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THE INFLUENCE OF PHOSPHOROUS, COPPER, ZINC AND ARBUSCULAR MYCORRHIZA ON GROWTH, PHOTOSYNTHETIC PROCESSES AND FINANCIAL VIABILITY OF *ARTEMISIA AFRA* GROWN IN A SIMULATED MARGINIAL SOIL OF THE WESTERN CAPE.

by

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DECLARATION

I, Robin Russell Koehorst, declare that the contents of this thesis represent my own unaided work, and that the thesis has not previously been submitted for academic examination towards any qualification. Furthermore, it represents my own opinions and not necessarily those of the Cape Peninsula University of Technology.

Signed **Date**

ABSTRACT

This study evaluated the effects of supplementary phosphorus, copper and zinc alone and in conjunction with arbuscular mycorrhiza on *Artemisia afra* grown in a simulated soil medium. The treatments consisted two groups. Group A had no mycorrhizal inoculation and 1) no supplementary fertilization, 2) supplementary zinc application, 3) supplementary copper 4) supplementary phosphorus 5) supplementary zinc and copper, 6) supplementary zinc and phosphorus, 7) supplementary copper and phosphorus, 8) supplementary zinc, copper, and phosphorus. Group B had mycorrhizal inoculation in combination with 9) no supplementary fertilization, 10) supplementary zinc application, 11) supplementary copper 12) supplementary phosphorus 13) supplementary zinc and copper, 14) supplementary zinc and phosphorus, 15) supplementary copper and phosphorus, 16) supplementary zinc, copper, and phosphorus.

There was also a pilot study into the pH range most suitable for the cultivation of *A. afra* in a hydroponic system, which was used to refine the mycorrhizal investigation, as pH has influences with regards to nutrient uptake of plants.

The objectives of this study were to assess the effects of supplementary nutrients alone, in combination with each other, and in combination with arbuscular mycorrhiza on the growth and development, photosynthetic processes, anthocyanin content, SPAD-502 levels, and marketability/economic feasibility of *A. afra* grown in a simulated marginal soil medium.

Each treatment was replicated 10 times. Photosynthetic processes were measured by analysing the photosynthetic rate, stomatal conductance, substomotal CO_2 concentration and the transpiration rate of *A. afra* at the start of growing, 6 weeks into the experiments and at week 12 of the experiment. Anthocyanin levels were recorded weekly using a CCM200A plus hand held anthocyanin meter, and SPAD-502 levels were recorded weekly using a hand held SPAD-502 meter. Growth and development was determined by measuring plant height, pre-planting and post harvesting root lengths, fresh and dry weight of roots and stems, and root: shoot ratios. Plant height and stem numbers were recorded at weekly intervals, root and shoot fresh weights were recorded pre planting, and dry weights were recorded at harvest. Root lengths were recorded before planting and after harvest. Economic feasibility of the harvested plants was calculated based on current market prices combined with the wet and dry yield of plant material and the plant's desirability (indicated by the overall appearance of the plants, based on SPAD-502 measurements).

The pilot investigation into pH showed that there was significant growth in the pH 6.5 range, and this indicated that a neutral pH is important for ideal growth of *A.* afra. In the main study,

the most favourable results were attained by the application of supplementary phosphorus, zinc and copper in conjunction with arbuscular mycorrhiza. This treatment produced plants with the highest rates of photosynthesis, the most desired levels of anthocyanin reading and highest SPAD-502 levels, and best growth and development. Before this was the supplementary phosphorus and copper combined with arbuscular mycorrhiza, followed by the supplementary phosphorus and zinc with arbuscular mycorrhiza. All treatments that included mycorrhiza outperformed the same treatments lacking mycorrhiza, regardless of nutrient application. The poorest results were noted in the control (no fertilization or mycorrhiza) as all the tested parameters in this treatment were the lowest.

The application of arbuscular mycorrhiza produced significant growth and development in *A. afra*, regardless of fertilization regime. It is postulated that the symbiosis of arbuscular mycorrhiza with the plant enhanced the plant's resistance to low nutrient levels, and contributed to the overall improved performance of the plant. This indicates that there is great potential in the use of arbuscular mycorrhiza in the cultivation of this widely utilized medicinal crop. More studies are recommended on the influence of arbuscular mycorrhiza on plant growth, specifically on the uptake of nutrients.

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CHAPTER ONE

LITERATURE REVIEW AND INTRODUCTION

Review

Mycorrhizal biotechnology in the cultivation of essential oil yielding Traditional African Medicinal plants.

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1.1 ABSTRACT

Mycorrhiza is the symbiotic association between plant roots and specific species of soil borne fungi. Mycorrhiza can play a large part in the cycling of mineral nutrients from the soil into the plant, and also can protect the host against stress, both environmental and cultural. The relationship of the fungi and host can take a variety of forms and can have a range of effects upon the growth and development of the host plant. The most commonly utilized mycorrhizal relationship type is arbuscular mycorrhiza (AM). AM is used in the cultivation of many important horticultural crops, and has been shown to have an effect upon both ornamental and medicinal crops. Because there is a large amount of information available on the use of AM in an ornamental horticultural situation, combined with a lack of research into the potential for indigenous traditional medicinal crop production, this paper outlines the current perspectives on the use of AM in the cultivation of traditional medicinal plants, with a specific focus on the use of AM in an African setting. Included is a short discussion of the history of traditional medicine and its cultivation/ harvesting in an African setting, specifically pertaining to nutrient applications and the potential of mycorrhiza to decrease reliance on alternate forms of nutrient supplementation. The paper also includes a brief discussion on *Artemisia afra*, of one of the most widely utilized traditional medicinal plants and the potential of utilizing mycorrhiza in its cultivation.

Key words: Medicinal crop production, *Artemisia afra,* soil symbiosis.

Abbreviations: AM = Arbuscular Mycorrhiza; $kg.ha^{-1}$ = kilograms per hectare; mg/L = milligram per litre; $t.ha^{-1}$ = tonne per hectare, TAM = Traditional African Medicine, VAM = Vesicular-Arbuscular Mycorrhiza

1.2 INTRODUCTION

1.2.1. Traditional Indigenous Medicinal Plant Usage

Taylor et al., (2001) has stated that indigenous cultures have relied on traditional medicinal plants to supply their healing needs for centuries. It has been estimated by Fennel et al., (2004) that South Africa alone has about 27 million people that are dependent on traditional medicine for their health care needs. The economic value of indigenous medicinal plants in South Africa is estimated at roughly $6 million annually (Keirungi & Fabricius, 2005). Most other countries in Africa also rely upon Traditional Medicine. In Mali, for example, it is estimated that over 80% of the population relies on traditional medicine as their only type of medicine (Diallo et al., 1996). Although there is cultivation of selected plants by traditional healers, the majority of these plants are collected from the wild (Van Andel & Havinga, 2008). Makunga et al., (2008) has estimated that 700,000 tonnes of medicinal plant material have been harvested in 2009 in South Africa alone. Rapidly expanding human populations and a resurgence of interest in the uses of indigenous knowledge has led to the rapid commercialization of traditional African medicines (Van Wyk, 2008).

Investigations into the medicinal potential of various African plants have yielded some interesting results. The oils of several medicinal plants have been shown to be anti fungal and anti bacterial, with many showing inhibitory effects on gram-positive bacteria. Rabe and van Staden, (1997) has shown that 12 of their 21 investigated plants can have positive effects against *Bacillus subtilis*. They have also found that a *Warburgia salutaris* methanol extract inhibited the growth of *Escherichia coli*. Cimanga et al., (2001) has investigated 15 African plants that showed antibacterial properties and found that many African plant's oils can have significant effects against bacteria. Lourens et al., (2004) has performed investigations into the *in vitro* biological activity of several plant species indigenous to South Africa, and found that the acetone extract of the indigenous *Helichrysum dasyanthum* had good results against *Staphylococcus aureus*. Boyom et al., (2003) has found that some plants native to Cameroon exhibit anti-plasmodial activity. They found that the essential oils of *Xylopia phloiodora*, *Pachypodanthium confine*, *Antidesma laciniatum*, *Xylopia aethiopica*, and *Hexalobus crispiflorus* all have good results against *Plasmodium falciparum*. It was surmised by the investigators that the various sesquiterpenoid compounds (such as α-copaene, γ-cadinene, δ-cadinene, α-cadinol, spathulenol, and caryophyllene oxide) found in the essential oils are the major contributors to the anti-plasmidial actions of these oils.

2. Arbuscular Mycorrhiza

2.1 General concepts

Mycorrhiza are symbiotic associations between the roots of certain plants and specific soil fungi (Kapoor et al, 2002). Almost all soil types have the ability to support mycorrhiza (Entry et al., 2002). Almost all of the plant families are able to form these relationships (Phosri et al., 2010), with the exception of most plants in the Cruciferae, Chenopodiaceae, Cyperaceae, Caryophyllaceae and Juncaceae families (Cardoso & Kuyper, 2006). However, there are some species in these families that do form mycorrhiza (Azcon-Aguilar & Barea, 1997). Because of the large variety of environments and host plant morphology, there is a large variety in the types of mycorrhizal relationships. Despite the variety of hosts, there is a distinct lack of species specificity of the fungi for its host (Feddermann et al., 2010).

There are a variety of types of mycorrhizal relationships (Phosri et al., 2010). The most commonly investigated and utilized of the mycorrhizal relationships is that of the arbuscular mycorrhiza (AM) (Zak et al., 1998). These AM form mutualistic relationships with the host plants, providing nutrients extracted from the soil in return for carbon that the host plant has produced through photosynthesis (Verkade & Hamilton, 1983). AM also provide protection to the plant roots from stressors, including environmental and cultural stressors (Feddermann et al., 2010; Kapoor et al., 2008). It is this mutualism that is utilized by plant growers to increase crop quality and quantity (Thompson, 1996).

The process in which the fungus establishes its relationship with the roots of its host plant begins when the fungus biotrophically colonises the root cortex of the plant. This colonization is a process that involves both the host plant as well as the ‘invading’ fungus (Feddermann et al., 2010). Although there is little experimental information on the actual processes of infection, it is generally accepted that colonization must include both signals from the fungus to the plant, as well as responses by the plant to the signals (Azcon-Aguilar & Barea, 1997; Feddermann et al., 2010). It has been proposed that there is a continuous molecular exchange or ‘dialogue’ between the host plant and the fungus (Feddermann et al., 2010). Once the fungus colonizes the root cortex it essentially becomes an integral part of the plant’s roots. The plant’s root morphology is significantly modified by the ‘invasion’ of the AM (Clark & Zeto, 1996). Once the fungus has established itself in the cortex of the plant root it then establishes an extra-matrical mycelium. The mycelium both protects the plant root from stressors such as water and cultural stress and assists in the acquisition of nutrients from the soil. (Azcon-Aguilar & Barea, 1997; Clark & Zeto, 1996; Feddermann et al., 2010; Kapoor et al., 2008).

The relationship between the fungus and the host plant is considered to be mutualistic because the host receives mineral nutrition that the mycelium ‘mines’ from the soil, while the

fungus receives both carbon compounds that are produced during photosynthesis and a habitat that is ecologically protected (Azcon-Aguilar & Barea, 1997).

2.2 Influences of mycorrhiza on uptake of P, Zn, and Cu

AM are known to contribute to enhanced plant growth for two primary reasons. The first is that they provide protection against various stressors (Azcon-Aguilar & Barea, 1997). The second (and generally larger) contribution of AM to the growth of the host plant is due to the increased uptake of various mineral nutrients (Azcon-Aguilar & Barea, 1997; Clark & Zeto, 1996).

