extend at which plant organs respond to treatment depends on concentration applied (Maila and Mashela, 2009) and as a result one hormone can stimulate one process, while inhibiting other processes within the same plant organ (Hartmann *et al*., 1988). Consequently, increased, concentration of the applied plant growth regulator beyond the optimum level inhibited 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, 1992). The DDG patterns are characterised by three phases, namely, stimulation, neutral and inhibition (Liu *et al*., 2003).

Using concentrations at the optimum level as observed in this study would result in concentrations accumulating towards the inhibition phase, which would inhibit plant growth instead of promoting it (Mashela *et al*., 2015). In generating the DDG patterns, plant growth regulators behaved as allelochemicals at low concentrations (Liu *et al*., 2003), suggesting that above the observed optimum values the products might be phytotoxic. Apparently, the Curve-fitting Allelochemical Response Dosage (CARD) computer-based model could be used to determine the concentration that would stimulate plant growth, while also establishing the overall sensitivity of *M. zeyheri* to these products.

3.5 Conclusion

In conclusion, results of this research demonstrated that application of synthetic plant growth hormones at low concentrations can improve growth of *M. zeyheri* seedling. The use of IAA, GA3 and 6-BAP as foliar sprays on *M. zeyheri* demonstrated for the first time the existence of DDG patterns of various organs of this plant species, with the possibility of using the CARD computer model to generate concentrations that would stimulate plant growth.

CHAPTER 4
RESPONSE OF *MIMUSOPS ZEYHERI* TO VESICULAR ARBUSCULAR MYCORRHIZA

4.1 Introduction

Mimusops zeyheri fruit trees are being assessed as alternative crops in marginal communities of Limpopo Province, Republic of South Africa where conditions are suboptimal (Mashela and Mollel, 2002). The introduction of indigenous trees, which are recorded to contain high nutritional values, is a practice that could ensure food security. However, *M. zeyheri* trees are slow growers. The use of vesicular arbuscular mycorrhiza (VAM) could be effective in improving growth of *M. zeyheri* due to its ability to enhance moisture and nutrient uptake (Gavito *et al*., 2002). However, the effect of VAM on growth and nutrient accumulation in leaves of *M. zeyheri* is not documented. The objective of this study was to investigate the effect of VAM fungi on growth and accumulation of essential nutrient elements in *M. zeyheri* trees.

4.2 Materials and methods

4.2.1 Growth conditions and preparations

The study would be conducted under greenhouse conditions at the Green Technology Research Centre (GTRC), University of Limpopo, South Africa (23°53'10''S, 29°44'15''E). Day/night temperatures averaged 28/21^{o}C, with maximum temperatures controlled using thermostatically-activated fans. Relative humidity will be maintained at a high level through wet walls.

4.2.2 Experimental design and inoculation

The VAM experiment contained eight treatments, namely, 0, 10, 20, 30, 40, 50, 60 and 70 g VAM, as recommended by the supplier (Mycoroot^{TM}, Pietermaritzburg). Treatments were replicated seven times and arranged in a randomised complete block design (legend 4.1). Uniform *M. zeyheri* seedlings were hardened-off by exposing seedlings to the natural environment for five hours per day for a week before transplanting. Seedlings were transplanted in 30-cm-diameter plastic pots containing steam-pasteurised loam soil and river sand at 3:1 (v\v) ratio. The seedlings were placed on greenhouse benches with an inter-row spacing of 45 cm and intra-row spacing of 45 cm.

4.2.3 Cultural practices

Fertilisers were applied a day after transplanting using 5g 2:3:2 (26) 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 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). Plants were irrigated with 500 ml chlorine-free tap water every other day. Pests were scouted and aphids were controlled using Malasol as per label instruction.