The process in which the mycorrhiza assists in the nutrient uptake of the plant is twofold. The major effect of the fungus is to increase the surface area of the absorption areas of the roots (Cardoso & Kuyper, 2006; Clark & Zeto, 1996). This is achieved by the development of the extra-matrical mycelium that forms after the colonization of the root cortex. The mycelium extends into the soil for up to 2 cm from the roots of the host (Feddermann et al., 2010). This enables the mycelium to reach areas of the soil that have not been depleted of nutrients by the unassisted roots of the host. Another aspect of the mycelium is that, as it is of very small dimensions, it can travel through soil pores that are too small for the roots of the host to travel through, enabling it to reach reserves of mineral nutrients that are inaccessible to the host's unaided roots (Gupta et al., 2002). These two features of AM are thought to particularly increase the uptake of minerals that are of low soil mobility, such as phosphorus, zinc and copper (Cardoso & Kuyper, 2006; Feddermann et al., 2005).

The second effect of the mutualism of the host and fungus is that the fungus has a 'damping' effect on the host plant's natural processes of nutrient uptake (Feddermann et al., 2010). Although this seems to be contradictory to the healthy growth of the host, the fact that the mycorrhiza enables the uptake of much more mineral nutrients than the unassisted host plant negates this.

The process by which the mycorrhiza restricts the nutrient uptake of the roots is not clearly understood, but it is assumed that the epidermal phosphate transporters of the host are down regulated (Feddermann et al., 2010). Instead of the host taking phosphorus from the soil, the fungal hyphae of the mycorrhiza deliver phosphorus directly to the root cortex as polyphosphate. Once in the root cortex the polyphosphate is taken up by cells through high affinity P-transporters. These are located in the periarbuscular membrane (Feddermann et al., 2010).

3. The effect of mycorrhizal relationships on the production and composition of the essential oils of various plants.

Toussaint et al., (2007) investigated the effects of vesicular-arbuscular mycorrhiza (VAM) upon the phytochemical levels of the essential oils of *Ocimum basilicum* and found that the presence of VAM increased levels of phytochemicals regardless of phosphorus addition. Their investigations showed that the presence of the AM fungus *Glomus caledonium* increased the amounts of rosmarinic and caffeic acid levels in the plant shoots, regardless of the levels of supplementary phosphorus. They also found that the presence of *Glomus mosseae* increased the levels of caffeic acid, regardless of suplimentary phosphorus nutrition. The fact that there was a positive effect in phytochemical levels irrespective of the levels of phosphorus nutrition indicates the potential of VAM in a setting with a lack of sufficient plant nutrition.

Kapoor et al., (2002) tested the effects of two strains of VAM (*Glomus macrocarpum* and *G. fasciculatum*) on the quality of essential oils from *Coriandrum sativum*, and found that the application of both varieties of AM significantly increased the concentrations of both geraniol and linalool in the essential oils. The application *of G. macrocarpum* VAM increased the yield of essential oil by 28%, while the use of *G. fasciculatum* inoculation increased the essential oil yield by 43%. They also discovered that the shoots of VAM inoculated plants had higher levels of phosphorus than the non-VAM inoculated plants. This, along with the fact that phosphorus is essential in the formation of essential oils indicates the potential of VAM in the cultivation of essential oil crops in areas lacking suitable levels of phosphorus.

Khaosaad et al., (2006) found that mycorrhiza can have an effect on both the yield and composition of the essential oils found in oregano. Their investigations showed that mycorrhization positively affected the essential oil concentration in *Oregenum basilicum* and *O. vulgare*. However, there were major variations in the increase in concentration of essential oils among genotypes. This illustrates the need to investigate the effects of mycorrhiza upon a variety of plants to ascertain the effects upon individual plant genomes.

3.1 *Artemisia afra*

Artemisia afra (Asteraceae) is undoubtedly one of the most widely collected and used of the traditional African medicinal plants. This is a highly aromatic perennial shrub, growing up to two meters tall with an equal spread. *A. afra* grows erect, with multiple stems. The leaves tend towards a light grey-green colour, and are feathery and finely divided. The flowers, borne on the branch ends, are pale yellow and are not significantly large. It is found on the African continent, occurring as far north as Ethiopia and tropical east Africa, and as far south as the Western Cape of South Africa.

A. afra has long been used to treat a large variety of conditions, ranging from colds and fevers, to open wounds, to intestinal worms. One of the major uses is to clear congested sinuses, either by the insertion of fresh leaves into the nostrils or by the use of the steam of the leaves plunged into boiling water (Liu et al., 2008; Thring & Weitz, 2006). Another traditional use is in the preparation of 'Wilde als brandy'. This has been a staple of the rural medicine chest for generations, and is used for the treatment of coughs, colds and chest infections, as well as a variety of stomach ailments (Liu et al., 2008; Thring & Weitz, 2006).

The volatile oils of *A. afra* have been shown to have definite anti-oxidative and antimicrobial actions. The oils contain mainly artimisyl acetate, 1,8-cineole, α-thujone, β-thujone, as well as camphor and borneol (Liu et al., 2008). The levels of these metabolites vary greatly in plants collected from different geographic locations. For example, plants that were collected from Ethiopia were to found to have levels of artimisyl acetate in the oil between 24.4 and 32.1 percent (Liu et al., 2008). However, oils from Kenya were found to consist of approximately 67.4% 1,8-cineole, while oils from South Africa were found to consist of roughly 54.2% α-thujone (Liu et al., 2008). It has also been found that the levels of various metabolites in the oils of cultivated *A. afra* versus wild populations can vary greatly. For example, the levels of artemisia ketone in the wild populations of Zimbabwe were found to vary from 6.3 – 41.9%, while the levels of artemisia ketone in the cultivated plants ranged from 32.1 – 34.8% (Liu et al., 2008). This is undoubtedly due to the application of specific fertilizer and cultivation regimes, such as the supply of sufficient irrigation water and required nutrients. The usefulness of any medicine is directly related to the certainty of finding stable levels of active agents (Canter et al., 2005), and it appears that cultivation is one method of ensuring that specific levels of various metabolites are constant throughout harvests.

3.2 Possible effects of mycorrhiza on the growth, development and yield of constituents of *A. afra*

The presence of mycorrhiza has been shown to have a significant effect on a large variety of growth parameters. This has been attributed to (among other factors): i) the improved rooting and establishment of 'infected' plants; ii) the improved uptake of nutrients, specifically those ions of low mobility; iii) increased resistance to various stressors, both biotic and abiotic (Azcon-Aguilar & Barea, 1997; Clark & Zeto, 1996; Feddermann et al., 2010; Zak et al., 1998). Each of these factors is interrelated, and they can be reduced to the lowest common denominator – the fact that the presence of mycorrhiza in the root cortex greatly assists with nutrient uptake and cycling (Azcon-Aguilar & Barea, 1997; Feddermann et al., 2010; Zak et al., 1998). This is significant to not only the growth of the plant as measured by an increase in the phytomass, but also to the fact that increased nutrient availability could lead to a stability in the levels of various metabolites in the oils of the plant (Canter et al., 2005).

It has been postulated that there is a direct link between the stress levels that a plant experiences during growth and the levels of metabolites found in the plant's oils (Zobayed et al., 2007). It is thought that the production of metabolites is many plants's response to situations of less than ideal growth parameters (Jahangir et al., 2009). This seems evident in the fact that, although there is a much larger variation in various levels of artemisia ketone in *A. afra* harvested from the wild, the plants under cultivation had significantly lower upper levels of artemisia ketone. It is apparent from this that there are factors that, if controlled, could increase the yield of this metabolite while still providing the low levels of variation in concentration that result from organized cultivation. Although artemisia ketone has been used as an example, it should be readily apparent that the yield of other desired metabolites could be manipulated by similar techniques.

If various stressors do have an impact upon the yields of various secondary metabolites, the effect that mycorrhiza has on the plant's ability to withstand stressors should result in a change in the yield of secondary metabolites. To discuss the various anticipated effects of mycorrhiza upon secondary metabolite yields each of the major effects of mycorrhizal infection will be discussed in relation to the anticipated result.

3.2.1 Enhanced uptake of nutrients

Phosphorus is regarded as a macro-nutrient. This is because it is found relatively large concentrations in plant tissues. It is part of many important organic compounds, such as ATP, phospholipids, nucleic acids and sugar phosphates (Rodríguez & Fraga, 1999). These compounds are essential for successful plant growth, and therefore the presence or absence of phosphorus can have a significant effect upon not only the growth, but also the yield of secondary metabolites of plant species.

Zinc is required in the formation of indoleacetic acid, as well as the activation of various enzymes (Nag et al., 1984). Indoleacetic acid is one of the primary plant auxins, and as such plays a vital role in cell elongation and cell division (Hartmann et al., 2002). The lack of zinc can have negative effects upon the growth and development of plants due to the fact that the plant is unable to produce sufficient levels of this hormone (Hartmann et al., 2002; Nag et at., 1984),

Copper plays a significant part in the photosynthetic process. It acts as an electron carrier and is also part of certain enzymes. The lack of copper can significantly reduce the performance of plants as copper deficient plants display reduced levels of photosynthesis (Hartmann et al., 2002).

In many plants one of the results of growing in a low nutrient situation is the release of secondary metabolites into the soil (Makoi et al., 2010). These metabolites provide a signal

for soil borne funguses to invade the cortex of the roots and form a mycorrhizal relationship. Once a mycorrhizal relationship has formed, the nutrient requirements of the plant are more successfully met (Azcon-Aguilar & Barea, 1997; Clark & Zeto, 1996; Feddermann et al., 2010; Kapoor et al., 2002;). The fact that the presence of mycorrhiza has a significant effect on nutrient uptake and cycling suggests that there will be a resultant impact upon the various growth factors of the plant. Although it is known that certain plants can be made to increase production of useful secondary metabolites by exposure to various stresses, such as low levels of nutrients, there is little information regarding the relationship between the increase in phytomass (as a result of all growth factors being satisfactorily met) and the yields of metabolites. It would be beneficial to growers to know that if, for example, the growth conditions are perfectly met the plant will increase in mass by a factor of two, while the yield of metabolites will increase by a factor of 1.5. This would indicate to the grower that it would be more economically feasible to cultivate more plants under less than ideal conditions, thereby decreasing phytomass but increasing the levels of useful metabolites.

3.2.2 Increased resistance to stressors

The fact that the presence of mycorrhiza not only assists in the uptake of nutrients but also protects the infected plant's root system from various stressors indicates that AM could have significant uses in the cultivation of medicinal plants in small scale situations. The reason that many small scale cultivators ultimately fail in the production of a high quality product can often be related to stressors that could have been alleviated with the use of mycorrhiza (Kaya et al., 2009).

One such stress is that of invasion of the root system by various soil biota (Cardoso & Kuyper, 2006). The roots of plants that have been infected by a mycorrhizal fungus have been shown to be resistant to many invading fauna (Cardoso & Kuyper, 2006). Mycorrhizal colonized plants have been shown to demonstrate a significant level of bioprotection against pathogens such as *Fusarium*, *Phytophthora* and Nematodes (Azcon-Aguilar & Barea, 1997; Feldmann et al., 1989; Vos et al., 2013). This increased resistance could be utilised by the small scale farmers to alleviate the impact of destructive soil biota, as well as lower the farmer's dependence upon traditional agricultural pest prevention methods such as the use of chemical pesticides.

Another use of mycorrhiza in the alleviation of stressors is the enhancement of the survival of plants in areas of poor soil quality. A mycorrhizal symbiosis may enhance the survival of plants in poor soil areas by the amelioration of negative soil structure and the mycorrhiza's contribution to soil aggregation (Haselwandter & Bowen, 1996).

One of the most pressing of plant stressors in Africa is the low levels of water in many areas. This can have a large impact upon the success or failure of any cultivation attempt. (Zak et

al., 1998). It has been shown that the presence of mycorrhiza can increase the resistance of the infected crop to low water situations (Asrar & Elhindi, 2010; Azcon-Aguilar & Barea, 1997; Kapoor et al., 2008). One proposed mechanism for this improved resistance is that the presence of mycorrhiza leads to an increase in the root hydraulic conductivity. Another mechanism is the enhanced water uptake as a result of the extra-radical hyphae. Asrar and Elhindi (2010) have shown in marigolds that the water uptake of plants inoculated with mycorrhiza is increased, as is the growth under drought conditions. Asrar & Elhindi (2010) also showed that, while the levels of phosphorus in drought affected plants were lower than the control, the levels of phosphorus in drought exposed plants that were inoculated with the mycorrhiza were higher than those in plants that were not inoculated with the fungus. This fact illustrates the synergistic effects that mycorrhizal infection can have on the growth and development of various plants. The enhanced resistance to drought is a feature of the mycorrhizal relationship that could also be utilised by small scale farmers to reduce the impact of low water levels.

4. Conclusion and recommendations

The use of mycorrhizal relationships has been shown to have a significant effect upon numerous growth factors of various plants. Although there has been a large amount of research with regards to the effects of mycorrhiza upon horticultural and medicinal crops, there is a distinct lack of experimental information regarding the use of mycorrhiza in the cultivation of traditional African medicinal plants. The potential uses of mycorrhizal relationships in the small scale cultivation of traditional crops are many, and its use could lead to more sustainable cultivation of high quality medicinal plants. The fact that mycorrhizal relationships provide enhanced nutrient uptake, protect the roots of infected plants from attack, and also increases the infected plant's resistance to drought stressors should be investigated further, along with the impacts that these benefits will have on the growth and development of medicinal plants. There is scope for investigations into the actions of mycorrhizal relationships, particularly with regards to the cultivation of indigenous African medicines. This is an area of plant cultivation that could benefit from any systems that would serve to ameliorate the need for expensive soil conditioners and nutrient applications. There is a need for further investigations into the actions of mycorrhizal relationships upon the yields of medicinal plants, both the plant matter yield, essential oil yield, and the yield of useful metabolites. There is also a need for investigations into the financial aspects of the utilization of arbuscular mycorrhiza in the cultivation of medicinal crops. Another area of investigation with regards to arbuscular mycorrhiza is its effects upon the photosynthetic processes of traditional medicinal plants.

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CHAPTER TWO

PROBLEM STATEMENT, AIMS, HYPOTHESIS AND OBJECTIVES

2.1 PROBLEM STATEMENT

The harvesting of traditional medicinal plants from the wild in Africa is leading to the threatened status of many important species. Cultivation of these plants is one method of maintaining populations, but many end users argue that plants in cultivation are not of the same quality as those found in nature. One of the factors in the growth of medicinally useful plants is the formation of symbiotic relationships with arbuscular mycorrhiza (AM). In the wild these relationships form naturally, but in cultivation there is often a lack of symbiotic relationships. The use of these relationships in cultivation has the potential of increasing output, while reducing reliance on chemical fertilizers.

Production of quality medicinal crops is reliant on the use of high quantities of inorganic fertilizers. This increases costs of production, which leads to reduction of profits. Without these inputs the quality of cultivated plants is lower, leading to end users preferring to harvest medicinal plants from the wild. This leads to the threatened status of many important species. One of the factors in the growth of medicinally useful plants is the formation of symbiotic relationships with arbuscular mycorrhiza (AM). In the wild these relationships form naturally, but in cultivation there is often a lack of symbiotic relationships. The use of these relationships in cultivation has the potential of increasing output, while reducing reliance on chemical fertilizers.

2.2 AIMS

This study aims at cultivating *Artemisia afra* using supplementary levels of phosphorus, zinc and copper alone and in conjunction with arbuscular mycorrhiza

2.3 HYPOTHESIS

The growth and development of *A. afra* will be influenced by the nutrient uptake capacities of the arbuscular mycorrhiza.

2.4 OBJECTIVES OF THE RESEARCH

2.4.1 MAIN OBJECTIVES

To assess *A. afra* growth when treated with supplementary nutrient applications alone and combined with arbuscular mycorrhiza.

2.4.2. SPECIFIC OBJECTIVES:

1) Assess an ideal pH range for the cultivation of *A. afra* to establish suitable growing conditions.

2) Assess the effect of phosphorus, zinc and copper supplementation with and without arbuscular mycorrhiza on growth and development of *A. afra* grown in simulated soil cultivation.

3) Evaluate the effect of supplementary phosphorus, zinc and copper with and without arbuscular mycorrhiza on *A. afra* with regards to photosynthesis and chlorophyll content of *A. afra* grown in simulated soil cultivation.

4) Assess the effects of different combinations of arbuscular mycorrhiza and supplementary fertilization on marketability and economic feasibility of *A. afra* grown in simulated soil.

CHAPTER THREE

GROWTH RESPONSE OF *ARTEMISIA AFRA* JACQ. TO DIFFERENT PH LEVELS IN A CLOSED HYDROPONICS SYSTEM.

GROWTH RESPONSE OF *ARTEMISIA AFRA* JACQ. TO DIFFERENT PH LEVELS IN A CLOSED HYDROPONICS SYSTEM.

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3.1 ABSTRACT

In this study the effect of varying levels of pH on the fresh and dried yield and chlorophyll content of *Artemisia afra* Jacq. was investigated. Five groups of plants received deionised water adjusted to a certain pH. The pH for the treatments were 4.5, 5.5, 6.5 (the control), 7.5, and 8.5. The fresh weight of the plants was highest for the pH treatment of 6.5, while the fresh weight of the 4.5, 5.5 and 7.5 treatments did not differ significantly. The plants grown with a pH of 8.5 were significantly reduced in fresh weight. The total dry weight, shoot dry weight and root dry weight of the plants did not show significant variation between the 5.5, 6.5 and 7.5 treatments, but was significantly lower for the 4.5 and 8.5 treatments. The chlorophyll content of the leaves of the plants showed a marked variation between treatments. The chlorophyll content of the plants grown at pH 6.5 was highest, followed by those grown at the pH of 5.5 and the pH 4.5. The chlorophyll content of the pH 7.5 treatment was the second lowest, while the content of the 8.5 treatment was the lowest. These results indicated that the optimum pH of water supplied to *A. afra* can have a significant effect on the fresh and dried yield of this plant. The study shows that, although *A. afra* can survive at a range of acidic pH's, it does not fare well with regards to chlorophyll content, fresh weight, root dry weight and shoot dry weight in an alkaline or acidic situation.

Key words: Chlorophyll content, traditional medicine, yield, *Artemisia afra*, pH level, acidic stress.

3.2 INTRODUCTION

Indigenous cultures have relied on plants to supply their medicinal needs for centuries (Taylor et al., 2001). In South Africa alone it is estimated that about 27 million people depend on traditional medicine for their health care needs (Fennell et al., 2004). One estimate of the economic value of medicinal plants in South Africa is that the trade generates roughly $6 million annually (Keirungi & Fabricius, 2005). By far the majority of these plants are collected from the wild (Van Andel & Havinga, 2008). It has been predicted that around 700,000 tonnes of medicinal plants will be harvested in 2009 (Makunga et al., 2008). In the last 10

years the commercialization of Traditional African Medicines has been rapidly gaining momentum (Van Wyk, 2008).

In the past, low population densities helped to limit the demands placed on the natural ecosystems by harvesters (Netshiluvhi, 1999), most of whom are traditional healers (Van Andel and Havinga, 2008). However, rising unemployment levels combined with the entry into a cash economy has led to a breakdown of traditional conservation methods (Netshiluvhi, 1999). As the effectiveness of medicinal plants is more widely acknowledged and accepted, over harvesting and extinction can result (Strangeland et al., 2008). According to McGeocha et al. (2008), “Over exploitation is a growing problem for many medicinal species in Africa”. As an example, in Tanzania alone there are nine plants of medicinal value that are reported to be of conservation concern (Strangeland et al., 2008). Although there is still a drive towards sustainable harvesting, increasing demand coupled with the loss of habitats is quickly leading to the only real solution being the cultivation of important medicinal plants (Fennell et al., 2004). According to Netshiluvhi (1999), the supply of the most commonly used plants could be ensured only by using a “...firm scientific basis for propagation”.

Although it is agreed that there is a need for the cultivation of medicinal plants, there is a lack of relevant information available as to the specific requirements of these plants (Makunga et al., 2008; Fennell et al., 2004; McGeocha et al., 2008). Little information exists on the effects of cultivation practices on the growth and biological activity of African medicinal plants (Fennell et al., 2004). There is a pertinent need to determine which plants would be suitable for cultivation on a medium to large scale (Van Wyk, 2008). Street et al. (2008) reported that 82% of traditional healers would cultivate the medicinal plants that they use. While there is information regarding specific crops it is generally circumstantial and general in nature, with little scientific justification.

The most widely utilized medicinal plant in southern Africa is undoubtedly *Artemisia afra* Jacq. (Liu et al., 2008; Diederichs, 2006; Van Wyk, 2008). The most common method of use is as either dry or fresh leaves and shoots boiled and then used as a tea. Sometimes the roots are also used (Diederichs, 2006; Liu et al., 2008). It is often also used fresh to pack around painful teeth, or as a decoction that is used against gum infections by holding in the mouth (Diederichs, 2006). *A. afra* Jacq. Contains many chemical compounds (Liu et al., 2008; Van Wyk, 2008). The most common components are scopoletin, found in the flower heads, and α–thujone, ß-thujone, artemisyl acetate and artemisia ketone. *A. afra* also contains camphor, santolina alcohol, and borneol, as well as a large number of secondary metabolites. (Bohlmann and Zdero, 1972; Liu et al., 2008). The main traditional uses of *A. afra* are to treat chest problems, such as coughs, asthma, pneumonia, croup, influenza and

upper respiratory tract infections. It can be used to treat stomach problems like gastritis, gastric derangement, dyspepsia, poor appetite, indigestion, constipation, flatulence, colic and intestinal worms (Diederichs, 2006; Liu et al., 2008; Gurib-Fakim, 2006). It is also used to treat gout, malaria, fevers, colds, chills, bladder and kidney disorders, diabetes, convulsions, heart inflammation, rheumatism, and sore throats and it is sometimes used as a purgative (Diederichs, 2006; Liu et al., 2008; Gurib-Fakim, 2006). *A. afra* has shown some antimicrobial and antioxidative activity in in vitro tests (Viljoen, 2007).

A. afra can tolerate a wide range of environments, (Diederichs, 2006) but is reported to grow best in a sandy loam soil (Grey, 2009). Although there have been studies that investigate the role of pH, nitrogen fertilization and other growth factors on this genus (Ozguven et al., 2008; Liu et al., 2003) there is little research available on *A. afra* in particular, specifically with regards to suitable pH ranges. The pH of the soil is an important factor influencing the choice of crop to grow (Diederichs, 2006; Hartmann et al., 2002; Stern, 2006). Although the pH of the soil can be manipulated via the addition of certain products, such as the application of sulphur to lower the pH or lime to increase the pH (Denisen, 1979), it is often not practical for the small scale subsistence farmer. pH is a critical variable in plant growth (Rengel, 2003). As well as affecting the availability of various elements to the plant (Kunh et al., 1995, Marschner 1995), research has indicated that pH can have a significant influence on the growth and essential oil yield of various plants (Ram et al., 1997). Research by Kuhn et al. (1995) has shown that pH can have an adverse effect on plant growth, particularly on those that are being cultivated in hydroponic cultures. As the pH approaches 5.5 and below calcium, magnesium, zinc and copper are less readily available for plant uptake (Brady and Weil, 2008). Despite the fact that these elements (with the exception of magnesium) do not play a direct part in chlorophyll formation, they do contribute to the action of enzymes and thereby affect the action of certain metabolic processes, which in turn influence plant weight (Stern, 2006). As the pH rises above 7.5 phosphorus, iron, manganese, boron and zinc are reduced in their availability to plants (Brady and Weil, 2008, Kunh et al., 1995; Marschner, 1995). According to Stern (2006) the lack of minerals such as phosphorus and iron can lead to a loss of chlorophyll. A lack of these nutrients, especially iron, could lead to the restricted development of chlorophyll in *A. afra*. Before a plant can be recommended for cultivation it is essential that the pH range that it will be most productive in is known. In this study the aim was to determine the optimum pH for the cultivation of *A. afra*, which could assist future growers with improved commercial success in the cultivation practices of this important medicinal plant species.