4.2.4 Data collection

At 84 days after initiation of treatments, stem diameter, plant height, number of leaves, number of shoots, chlorophyll content, dry shoot mass, dry root, leaf length, leaf width, petiole length and leaf. Tops were severed at the soil surface and oven-dried at 70°C for 72 h. Mature dried leaves were separated from the stem and ground into powder using pestle and mortar. The powdered sample was digested in 5% nitric acid, which was then mixed with a vortex meter. The samples were then incubated in a warm water bath for an

hour at 95°C, cooled at room temperature and filtered, when the containers were each covered with a foil (SW-846 EPA Method 3050B). Samples were then submitted to the Limpopo Agrofood Station (LATS), where they were quantified using the Inductively coupled plasma atomic emission spectrometry (ICP) for mineral elements.

4.2.5 Data analysis

Data were subjected to analysis of variance through the SAS software version 10.0 (SAS Institute, 2008). Mean separation was achieved using Waller-Duncan Multiple Range test.

4.3 Results

4.3.1 Responses of *Mimusops zeyheri* growth to VAM

Application of VAM had no significant effects on plant height, stem diameter, leaf number, leaf width, leaf length, petiole length, chlorophyll content, dry root and dry shoot mass (Table 4.1).

Legend 4.1: Effect of vesicular arbuscular mycorrhiza on growth of six-month old *Mimusops zeyheri* seedlings under greenhouse conditions.

4.3.2 Responses of *Mimusops zeyheri* foliar nutrient elements to VAM

The VAM treatments did not affect the accumulation of the four macronutrients and two micro nutrient elements in the study. However, means were provided for future records (Table 4.2).

Table 4.1 Effect of vesicular arbuscular mycorrhiza on selected growth variables in *Mimusops zeyheri* seedlings.

Treatment (%)	Stem diameter (cm)[y]	Leaf number	Dry shoot mass (g)	Plant height (cm)	Leaf length (cm)
0	1.3014	123.71	43.833	73.829	11.457
10	1.2786	108.00	43.556	61.057	12.000
20	1.2771	106.43	38.079	59.257	11.900
30	1.1957	104.14	41.583	62.000	11.557
40	1.2286	121.29	36.633	58.557	10.629
50	1.4557	115.71	47.790	68.571	11.157
60	1.3329	122.57	42.110	58.214	10.914
70	1.2629	82.14	41.336	63.457	11.671
$LSD_{0.05}$	0.2453	37.055	12.405	13.954	1.4818

[y]Column means followed by the same letter were not different according to Waller-Duncan Multiple range test.

Table 4.2 Effect of vesicular arbuscular mycorrhiza on selected essential nutrients elements in leaves of *Mimusops zeyheri* seedlings.

Treatments (%)	Magnesium ($mg.ml^{-1}$)[y]	Calcium ($mg.ml^{-1}$)	Phosphorus ($mg.ml^{-1}$)	Potassium ($mg.ml^{-1}$)	Zinc ($mg.ml^{-1}$)
0	17.786	31.629	23.229	22.514	0.3209
10	18.943	39.586	26.700	24.386	0.4539
20	16.34	39.729	25.586	25.857	1.4967
30	19.100	36.100	21.857	22.921	1.4343
40	19.743	40.200	26.114	25.771	0.4223
50	18.600	36.957	19.229	18.157	0.5376
60	18.757	37.300	26.957	24.357	0.3503
70	18.557	38.871	17.714	27.543	0.4630
$LSD_{0.05}$	4.5024	16.153	7.7427	6.6154	1.6161

[y]Column means followed by the same letter were not different according to Waller-Duncan Multiple range test.

4.4 Discussion

Application of VAM had no significant effect on plant height, stem diameter, leaf number, leaf width, leaf length, petiole length, chlorophyll content, dry root mass and shoot mass of *M. zeyheri* seedlings. The observations in the current study did not support those in woody plants (Sidhoum and Fortas, 2013; Powell and Nakrishnan, 1986; Świerczyński and Stachowiak, 2010). The VAM treatments resulted in depressed growth of sour orange (*Citrus aurantium*) seedlings under high phosphorus supply (Jifon *et al*., 2002). Contradictory results were observed when a significant increase in leaf area, plant height, stem diameter and plant biomass was observed in Orange (*Citrus sinensis*) seedlings during screening of five *Glomus* species of VAM, namely, *Glomus, mosseae (UK), G. mosseae (USA), G. clarium, G. caledonium and G. etunicatum* (Ortas, 2002). Mycorrhiza symbiosis in plants results in enhanced moisture and nutrient uptake, which can be observed through increased plant height, stem diameter, leaf number and total biomass (Sharma *et al*., 2012; Usha *et al*., 2004). Extensive experimental trials demonstrated improved tree growth when VAM symbiosis was successful (Świerczyński and Stachowiak, 2010; Tong *et al*., 2006). However, lack of a significant effect on all tested plant growth variables of *M. zeyheri* seedlings in the current study could not be exclusively attributed to lack of mycorrhiza symbiosis. Generally, VAM treatment is effective under stressful conditions such as nutrient deficiency and salinity (Juniper and Abbott, 1993). Apparently, in the current study there were no stressful conditions.

Increasing the levels of VAM applied on *M. zeyheri* trees had no significant effect on tree growth and neither accumulation of essential nutrient elements, which could imply that the relation was at the neutral phase, where there was no observed stimulation nor

inhibition of plant growth. The levels of VAM used in this experiment should be adjusted in another experiment to investigate the effect on plant growth under stressful conditions.

Generally, VAM is used to enhance the absorption of phosphorus (Calvet *et al*., 2004), particularly in South Africa where the soils are deficient in this element (DOA, 2003). In the current study, phosphorus or any essential element was not deficient. Apparently, under luxurious soil conditions, the efficacy of VAM on absorption of nutrients is curtailed. Extensive studies had proven that mycorrhiza application on trees enhance nutrient uptake especially of scarce and immobile nutrients such as phosphorus and nitrogen (Bolan, 1991; Sidhoum and Fortas, 2013).

4.5 Conclusion

The findings of the current study demonstrated that *M. zeyheri* seedlings had no symbiotic association with the used VAM. A screening experiment should be conducted using various species of the VAM on *M. zeyheri* seedlings to observe the suitable fungus under different stressful conditions such as in phosphorus- deficient and/or saline soils.

CHAPTER 5
SUMMARY, SIGNIFICANCE OF FINDINGS, RECOMMENDATIONS AND CONCLUSIONS

5.1 Summary

At 84 days after initiation and application of treatments plant height, stem diameter, leaf number, leaf area, petiole length, dry root mass and dry shoot mass were collected. The IAA concentrations had significant effects on stem diameter and leaf number. Stem diameter and leaf number each over increasing concentration of IAA exhibited positive curvilinear quadratic relations, with the relationships explaining the model by 98 and 94% in respective variables. Leaf number and plant height over increasing GA3 concentrations exhibited positive curvilinear quadratic relations, with the relationships being explained by 94 and 92% of the model, respectively. Leaf number, dry root mass, stem diameter and dry shoot mass over increasing 6-BAP concentrations exhibited positive curvilinear quadratic relations, with the relationships explaining 97, 98, 94 and 87%, respectively, of the model. Applying IAA, GA3 and 6-BAP at 14.3, 2.5 and 14.63%, respectively, would improve growth of *M. zeyheri* seedlings. Application of vesicular arbuscular mycorrhiza (VAM) fungi had no significant effects on all plant variables and accumulation of selected essential nutrient elements.

5.2 Significance of findings

The observed quadratic relations between various plant variables of *M. zeyheri* and the increasing concentrations of plant growth regulators (PGRs) allowed the determination of optimum quantities of PGRs for improving growth of *M. zeyheri* seedlings under nursery conditions. Also, lack of growth and nutrient accumulation in *M. zeyheri* leaves was important since this provided some clue as to where VAM should be used to improve growth in this plant species.

5.3 Recommendations

Synthetic plant growth regulators could be effectively used in enhancing growth of the slow growing *M. zeyheri* seedlings at the obtained optimum levels. The recommended optimum levels are 14.3, 2.5 and 14.63% for IAA, GA3 and 6-BAP, respectively, should be validated under various conditions, including field conditions. Another experiment should be conducted using VAM on *M. zeyheri* seedlings under stressful conditions such as phosphorus-deficient or salinity-affected soils. Additionally, screening for suitable *Glomus* species strain could be considered for future studies.