3.3 MATERIALS AND METHODS

3.3.1 Experimental design

3.3.2 Glasshouse experiment

The experiment was conducted from July to October 2009. It was located in the research greenhouse of the Cape Peninsula University of Technology in the Western Cape of South Africa. The latitude and longitude are S33°55' 58 E18°25' 57. The climate controlled greenhouse had temperatures ranging from 16 - 36°C during the days, and 10 - 18°C at night. The relative humidity of the glasshouse averaged 35%. There is a 40% Alunet shade cloth suspended 2 m above the ground of the glasshouse. The light intensities ranged from 030 lux to 600 lux, as measured by a Toptronic T630 light meter. Irrigation water was supplied from a Hager IP65 Water Filtration Plant de-ioniser, and had an average temperature of 16°C.

3.3.3 Hydroponic experiment

A recirculation soilless medium setup was used to supply the treatments to the plants. 15 cm plastic pots were filled with approximately 220 g of medium grade horticultural perlite. This medium was chosen due to its neutral pH and lack of nutrients. The fluoride content of the perlite was reduced by a series of flushes with deionised water. The pots were lined at the bottom with discs of shade cloth to prevent any medium leaving through the drainage holes. Each treatment had 20 pots, each containing one plant. Every pot functioned as an experimental unit and was placed randomly in one of the five treatments. The treatments were placed on galvanized steel tables which were divided into five separate compartments (each of which was 40 cm × 100 cm), one for each of the treatments. Each treatment drained into its own plastic 65 L container which was used as a reservoir to hold the water treatment. Each reservoir contained its own 1350 L/h hour Boyu submersible pump. The water was supplied to the pots via spaghetti tubing inserted into the medium. A TopTronic TMT24 analogue timer was used to activate the pumps used to irrigate the plants. The timer was set to provide water for 15 min every 90 min. This resulted in each pot receiving 2L of water every 90 min, ensuring that the medium was wet to carrying capacity and then had time to drain. As the water drained out of the pots it drained back into the reservoirs.

3.3.4 Factors controlled in the experiment

After setup but before planting the system was turned on and allowed to run for 24 h with a 1 ml per 2 L of water concentration of SporeKill (supplied by Hygrotech) (active ingredient Didecyl Dimethyl Ammonium Chloride 120 g/l) to disinfect the medium and system. After the 24 h period the whole system was flushed three times with deionised water and allowed to

run for another 24 hour period with deionised water, before being filled the final time with the prepared treatments.

3.3.5 Plant selection and planting process

Two month old *A. afra* Jacq. plants were obtained from Good Hope Nursery. They all originated from one mother stock plant identified as a suitable phenotype for medicinal use by a group of local traditional healers (Grey, 2009). Prior to planting the plants in the hydroponics system they were thoroughly washed in deionised water to remove any foreign matter from their roots.

3.3.6 Treatment preparation

The experiment was laid in a randomised complete block design. There were 5 treatments, each of which was applied to 20 plants. The treatments were pH 4.5, 5.5, 6.5 (the control), 7.5, and 8.5. Hydroponic pH was maintained by the addition of NaOH to raise or HCl to lower the pH. The application of the treatments was via the hydroponic nutrient solution adjusted to the required pH. The plants were fed by adding 2 g/L of the commercially available hydroponic fertilizer Chemicult® [Chemicult Products (Pty) ltd, 133 Camps Bay, South Africa, 8040] to the water supply. The first dose of fertilizer was prepared according to the instructions supplied with the fertilizer, and the electrical conductivity (EC) of the water was tested. The EC of the water was determined to be 1600 µs at the recommended dose of fertilizer. After the first dose the EC was maintained at 1600 µs by the addition of Chemicult dissolved in a small amount of deionised water. The pH and EC were monitored every two days using Martini Instrument's PH55 handheld pH meter and a DIST handheld EC meter respectively.

3.3.7 Data collection

The plants were grown in the hydroponic system for 2 months. They were planted on the 24th of July, and were harvested on the 2nd of October, when the plants were still in their active stage of growth. On the day before harvesting the chlorophyll content of the leaves was measured. Average chlorophyll content (SPAD value) of four leaves was taken for each plant, using a Chlorophyll Meter (Konica Minolta SPAD-502, Spectrum Technologies, Plainfield, Illinois). Immediately after harvesting, the fresh plant weights were determined, after which they were sun-dried to constant weight for 4 weeks and the roots and shoots separated and their respective weights determined once again (Diederichs, 2006; Liu et al., 2008).

3.3.8 Statistical analysis

Mean values of data collected of yield components were analyzed statistically using a one way analysis of variance (ANOVA). These computations were performed with the software program Statistica version 8 (Hill & Lewicki, 2006) Fisher's Least Significant Difference (LSD) was used to compare treatment means at $P \leq 0.05$ (Steel & Torrie, 1980).

3.4 RESULTS AND DISCUSSION

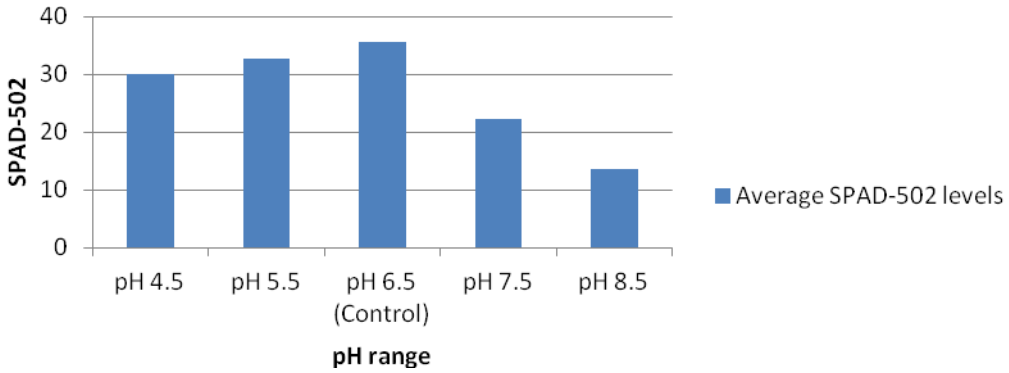

Figure 3.1 Effect of pH on average chlorophyll (SPAD-502) content *A. afra.*

Results of the determination of the effect of pH on the chlorophyll content of the plants are shown in Figure 3.1. The chlorophyll content was significantly affected ($P \leq 0.001$) by the variations of pH. Results showed that plants grown at a pH 6.5 (control) had significantly higher levels of chlorophyll content, followed by those grown at pH 5.5 and 4.5 respectively. Results also showed that at pH 7.5, the chlorophyll content of the plants was significantly reduced when compared with the control. The pH 8.5 plants showed the lowest levels of chlorophyll and were significantly lower than those of the control and all other treatments. In this study, nutrient availability was not measured. However, it seems that nutrient availability was adversely affected by pH extremes and this was in agreement with the findings of Edmond et al. (1975), Reed (1996); Preece and Read (2005). (Table 3.1)

At the pH of 5.5 and below calcium, magnesium, zinc and copper are less readily available for plant uptake (Brady & Weil, 2008). The reduction of the availability of these minerals is due to the impairment of the net extrusion of H+, combined with the displacement of the various nutrients' bivalent cations from adsorption sites such as cell walls and membranes by aluminium (Kunh et al., 1995, Marschner, 1995). Although these elements (with the exception of magnesium) do not play a direct part in chlorophyll formation, they do contribute to the action of enzymes, which in turn affects the action of metabolic processes, and thereby the creation of plant weight (Stern, 2006). Magnesium does play a part of chlorophyll

synthesis, and this could explain the low chlorophyll content of the plants in the pH 4.5 and 5.5 treatment when compared with the control (Table 3.1).

At a pH above 7.5 phosphorus, iron, manganese, boron and zinc are reduced in their availability to plants (Brady and Weil, 2008). In an alkaline situation phosphorus becomes unavailable to the plants due to adsorption and precipitation reactions (Bertrand et al., 2003). The precipitation of ferric oxide is the major factor influencing the availability of iron in alkaline soils. With a soil pH that is in the alkaline range, zinc becomes less available to the plants due to the adsorption of zinc by soil constituents. Manganese is less available for plants in a soil with an alkaline pH due to the manganese forming into insoluble oxide forms. (Wilkinson, 2000). Although not measured, it is proposed that the lack of minerals such as phosphorus and iron can lead to a loss of chlorophyll (Stern, 2006). The deficit of these nutrients, especially iron, could lead to the restricted development of chlorophyll in the pH 7.5 and 8.5 treatments (Table 3.1).

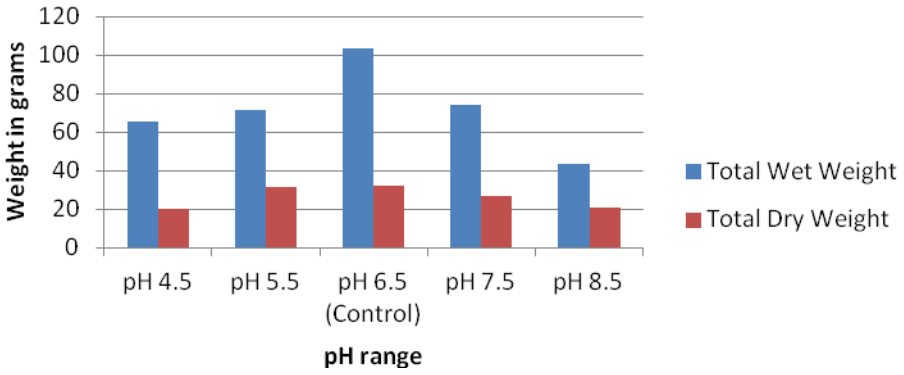

Figure 3.2 Effect of pH on average total wet and average total dry weights of *A. afra.*

The manipulation of the pH significantly ($P \leq 0.001$) affected the average fresh weight of the plants (Figure 3.2). The highest measurement was obtained in the control treatment of pH 6.5 (Table 3.1). The plants that were grown in pH adjusted to 4.5, 5.5 and 7.5, all had fresh weights that were significantly lower than the control. However, they did not vary in a statistically significant way from each other. The plants exposed to the pH 8.5 treatment were significantly lower in fresh weight when compared with the control. They were also significantly lower than the 4.5, 5.5 and 7.5 treatments (Table 3.1). The pH 5.5 and 7.5 treatments producing similar fresh weights may be attributed to the fact that at these pH values there is no major impact on nutrient availability (Brady and Weil, 2008; Van Oorschot et al., 1997). As pH approaches 4.5, calcium, magnesium and copper become less available. As Reed (1996) has shown, these are needed in large quantities in the development of the plants. This could explain the fact that the 4.5 treatment differed significantly from the control in fresh weight, which is probably due to the unavailability of magnesium, copper and

calcium. Research has shown that as pH is raised above 7.5 minerals such as phosphorus, iron, manganese, and boron begin to become unavailable to the plants (Edmond et al., 1975; Reed, 1996; Preece & Read, 2005; Brady & Weil, 2008). These minerals are essential for plant development, and this could contribute to the significantly lower fresh weight of the plants grown at a pH of 8.5 as compared to near neutral pH.