5.4 Conclusions

Application of indole-3-acetic acid, gibberellic acid and 6-benzylaminopurine could be used to improve growth of *M. zeyheri* seedlings using the derived optimum concentrations. The use of PGRs and VAM to stimulate growth of *M. zeyheri* seedlings, should promote the potential commercialisation of this important alternative underutilised indigenous fruit tree.

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APPENDICES

Appendix 3.1 Analysis of variance for leaf number of *Mimusops zeyheri* in response to indole-3-acetic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.02469	0.00617		
Treatment	9	0.11367	0.01263	3.00	0.0091
Error	36	0.15177	0.00422		
Total	49	0.29014			

Appendix 3.2 Analysis of variance for stem diameter of *Mimusops zeyheri* in response to indole-3-acetic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.91525	0.22881		
Treatment	9	1.93261	0.21473	2.18	0.0470
Error	36	3.54199	0.09839		
Total	49	6.38985			

Appendix 3.3 Analysis of variance for plant height of *Mimusops zeyheri* in response to indole-3-acetic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	22.661	5.6652		
Treatment	9	94.166	10.4629	1.58	0.1592
Error	36	238.695	6.6304		
Total	49	355.522			

Appendix 3.4 Analysis of variance for leaf length of *Mimusops zeyheri* in response to indole-3-acetic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	8.5781	2.14451		
Treatment	9	16.7412	1.86013	1.37	0.2398
Error	36	49.0404	1.36223		
Total	49	74.3596			

Appendix 3.5 Analysis of variance for leaf width of *Mimusops zeyheri* in response to indole-3-acetic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.6595	0.16486		
Treatment	9	4.1110	0.45677	1.30	0.2732
Error	36	12.6920	0.35256		
Total	49	17.4624			

Appendix 3.6 Analysis of variance for petiole length of *Mimusops zeyheri* in response to indole-3-acetic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	8.5212	2.13030		
Treatment	9	17.4152	1.93502	0.96	0.4914
Error	36	72.9028	2.02508		
Total	49	98.8392			

Appendix 3.7 Analysis of variance for dry shoot mass of *Mimusops zeyheri* in response to indole-3-acetic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.10421	0.02605		
Treatment	9	0.48947	0.05439	0.67	0.1331
Error	36	1.17327	0.03259		
Total	49	1.76695			

Appendix 3.8 Analysis of variance for dry root mass of *Mimusops zeyheri* in response to indole-3-acetic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.17372	0.04343		
Treatment	9	0.68480	0.07609	1.20	0.3270
Error	36	2.28908	0.06359		
Total	49	3.14760			

Appendix 3.9 Analysis of variance for chlorophyll content of *Mimusops zeyheri* in response to indole-3-acetic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	243.09	60.7727		
Treatment	9	420.34	46.7041	0.94	0.5003
Error	36	1780.98	49.4716		
Total	49	2444.40			

Appendix 3.10 Analysis of variance for Magnesium (Mg) accumulation in *Mimusops zeyheri* in response to indole-3-aceticacid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	2.3372	0.58430		
Treatment	9	17.9682	1.99647	1.30	0.2710
Error	36	55.2948	1.53597		
Total	49	75.6002			

Appendix 3.11 Analysis of variance for iron (Fe) accumulation in *Mimusops zeyheri* in response to indole-3-aceticacid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.79896	0.19974		
Treatment	9	0.79473	0.08830	0.72	0.6842
Error	36	4.39308	0.12203		
Total	49	5.98677			

Appendix 3.12 Analysis of variance for phosphorus (P) accumulation in *Mimusops zeyheri* in response to indole-3-aceticacid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	999.11	249.778		
Treatment	9	1284.01	142.668	1.00	0.4559
Error	36	5123.85	142.329		
Total	49	7406.97			

Appendix 3.13 Analysis of variance for potassium (K) accumulation in *Mimusops zeyheri* in response to indole-3-aceticacid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	156.05	39.0125		
Treatment	9	220.72	24.5244	0.61	0.7774
Error	36	1439.15	39.9764		
Total	49	1815.92			

Appendix 3.14 Analysis of variance for calcium (Ca) accumulation in *Mimusops zeyheri* in response to indole-3-aceticacid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	243.62	60.9057		
Treatment	9	656.05	72.8943	0.88	0.5519
Error	36	2982.77	82.8548		
Total	49	3882.44			

Appendix 3.15 Analysis of variance for Zinc (Zn) accumulation of *Mimusops zeyheri* in response to indole-3-aceticacid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	2.6278	0.65694		
Treatment	9	2.3550	0.26166	0.67	0.7273
Error	36	13.9908	0.38863		
Total	49	18.9736			

Appendix 3.16 Analysis of variance for leaf number of *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.12246	0.03061		
Treatment	9	0.18972	0.02108	2.87	0.0117
Error	36	0.26456	0.00735		
Total	49	0.57673			

Appendix 3.17 Analysis of variance for stem diameter of *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	1.98117	0.49529		
Treatment	9	2.52679	0.28075	1.99	0.0697
Error	36	5.08047	0.14112		
Total	49	9.58843			

Appendix 3.18 Analysis of variance for plant height of *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	122.795	30.6988		
Treatment	9	427.856	47.5396	4.87	0.0003
Error	36	351.613	9.7670		
Total	49	902.264			

Appendix 3.19 Analysis of variance for leaf length of *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	23.0689	5.76722		
Treatment	9	10.0238	1.11376	0.89	0.5411
Error	36	44.9018	1.24727		
Total	49	77.9945			

Appendix 3.20 Analysis of variance for leaf width of *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	1.7876	0.44690		
Treatment	9	4.1203	0.45781	2.00	0.0681
Error	36	8.2369	0.22880		
Total	49	14.1448			

Appendix 3.21 Analysis of variance for petiole length of *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	8.9148	2.22870		
Treatment	9	17.3368	1.92631	1.06	0.4147
Error	36	65.4412	1.81781		
Total	49	91.6928			

Appendix 3.22 Analysis of variance for dry shoot mass of *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.42669	0.10667		
Treatment	9	0.12986	0.01443	0.46	0.8901
Error	36	1.12391	0.03122		
Total	49	1.68046			

Appendix 3.23 Analysis of variance for dry root mass of *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.07701	0.01925		
Treatment	9	0.09395	0.01044	0.85	0.5804
Error	36	0.44455	0.01235		
Total	49	0.61551			

Appendix 3.24 Analysis of variance for chlorophyll content of *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	516.46	129.114		
Treatment	9	329.97	36.664	0.51	0.8600
Error	36	2606.01	72.389		
Total	49	3452.44			

Appendix 3.25 Analysis of variance for magnesium (Mg) accumulation in *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	1.6972	0.42430		
Treatment	9	3.8802	0.43113	1.26	0.2921
Error	36	12.3268	0.34241		
Total	49	17.9042			

Appendix 3.26 Analysis of variance for iron (Fe) accumulation in *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	2.8312	0.70781		
Treatment	9	3.3857	0.37618	1.59	0.1557
Error	36	8.5219	0.23672		
Total	49	14.7388			

Appendix 3.27 Analysis of variance for phosphorus (P) accumulation in *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	1199.37	299.844		
Treatment	9	1146.34	127.371	0.96	0.4876
Error	36	4773.99	132.611		
Total	49	7119.71			

Appendix 3.28 Analysis of variance for potassium (K) accumulation in *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	218.20	54.5503		
Treatment	9	252.67	28.0742	1.59	0.1548
Error	36	634.68	17.6301		
Total	49	1105.55			

Appendix 3.29 Analysis of variance for calcium (Ca) accumulation in *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	231.41	57.8513		
Treatment	9	143.55	15.9497	0.85	0.5738
Error	36	672.93	18.6926		
Total	49	1047.89			