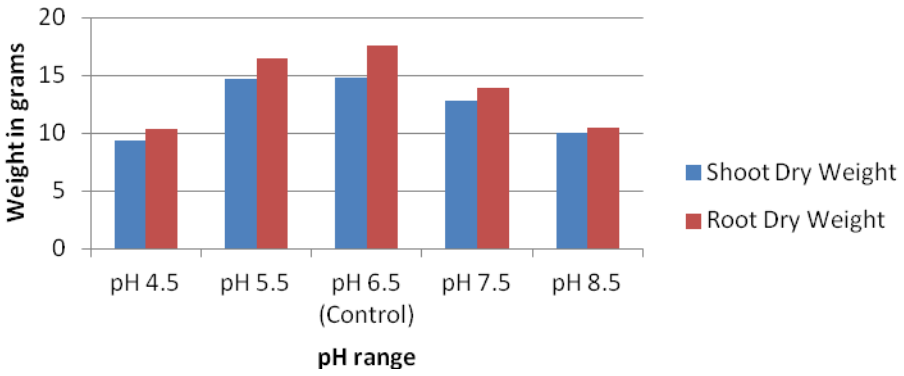

Figure 3.3 Effect of pH on average root and average shoot dry weights of *A. afra.*

The total dry weight was significantly affected ($P \leq 0.001$) by pH treatments (Figure 3.2). The average total dry weight of the control was not significantly different to the average total dry weight of the plants grown at the pH values of 5.5 and 7.5. The pH 4.5 and 8.5 had an effect upon the total dry weight of the plants, which was significantly lower than that of the control. It is likely that this is also an effect of the lower availability of nutrients at these pH levels. It is interesting to note that while the fresh weight of the plants grown at the control of 6.5 was significantly higher than that of the 4.5, 5.5 and 7.5, the total dry weight of the control, 5.5 and 6.5 treatments was not significantly different. Shoot dry weight was significantly influenced ($P \leq 0.001$) by different pH treatments (Figure 3.3). When compared with the control of pH 6.5, the plants at a pH of 5.5 and 7.5 were not significantly different in terms of shoot dry weight (Figure 3.3). However, the plants grown in the medium adjusted to pH 4.5 and pH 8.5 had significantly lower shoot dry weights than those of the control. A similar significant ($P \leq 0.001$) trend with pH adjustment was noticed with the dry weight of the roots (Table 3.1). The control was not significantly varied from the pH 5.5 and pH 7.5 treatments in terms of root dry weight. However, the pH 4.5 and pH 8.5 treatments produced significantly lower weights of dry roots than the control (Figure 3.3). When a comparison between the total dry weights and the chlorophyll content of the different treatments is made it can be seen that there is a relationship between chlorophyll content and dry weights. The lower average dry shoot and root weights and chlorophyll content of the pH 4.5, and 8.5 pH values could be attributed to the lower levels of nutrients such as iron, manganese and boron that are available at these pHs (Edmond et al., 1975; Reed, 1996; Preece & Read, 2005).

As the nutrients become unavailable to the plant, various metabolic processes such as chlorophyll synthesis, photosynthesis and respiration are restricted (Stern, 2006). As pH is raised above 7.5, minerals such as iron, manganese and boron become unavailable to the plants (Edmond et al., 1975; Reed, 1996; Preece and Read, 2005). Below a pH of 5.5, nitrogen, phosphorus and many others begin to become unavailable to the plants (Edmond et al., 1975; Reed, 1996; Preece & Read, 2005; Brady & Weil, 2008). The lack of minerals such as phosphorus at a low pH and iron at a high pH can lead to chlorosis and hence a loss of chlorophyll (Stern, 2006). This could contribute to the chlorophyll levels of the plants exposed to the pH 4.5 treatment being significantly lower than that of the pH 5.5 and 6.5 treatments, while the total dry weight is significantly lower than that of the control, but similar to the pH 7.5 and 8.5.

The results clearly indicated that there is a relationship between the pH of supplied irrigation water and the yield and chlorophyll content of *A. afra*. Although there was a significant difference between the fresh weights of all the treatments, with the highest weight being that of the control treatment, the dry yield was not significantly different between the treatments below pH 7.5. In the South African context, information regarding *A. afra* response to pH is important knowledge, because many of the small scale cultivators of this medicinal plant cannot afford soil amendment products (Makunga et al., 2008). In conclusion, this pilot study has demonstrated that pH can play a significant part in the growth and yield of *A. afra*. It has indicated that this plant is tolerant of a wide range of pH levels, but performs best (in terms of fresh yield and chlorophyll content) in a pH range from 5.5 to 7.5. Although the yield of the plant is the primary focus of most small scale growers, to the medicinal industry the most important factor is the yield of useful metabolites (Fennell et al., 2004). Further studies are recommended as to the effect of varying pH levels on the production of secondary metabolites and other chemical components with medicinal values. In-depth studies as to the relationship between mineral requirements of *A. afra* and its production of useful secondary metabolites would yield useful data pertaining to the commercial cultivation of this plant. It would also be relevant to investigate the effect that the combination of factors such as pH and nutrient availability would have on the metabolite content of the plant.

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Table 3.1: Mean yield results of total fresh and dry weight, shoots and roots dry weight and chlorophyll for A. afra grown at various pH concentrations.

Treatment	Total Fresh Weight (grams)	Total Dry Weight (grams)	Shoots Dry Weight (grams)	Roots Dry Weight (grams)	Chlorophyll (SPAD units)
pH 4.5	65.5 ± 5.7b	19.8 ± 2.0b	9.4 ± 0.72b	10.4 ± 1.5b	30.1 ± 0.3c
pH 5.5	71.2 ± 4.5b	31.2 ± 2.7a	14.7 ± 1.5a	16.5 ± 2.2a	32.7 ± 0.3b
pH 6.5 (Control)	103.5 ± 4.8a	32.4 ± 2.2a	14.8 ± 0.8a	17.6 ± 1.9a	35.5 ± 0.2a
pH 7.5	73.8 ± 4.2b	26.8 ± 2.1a	12.8 ± 0.7a	13.9 ±1.7ab	22.2 ± 0.3d
pH 8.5	43.6 ± 1.3c	20.5 ± 1.1b	10.0 ± 0.6b	10.5 ± 0.7b	13.7 ± 0.3e
One-way ANOVA (F-Statistic)	24.1***	7.97***	7.67***	3.9**	1093.4***

Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at ***: P≤0.001, ns: not significant

CHAPTER FOUR:

EFFECTS OF DIFFERENT COMBINATIONS OF ARBUSCULAR MYCORRHIZA AND SUPPLEMENTARY FERTILIZATION ON GROWTH AND DEVELOPMENT AND MARKETABILITY OF *ARTEMISIA AFRA* JACQ. GROWN IN A SIMULATED SOIL.

CHAPTER FOUR: EFFECTS OF DIFFERENT COMBINATIONS OF ARBUSCULAR MYCORRHIZA AND SUPPLEMENTARY FERTILIZATION ON GROWTH AND DEVELOPMENT AND MARKETABILITY OF *ARTEMISIA AFRA* JACQ. GROWN IN A SIMULATED SOIL.

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4.1 ABSTRACT

Artemisia afra is a widely utilised traditional African medicinal plant, harvested from the wild between South Africa and Kenya. The wild harvesting of commercial plants is having a detrimental effect upon their wild populations, but traditional healers are reluctant to cultivate these plants due to the quality of cultivated plants not mirroring that of wild grown specimens. Cultivation of medicinal plants also relies on the use of inorganic fertilizers, which can raise costs. The use of symbiotic relationships such as arbuscular mycorrhiza has been shown to contribute to the production of plants of a high quality. This study assessed plant height, root lengths, fresh and dry weight of roots and shoots and root: shoot ratios of *Artemisia afra* grown in a simulated soil as influenced by supplementary nutrition and the application of arbuscular mycorrhiza. The aim of the study was to establish the effects of different combinations of arbuscular mycorrhiza and supplementation fertilization on the growth and development of *A. afra*. Results showed that plant height, fresh and dry weight of roots and shoots, root lengths, and root: shoot ratios were generally higher in the treatments consisting of mycorrhiza and supplementary fertilization.

Keywords: Medicinal plant, plant production, low input, plant growth.

Abbreviations: mg = milligrams; mg / L= milligram per liter

4.2 INTRODUCTION

Artemisia afra is a widely utilised Traditional African Medicinal Plant, harvested from the wild between South Africa and Kenya (Liu et al., 2008; Thring & Weitz, 2006) used to treat a number of ailments, including skin irritations, chest infections and congestion (Keirungi & Fabricius, 2005). The wild harvesting of commercial plants is having a detrimental effect

upon their wild populations (Makunga et al., 2008), but traditional healers are reluctant to cultivate these plants due to the quality of cultivated plants not mirroring that of wild grown specimens (Van Andel & Havinga, 2008; Zobayed et al., 2007). Cultivation of medicinal plants also relies on the use of inorganic fertilizers, which can raise costs (Zak et al., 1998).

Many areas of Africa are classified as marginal land- that is land that is low in available nutrition for plant growth (Gupta et al., 2002). Cultivation in these areas is handicapped by the lack of available nutrition (McGeocha et al., 2008), and the cost of fertilizers can make cultivation in these areas prohibitively expensive (Kaya et al., 2009). The use of sustainable crop production systems combined with low input is one method of increasing the output of these areas (Netshiluvhi, 1999).

The use of symbiotic relationships has been shown to contribute to the production of plants of a high quality (Thompson, 1996). Arbuscular mycorrhiza (AM) is a symbiotic relationship between soil borne fungus and the host plant's roots. AM relationships have been shown to have a large influence on the growth and development of many valuable crops (Phosri et al., 2010), and their usefulness as a part of a sustainable crop production system has been utilized for the production of many high value crops (Azcon-Aguilar & Barea, 1997; Clark & Zeto, 1996; Feddermann et al., 2010; Khaosaad et al., 2006; Zak et al., 1998).

Symbiotic relationships such as AM are commonly found in the wild and contribute to sustainable plant growth (Azcon-Aguilar & Barea, 1997), but have been found to be lacking in cultivated soils unless there has been specific care given to encouraging them (Haselwandter & Bowen 1996). The application of AM inoculations can help in the re-establishment of beneficial symbiotic soil relationships (Feldmann et al.,1989). This is often done commercially using a specific species of AM, but in the wild there is usually a varied collection of AM. There exist many studies of responses of host plant to mycorrhizal inoculation, but these studies have focused on single AM fungal isolate. Because of the need to simulate the natural growth conditions to satisfy wild harvesters, this study will use a mixed inoculum of AM fungi (Cardoso & Kuyper, 2006).

To most harvesters and producers of TAM, there are certain characteristics of growth that are sought after (Diederichs, 2006). The most predominant one is the weight of yield of product (Makunga et al., 2008). The cultivation of medicinal plants for use in TAM focuses on high production, judged by dry weight of product (Fennell et al., 2004), and so the aim of this investigation was to establish the effects of a blend of AM on the growth and development of *A. afra*, alone and in conjunction with supplementary application of phosphorus, copper and zinc. Marketability of plant product destined for traditional medicine is dependent on the

visual quality of the plants (Gurib-Fakim, 2006), and as SPAD-502 levels correspond closely with the visual heath of the plant these readings will be used to calculate the marketability of the plants.

4.3 MATERIAL AND METHODS

4.3.1 Glasshouse experiment

The experiment was conducted from August to October 2012. It was located in the research greenhouse of the Cape Peninsula University of Technology in the Western Cape of South Africa. The latitude and longitude are S33°55' 58 E18°25' 57. The climate controlled greenhouse had temperatures ranging from 20 - 29°C during the days, and 14 - 18°C at night. The relative humidity of the glasshouse averaged 35%. There is a 40% Alunet shade cloth suspended 2 m above the ground of the glasshouse. The light intensities ranged from 8 kLux to 11 kLux, as measured by a Toptronic T630 light meter. Irrigation water was supplied from a Hager IP65 Water Filtration Plant de-ioniser, and had an average temperature of 16°C.

4.3.2 Plant selection and planting process

Two month old *A. afra* Jacq. plants were obtained from Good Hope Nursery. They all originated from one mother stock plant identified as a suitable phenotype for medicinal use by a group of local traditional healers (Grey, 2009).

Plants for the experiment were propagated by cuttings taken from an individual. 6cm tip cuttings were rooted in a perlite medium on a hotbed with intermittent misting for 3 weeks. After being placed in cutting trays the cuttings were sprayed with the fungicide 'Funginex' (active ingredient triforine). This was to ensure that there was no contamination of the cuttings with any fungus. A second application of 'Funginex' was given at 2 weeks. During the rooting period there was no application of fertilizers. When the plants were rooted they were removed from their rooting medium and were washed with reverse osmosis filtered water to remove all traces of the perlite.