Appendix 3.30 Analysis of variance for Zinc (Zn) accumulation in *Mimusops zeyheri* in response to gibberellic acid under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.19357	0.04839		
Treatment	9	0.90239	0.10027	0.89	0.5415
Error	36	4.04426	0.11234		
Total	49	5.14022			

Appendix 3.31 Analysis of variance for leaf number of *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.08865	0.02216		
Treatment	9	0.09578	0.01064	2.50	0.0246
Error	36	0.15326	0.00426		
Total	49	0.33769			

Appendix 3.32 Analysis of variance for stem diameter of *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.47547	0.11887		
Treatment	9	2.28092	0.25344	2.31	0.0360
Error	36	3.94349	0.10954		
Total	49	6.69988			

Appendix 3.33 Analysis of variance for plant height of *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	76.517	19.1293		
Treatment	9	35.764	3.9738	0.61	0.7837
Error	36	236.179	6.5605		
Total	49	348.460			

Appendix 3.34 Analysis of variance for leaf length of *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	357.88	89.4701		
Treatment	9	738.02	82.0022	1.00	0.4604
Error	36	2962.95	82.3042		
Total	49	40.58.85			

Appendix 3.35 Analysis of variance for leaf width of *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	1.9947	0.49867		
Treatment	9	1.6382	0.18202	0.61	0.7833
Error	36	10.8079	0.30022		
Total	49	14.4407			

Appendix 3.36 Analysis of variance for petiole length of *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.12920	0.03230		
Treatment	9	0.14020	0.01558	0.68	0.7231
Error	36	0.82680	0.02297		
Total	49	1.09620			

Appendix 3.37 Analysis of variance for dry shoot mass of *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	5.3912	1.34780		
Treatment	9	4.7232	0.52480	2.21	0.0446
Error	36	8.5528	0.23758		
Total	49	18.6672			

Appendix 3.38 Analysis of variance for dry root mass of *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.58600	0.14650		
Treatment	9	1.77700	0.19744	3.74	0.0021
Error	36	1.90200	0.05283		
Total	49	4.26500			

Appendix 3.39 Analysis of variance for chlorophyll content of *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	156.89	39.2213		
Treatment	9	541.10	60.1218	1.03	0.4370
Error	36	2104.88	58.4689		
Total	49	2802.86			

Appendix 3.40 Analysis of variance for magnesium (Mg) accumulation in *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	37.843	9.46070		
Treatment	9	39.546	4.39398	0.67	0.7323
Error	36	237.017	6.58381		
Total	49	314.406			

Appendix 3.41 Analysis of variance for iron (Fe) accumulation in *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	1.14958	0.28739		
Treatment	9	1.66318	0.18480	1.07	0.4088
Error	36	6.22734	0.17298		
Total	49	9.04009			

Appendix 3.42 Analysis of variance for phosphorus (P) accumulation in *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	173.52	43.379		
Treatment	9	1124.67	124.964	1.70	0.1257
Error	36	2650.31	73.620		
Total	49	3948.50			

Appendix 3.43 Analysis of variance for accumulation of potassium (K) in *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	183.73	45.9333		
Treatment	9	272.06	30.2294	0.86	0.5710
Error	36	1270.50	35.2917		
Total	49	1726.30			

Appendix 3.44 Analysis of variance for accumulation calcium (Ca) in *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	187.64	46.9098		
Treatment	9	558.43	62.0475	0.62	0.7682
Error	36	3576.23	99.3398		
Total	49	4322.30			

Appendix 3.45 Analysis of variance for accumulation of zinc (Zn) in *Mimusops zeyheri* in response to 6-benzylaminopurine under greenhouse conditions at 84 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	2.3879	0.59697		
Treatment	9	7.1645	0.79605	1.19	0.3315
Error	36	24.1032	0.66953		
Total	49	33.6556			

Appendix 4.1 Analysis of variance for leaf number of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after initiation, inoculation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.39136	0.06523		
Treatment	9	0.17600	0.02514	0.77	0.6131
Error	36	1.36595	0.03252		
Total	49	1.93330			

Appendix 4.2 Analysis of variance for plant height of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after initiation, inoculation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	6208.9	1034.82		
Treatment	9	1468.8	209.82	1.25	0.2962
Error	36	7028.3	167.34		
Total	49	14706.0			

Appendix 4.3 Analysis of variance for stem diameter of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after initiation, inoculation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.45914	0.07652		
Treatment	9	0.30173	0.04310	0.84	0.5611
Error	36	2.15649	0.05134		
Total	49	2.91736			

Appendix 4.4 Analysis of variance for leaf length of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after initiation, inoculation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	19.011	3.16851		
Treatment	9	11.205	1.60071	0.85	0.5544
Error	36	79.258	1.88708		
Total	49	109.474			

Appendix 4.5 Analysis of variance for leaf width of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after initiation, inoculation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	2.6786	0.44643		
Treatment	9	2.6571	0.37959	1.37	0.2439
Error	36	11.6529	0.27745		
Total	49	16.9886			

Appendix 4.6 Analysis of variance for petiole length of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after initiation, inoculation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	0.14750	0.02458		
Treatment	9	0.45839	0.06548	1.29	0.2797
Error	36	2.13536	0.05084		
Total	49	2.74125			

Appendix 4.7 Analysis of variance for dry shoot mass of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after initiation, inoculation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	3216.03	536.005		
Treatment	9	587.79	83.970	0.63	0.7243
Error	36	5554.15	132.242		
Total	49	9357.97			

Appendix 4.8 Analysis of variance for dry root mass of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after initiation, inoculation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	3255.9	542.642		
Treatment	9	1230.5	175.791	0.65	0.7150
Error	36	11420.5	271.917		
Total	49				

Appendix 4.9 Analysis of variance for chlorophyll content of *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza fungi under greenhouse conditions at 90 days after initiation, inoculation and application of treatments.

Source	DF	SS	MS	F	P
Replication	4	770.54	128.424		
Treatment	9	653.18	93.311	0.55	0.7889
Error	36	7081.99	168.619		
Total	49	8505.71			

Appendix 4.10 Analysis of variance for magnesium (Mg) accumulation in *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza under greenhouse conditions at 90 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	6	185.65	30.9420		
Treatment	7	74.56	10.6514	0.57	0.7749
Error	42	782.99	18.6427		
Total	55	1043.21			

Appendix 4.11 Analysis of variance for iron (Fe) accumulation in *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza under greenhouse conditions at 90 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	6	1.04144	0.17357		
Treatment	7	0.79147	0.11307	0.97	0.4673
Error	42	4.90986	0.11690		
Total	55	6.74277			

Appendix 4.12 Analysis of variance for phosphorus (P) accumulation in *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza under greenhouse conditions at 90 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	6	782.85	130.475		
Treatment	7	607.53	86.790	1.68	0.1403
Error	42	2169.79	51.662		
Total	55	3560.17			

Appendix 4.13 Analysis of variance for accumulation of potassium (K) in *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza under greenhouse conditions at 90 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	6	85.78	14.2973		
Treatment	7	422.77	60.3963	1.40	0.2304
Error	42	1810.30	43.1024		
Total	55	2318.86			

Appendix 4.14 Analysis of variance for accumulation calcium (Ca) in *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza under greenhouse conditions at 90 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	6	1012.2	168.694		
Treatment	7	457.8	65.395	0.29	0.9537
Error	42	9431.8	224.566		
Total	55	10901.7			

Appendix 4.15 Analysis of variance for accumulation of zinc (Zn) in *Mimusops zeyheri* in response to vesicular arbuscular mycorrhiza under greenhouse conditions at 90 days after initiation and application of treatments.

Source	DF	SS	MS	F	P
Replication	6	9.632	1.60535		
Treatment	7	11.860	1.69426	0.76	0.6246
Error	42	93.798	2.23327		
Total	55	115.289			