The rooted cuttings were planted into 23cm round plastic pots which had been each filled with 1.4 kg of sterilized medium. The medium consisted of 2 parts washed perlite, 1 part polystyrene balls, 2 parts coco coir, 2 parts sifted bark, and 1 part silica sand. This medium was chosen to represent a mineral deficient soil. The medium was sterilized using a steam sterilizer at 100°C for 2 hours to destroy any spores and pathogens.

4.3.3 Treatment preparation

Sixteen treatments were tested. The treatments consisted of a randomised factorial design, made up of two groups. Group A had no mycorrhizal inoculation and 1) no supplementary fertilization, 2) supplementary zinc application, 3) supplementary copper 4) supplementary phosphorus 5) supplementary zinc and copper, 6) supplementary zinc and phosphorus, 7) supplementary copper and phosphorus, 8) supplementary zinc, copper, and phosphorus. Group B had mycorrhizal inoculation in combination with 9) no supplementary fertilization, 10) supplementary zinc application, 11) supplementary copper 12) supplementary phosphorus 13) supplementary zinc and copper, 14) supplementary zinc and phosphorus, 15) supplementary copper and phosphorus, 16) supplementary zinc, copper, and phosphorus.

There were 10 plants per treatment, and the treated plants were placed in mixed blocks on raised steel tables in the greenhouse. Nutrient applications consisted of phosphoric acid 85% to give 40 mg/kg phosphorus, zinc chelate AAC 10% to give 2.5 mg/kg, and copper AAC chelate 10% to give 1.8 mg/kg. Each of the nutrient supplementations was applied weekly in the irrigation water. The plants were watered twice weekly, using 600ml of reverse osmosis filtered water.

Mycorrhizal inoculation was performed by the application of 30g of the commercially available product Mycoroot™. The applications were made at planting, and a second application was made 2 weeks into the experiment.

4.3.4 Data collection

The growth and development of *A. afra* was determined by plant height, root length, fresh and dry weight of shoots and roots and root: shoot ratios. Plant height was recorded in millimetres (mm) from the soil level to the apex of the tallest stem once per week. Root length was recorded in mm pre planting and post harvest using a vernier calliper, measuring from soil level to the tip of the longest root. Total plant wet weights were recorded pre planting, and wet weights of shoots and roots was recorded at harvest. Plants were dried at 55°c for 48hrs after harvest, and dry shoots and root weights were recorded. Root: shoot wet and dry ratios were calculated by dividing the weights of the roots by the weights of the shoots. Marketability was calculated by dividing the cost of the treatment per plant by the yield of dry material to get cost per gram, and then adding to the cost per gram the SPAD-502 multiplied by the cost per gram. This resulted in a marketability value out of 10 that

represented the higher desirability of plants with a higher level of visual health as indicated by SPAD-502.

4.3.5 Statistical analysis

Data collected were analysed using a One-Way analysis of variance (ANOVA). The analysis was performed using STASTICA Software Programme 2010 (StatSoft Inc., Tulsa OK, USA). Where F-value was found to be significant, Fisher's least significant difference (LSD) was used to compare the means at P≤0.05 level of significance (Steel & Torrie, 1980).

4.4 RESULTS

4.4.1 Effect of different combinations of mycorrhiza and nutrient supplementation on height of *A. afra.*

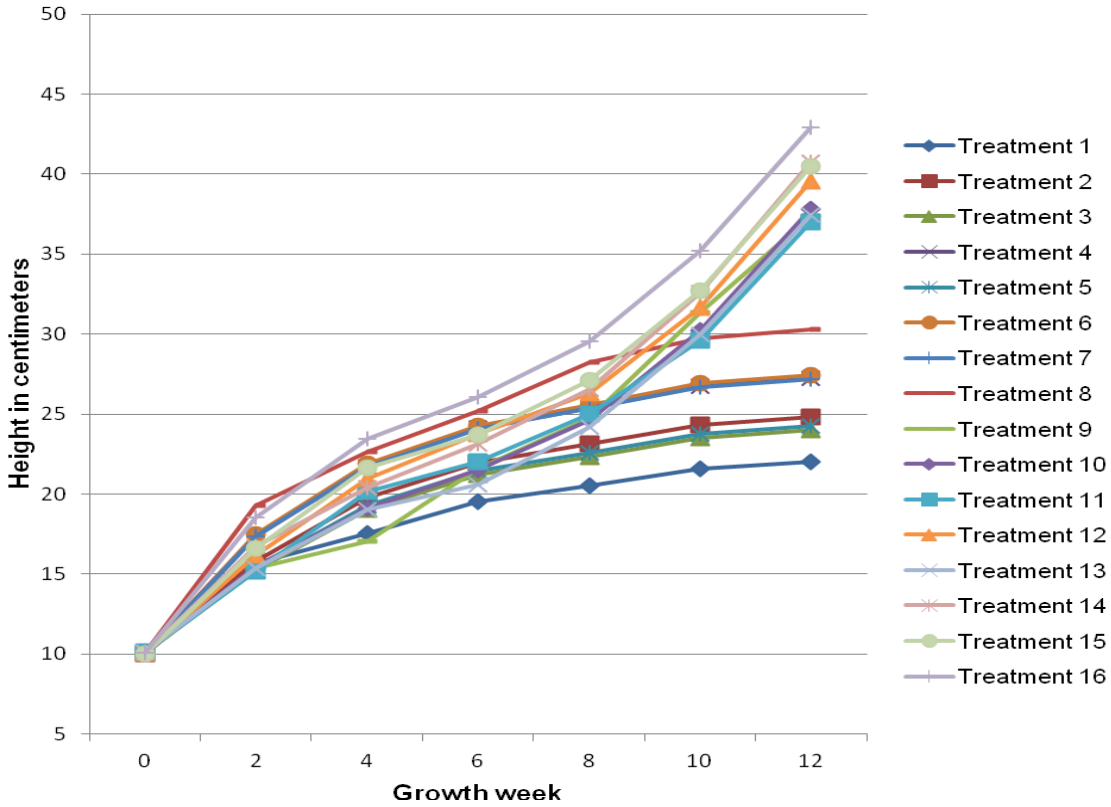

Figure 4.1: Average weekly height measurements of *A. afra* (treatments 1-8 without mycorrhiza, treatments 9-16 with mycorrhiza).

At the second week of the experiment there was little difference between the mycorrhizal and non-mycorrhizal treatments, with significant differences (P≤ 0.001) only between those plants that received nutrient supplementation and those that did not (Figure 4.1). In this week the plants that exhibited the tallest heights were those that received phosphorus supplementation- either alone or in conjunction with the zinc and copper applications. At week 4 there was a noticeable increase in the heights of those plants that received both

mycorrhizal inoculation and applications containing phosphorus, although those plants not receiving the inoculation but receiving phosphorus applications performed similarly. At weeks 6 and 8 this trend continued, but by week 10 all plants receiving mycorrhizal inoculations either alone or in conjunction with the nutrient applications began to significantly (P≤ 0.001) outperform those that did not receive the treatments, with the greatest height being in those plants that received the mycorrhizal inoculations in combination with the zinc, copper and phosphorus supplementation, followed by those that received the inoculations in combination with wither zinc and phosphorus or copper and phosphorus. At week 10 the plants with the lowest heights were those that did not receive the inoculations, regardless of nutrient applications. At the end of the experiment on week 12 all treatments receiving mycorrhizal inoculations were significantly (P≤ 0.001) outperforming those that did not receive the inoculations, with the significantly (P≤ 0.001) highest growth being found in those that received both the inoculations and the nutrient applications of zinc, copper and phosphorus together. As with week 10, the lowest growth was found in the treatments that did not include the inoculations of mycorrhiza (Table 4.1).

4.4.2 Effect of different combinations of mycorrhiza and nutrient supplementation on wet and dry weight of shoots of *A. afra.*

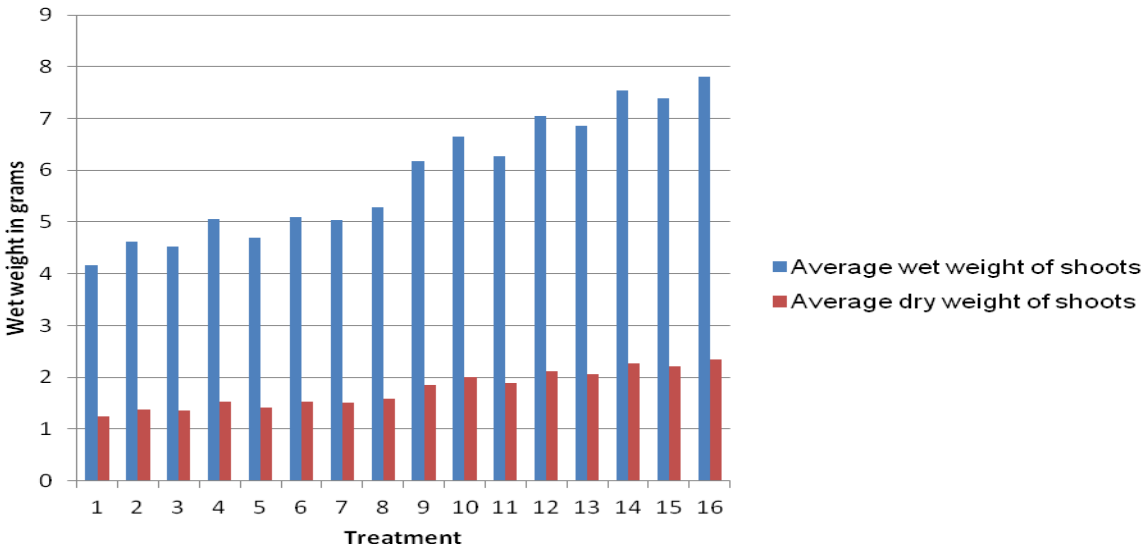

Figure 4.2: Average Wet and dry weights of shoots *A. afra* (treatments 1-8 without mycorrhiza, treatments 9-16 with mycorrhiza).

Both wet and dry weights of shoots displayed significant differences (P≤ 0.001) between treatments of mycorrhiza and supplementary nutrition addition (Figure 4.2). The treatments that contained mycorrhiza all had higher wet and dry weights of shoots when compared to those that did not receive mycorrhizal inoculation. The highest growths in terms of weight

were found in the plants that received the combination of mycorrhizal inoculation and supplementary zinc, copper and phosphorus. The plants that displayed the lowest wet and dry weights of shoots were those that either received no mycorrhizal inoculation combined with no nutrient supplementation, or received no inoculation in combination with zinc, copper but lacked phosphorus applications (Table 4.2).

4.4.3 Effect of different combinations of mycorrhiza and nutrient supplementation on wet and dry weights of roots of *A. afra.*

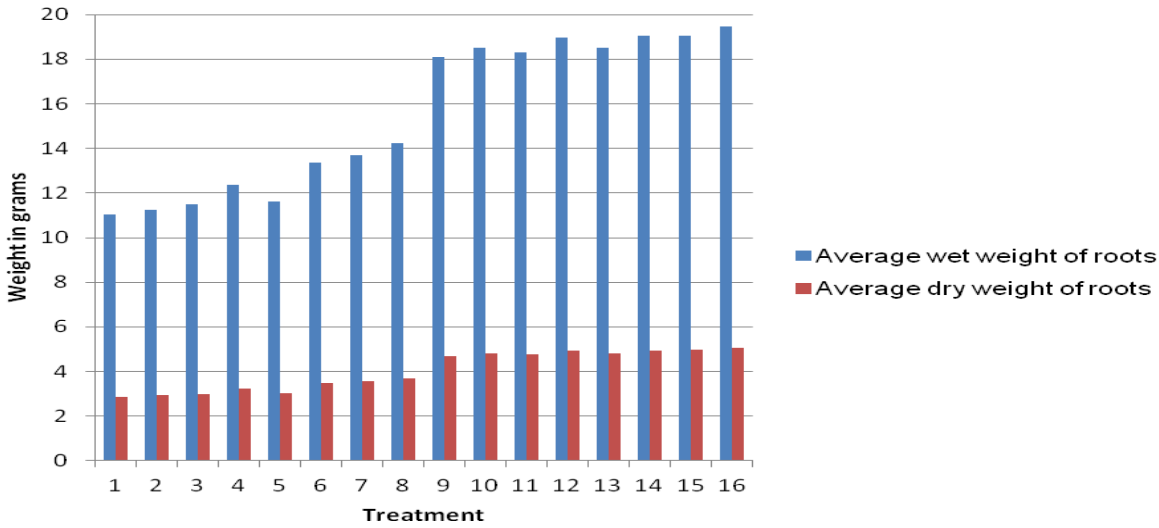

Figure 4.3: Average wet and dry weights of roots of *A. afra* (treatments 1-8 without mycorrhiza, treatments 9-16 with mycorrhiza).

The plants that received the mycorrhizal inoculations in combination with supplementary nutrient application all had significantly ($P \leq 0.001$) higher wet and dry weights of roots (Figure 4.3). Those plants that did not receive mycorrhizal inoculation had significantly lower wet and dry root weights, regardless of nutrient applications. The plants that received both mycorrhizal inoculation and supplementary zinc, copper and phosphorus all had the highest wet and dry weights of roots, and those that did not receive inoculation or supplementary nutrients had the lowest wet and dry weights of roots. The treatments that consisted of mycorrhizal inoculation in conjunction with nutrient supplementations containing phosphorus all had higher wet and dry weights of roots (Table 4.3).

4.4.4 Effect of different combinations of mycorrhiza and nutrient supplementation on root lengths of *A. afra.*

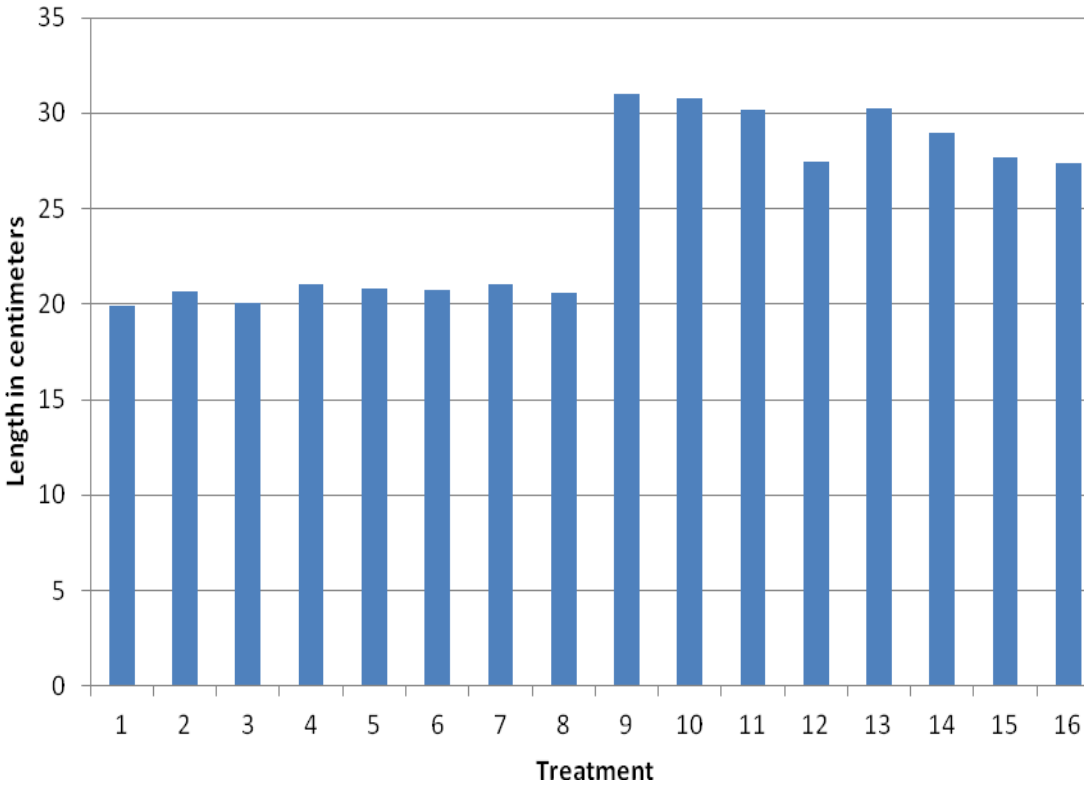

Figure 4.4: Average root lengths of *A. afra* (treatments 1-8 without mycorrhiza, treatments 9-16 with mycorrhiza).

There was a significant (P≤ 0.001) effect upon the root lengths of *A. afra* caused by the treatments (figure 4.4). The treatments that included mycorrhizal inoculations all resulted in significantly (P≤ 0.001) longer root lengths, while those lacking the inoculations all had significantly lower roots lengths. The treatments that included mycorrhizal inoculation but lacked phosphorus all had the longest root lengths when compared to those inoculated plants that received phosphorus supplementation. The treatment that received mycorrhizal inoculation bud did not receive nutrient supplementation had the longest roots out of all treatments (Table 4.5).

4.4.5 Effect of different combinations of mycorrhiza and nutrient supplementation on total wet and total dry weights of *A. afra.*

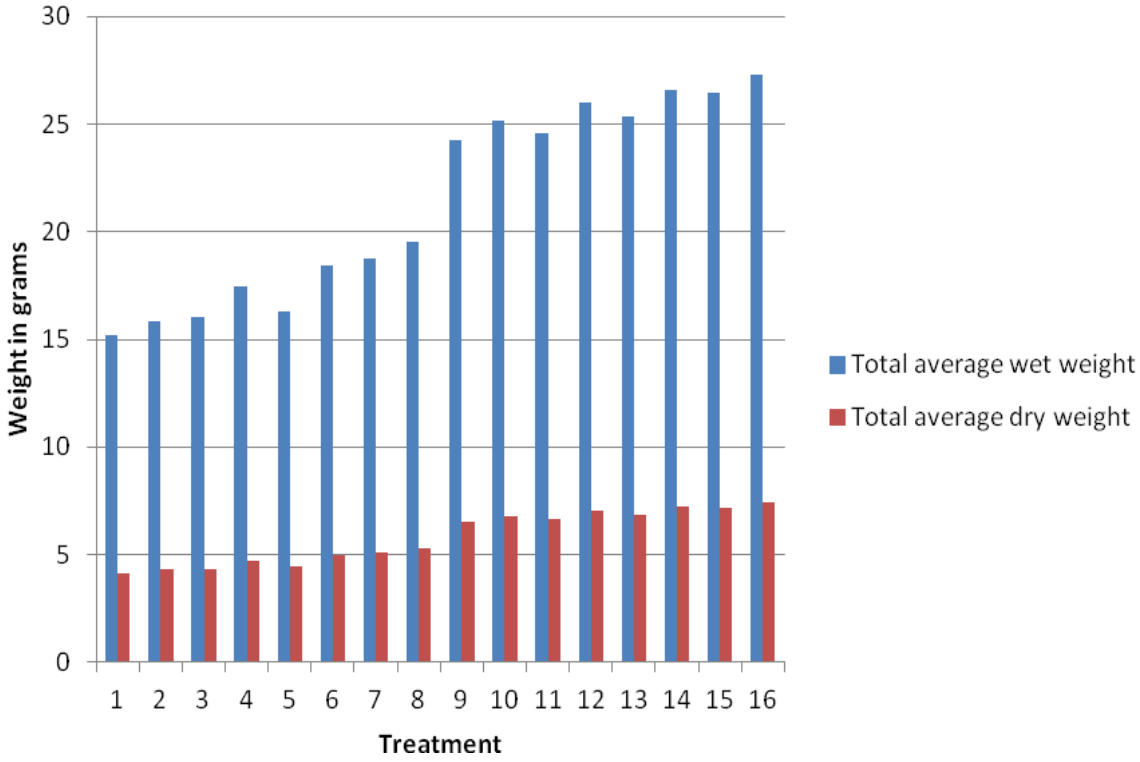

Figure 4.5: Average total wet and dry weights of *A. afra* (treatments 1-8 without mycorrhiza, treatments 9-16 with mycorrhiza).

Those plants that received the treatments consisting of mycorrhizal inoculation and nutrient supplementation all had significantly (P≤ 0.001) higher total wet and dry weights when compared to those that did not receive the inoculation (Figure 4.5). The treatments consisting of mycorrhizal inoculation, zinc, copper and phosphorus had significantly (P≤ 0.001) higher total wet and dry weights when compared to those that received the inoculation combined with supplementation lacking phosphorus. The lowest total wet and dry weights was found in the plants that did not receive any inoculations or nutrient supplementation, with significantly (P≤ 0.001) lower weights found in those treatments lacking inoculation and phosphorus (Table 4.5).

4.4.6 Effect of different combinations of mycorrhiza and nutrient supplementation on SPAD-502 levels of *A. afra.*

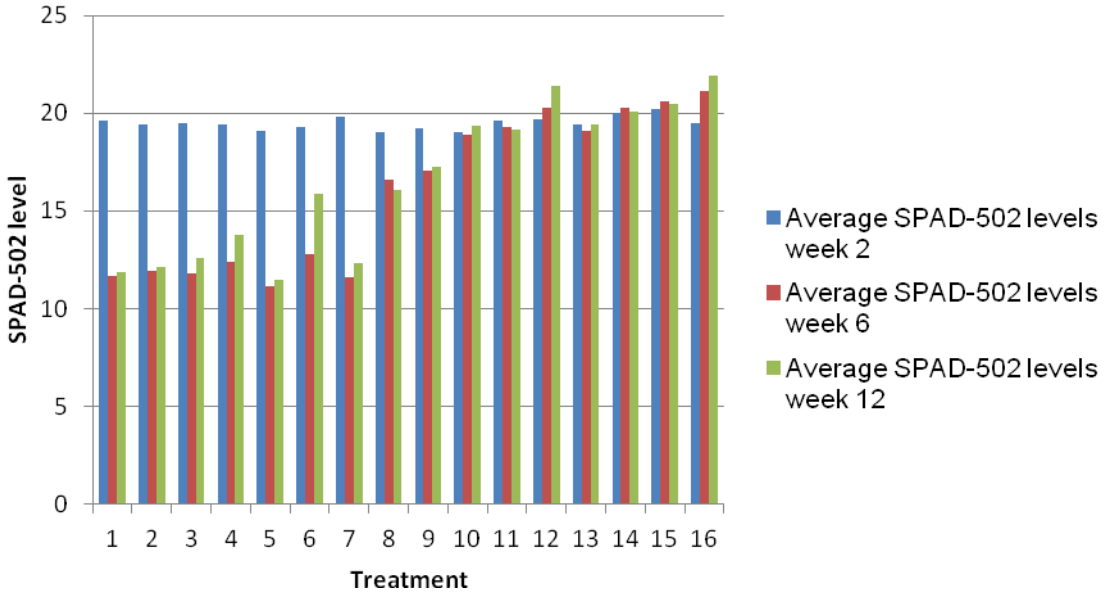

Figure 4.6: Average SPAD-502 levels of *A. afra* (treatments 1-8 without mycorrhiza, treatments 9-16 with mycorrhiza).

At week 2 there was no variation between the treatments in regards to chlorophyll levels as indicated by SPAD 502 readings (Figure 4.6).

During the 6th week there were significant (P≤ 0.001) differences between the treatments, with the plants receiving inoculations in combination with nutrient supplementation all showing significantly (P≤ 0.001) higher SPAD 502 readings when compared to those plants that did not receive the inoculations.

At the end of the experiment the plants that received the mycorrhizal inoculations in combination with supplementary nutrient application all had significantly (P≤ 0.001) higher levels of chlorophyll according to the SPAD 502 readings when compared to those that did not receive inoculation or nutrient supplementation. Treatments that included mycorrhizal inoculations and phosphorus supplementation had higher SAPD-502 levels than those that did not receive the supplementations (Table 4.6).

4.4.6 Effect of different combinations of mycorrhiza and nutrient supplementation on marketability of *A. afra.*

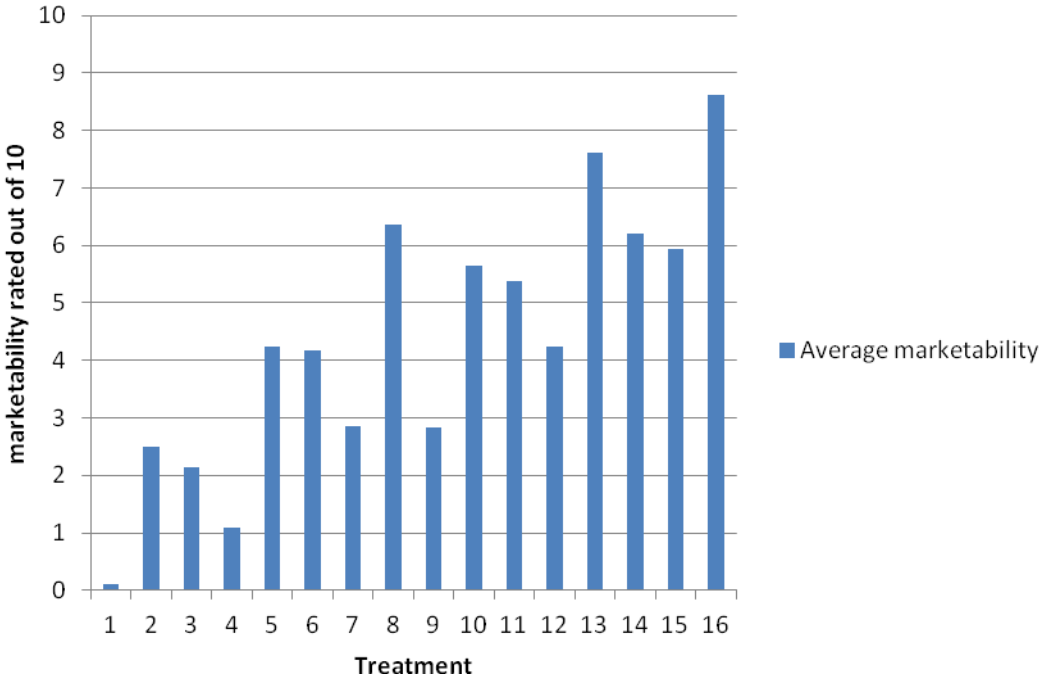

Figure 4.7: Average marketability of *A. afra* (treatments 1-8 without mycorrhiza, treatments 9-16 with mycorrhiza).

The marketability of the plants was on average much higher in those plants that received the mycorrhizal inoculations, whether alone or in conjunction with nutrient supplementation (Figure 4.7). The highest levels of marketability were found in the treatment that included both the mycorrhizal inoculations and phosphorus application. This was due to the vastly reduced costs involved by not applying the copper or zinc applications, offset by the SPAD-502 levels. The lowest marketability was found in the treatment consisting of no mycorrhizal inoculation combined with a copper application. This is due to the high cost of the copper supplementation and low SPAD-502 levels. The presence of phosphorus in treatments resulted in significantly higher SPAD-502 levels, in turn resulting in a higher marketability when compared to those treatments that did not receive the phosphorus, either alone or in conjunction with the mycorrhizal inoculations.

4.5 DISCUSSION

The growth over time of the treatments that included mycorrhizal inoculations was significantly higher when compared to those that did not receive the treatments. Up until week 8 the growth of those that did not receive the inoculation was close to those that received the mycorrhiza, but past week 8 there was a very significant increase in the growth

of those that received the inoculation. This indicates that in the long term the applications of mycorrhiza can provide useful height increases, but on a short term there is little difference between inoculation or non-inoculation. This could be attributed to the fact that there was no other supplementation apart from the applications of zinc, copper and phosphorus, and that the 8 weeks is the time that the plants took to show the effects of low levels of nutrients. It also indicates that the use of mycorrhizal inoculation has an effect upon the plant's utilization of nutrients other than the supplementary zinc, copper and phosphorus (Azcon-Aguilar & Barea, 1997).

In this study it was found that there was significant differences between the inoculated plants that received supplementary nutrition in the form of phosphorus and those that were inoculated but did not receive the nutrient supplementation. However, there was also a significant difference in height between those plants that received the phosphorus supplementation in conjunction with the inoculation and those that received nutrition supplementation without the inoculation. This indicates that the mycorrhiza has a positive effect upon the utilization of phosphorus available to the plant (Feddermann et al., 2010).

The higher wet and dry weights of those plants receiving the inoculation when compared to those that did not receive it shows that the mycorrhizal inoculation has a positive effect on the accumulation of mass by the plants. The higher weights of those plants receiving both mycorrhizal inoculation and phosphorus supplementation when compared to those that did not receive the inoculation shows that the mycorrhiza has a positive impact upon the plant's utilization of available phosphorus (Thompson, 1996).

On average those plants receiving the inoculations had higher levels of marketability when compared to those that did not receive the inoculations, regardless of nutrient supplementation. This indicates that there is significant financial motivation for the use of mycorrhizal inoculations in the cultivation of *A. afra (*Makunga et al., 1998). The highest marketability of those plants that received the inoculation in conjunction with phosphorus applications indicates that the most profitable method of cultivation is with the combination of mycorrhizal inoculation and phosphorus supplementation.

The application of mycorrhiza had positive impacts upon all aspects of plant growth, regardless of nutrient application. This showed that the symbiosis was having significant effects upon the nutrient uptake and utilization of soil nutrients by the plants (Cardoso & Kuyper, 2006). From the data collected it is apparent that there is much to be gained in terms of yield of plant material from the utilization of mycorrhizal relationships *(*Makunga et al., 1998). The fact that traditional growers and harvesters of medicinal plants primarily seek out

increasing yields and visual high signs of health indicates that there is potential for the increasing of cultivation of medicinal plants through the use of mycorrhiza. Due to the fact that there was only a small difference between the treatments that received mycorrhiza and the various nutrient supplementations in terms of wet and dry yield this experiment indicates that a grower could increase plant yield by the addition of mycorrhiza, instead of the use of nutrient supplementation. The high levels of marketability demonstrated by those plants that received mycorrhizal inoculation in conjunction with supplementary phosphorus indicated the potential for traditional healers to cultivate plants of desirable plants through the use of mycorrhizal inoculation This is especially relevant in an African setting, as the cost of nutrient supplementation can be prohibitive to the small scale cultivator.

4.6 CONCLUSION

In conclusion, the use of mycorrhiza inoculations to increase yield and marketability of *A. afra* showed that there is a significant advantage to using the symbiotic relationship, and that mycorrhizal inoculation outperforms supplementation of zinc, copper and phosphorus in producing plants of a high yield and marketability after a three month growth period.

7 ACKNOWLEDGEMENTS

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Table 4.1: Effect of different combinations of mycorrhiza and nutrient supplementation on plant height of *A. afra* in centimetres							
Treatment	**Week 0**	**Week 2**	**Week 4**	**Week 6**	**Week 8**	**Week 10**	**Week 12**
1 No mycorrhiza, no supplementation	10.1±0.06a	15.7±0.31gh	17.6±0.33h	19.5±0.36h	20.5±0.38h	21.6±0.40h	22.1±0.41h
2 No mycorrhiza +Zn	10.0±0.07a	15.8±0.24fg	19.8±0.30efg	22.0±0.33f	23.1±0.35g	24.3±0.37g	24.8±0.37g
3 No mycorrhiza +Cu	10.1±0.07a	15.3±0.23hi	19.1±0.29g	21.2±0.28fg	22.3±0.30g	23.5±0.31g	24.0±0.32g
4 No mycorrhiza +P	10.1±0.06a	17.4±0.20c	21.7±0.26bc	24.1±0.28bcd	25.4±0.29de	26.7±0.31f	27.3±0.32f
5 No mycorrhiza +Zn+Cu	10.0±0.08a	15.5±0.22ghi	19.3±0.28fg	21.4±0.32fg	22.6±0.33g	23.7±0.35g	24.2±0.35g
6 No mycorrhiza +Zn+P	10.0±0.08a	17.5±0.12c	21.9±0.15bc	24.3±0.16bc	25.6±0.17de	26.9±0.19f	27.5±0.20f
7 No mycorrhiza +Cu+P	10.1±0.07a	17.4±0.15c	21.7±0.19bc	24.1±0.21bcd	25.4±0.23de	26.7±0.24f	27.2±0.25f
8 No mycorrhiza +Zn+P+Cu	10.0±0.06a	19.3±0.14a	22.6±0.64ab	25.2±0.50ab	28.2±0.20b	29.7±0.20e	30.3±0.20e
9 Mycorrhiza, no supplementation	10.1±0.08a	15.4±0.11ghi	17.1±0.11h	21.6±0.48fg	24.8±0.42ef	31.4±0.73d	37.4±0.23d
10 Mycorrhiza +Zn	10.1±0.09a	15.5±0.12ghi	19.1±0.34fg	21.5±0.40fg	24.6±0.31ef	30.3±0.21e	37.8±0.27d
11 Mycorrhiza +Cu	10.1±0.08a	15.2±0.15i	20.2±0.34def	22.0±0.44ef	25.0±0.35ef	29.6±0.28e	37.0±0.34d
12 Mycorrhiza +P	10.0±0.08a	16.2±0.12ef	21.0±0.46cd	23.8±0.52cd	26.3±0.38cd	31.6±0.24cd	39.5±0.29c
13 Mycorrhiza +Zn+Cu	10.1±0.08a	15.4±0.11ghi	19.0±0.53g	20.6±0.47gh	24.2±0.28f	30.0±0.20e	37.4±0.26d
14 Mycorrhiza +Zn+P	10.0±0.10a	16.7±0.13d	20.4±0.59de	23.1±0.64de	26.6±0.36c	32.6±0.25bc	40.7±0.31b
15 Mycorrhiza +Cu+P	10.0±0.09a	16.6±0.19de	21.7±0.42bc	23.7±0.42cd	27.1±0.59c	32.7±0.46b	40.5±0.47b
16 Mycorrhiza +Zn+P+Cu	10.1±0.10a	18.6±0.16b	23.4±0.32a	26.1±0.30a	29.6±0.32a	35.2±0.42a	42.9±0.50a
One-way ANOVA (F-Statistic)	**0.2ns**	**49.8*** **	**22.62*** **	**19.93*** **	**44.41*** **	**124.4*** **	**482.9*** **
Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at ***: P≤0.001, ns: not significant							

Table 4.2: Effect of different combinations of mycorrhiza and nutrient supplementation on wet and dry weights of *A. afra* shoots in grams.		
Treatment	**Wet weight**	**Dry weight**
1 No mycorrhiza, no supplementation	4.17±0.24g	1.25±0.07g
2 No mycorrhiza +Zn	4.61±0.21efg	1.39±0.06efg
3 No mycorrhiza +Cu	4.53±0.30fg	1.36±0.09fg
4 No mycorrhiza +P	5.06±0.35ef	1.52±0.10ef
5 No mycorrhiza +Zn+Cu	4.69±0.12efg	1.41±0.04efg
6 No mycorrhiza +Zn+P	5.09±0.17ef	1.53±0.05ef
7 No mycorrhiza +Cu+P	5.04±0.21ef	1.51±0.06ef
8 No mycorrhiza +Zn+P+Cu	5.29±0.26e	1.59±0.08e
9 Mycorrhiza, no supplementation	6.18±0.17d	1.85±0.05d
10 Mycorrhiza +Zn	6.64±0.30cd	1.99±0.09cd
11 Mycorrhiza +Cu	6.26±0.28d	1.88±0.09d
12 Mycorrhiza +P	7.04±0.20bc	2.11±0.06bc
13 Mycorrhiza +Zn+Cu	6.86±0.15bcd	2.06±0.05bcd
14 Mycorrhiza +Zn+P	7.53±0.26ab	2.26±0.08ab
15 Mycorrhiza +Cu+P	7.39±0.31ab	2.22±0.09ab
16 Mycorrhiza +Zn+P+Cu	7.81±0.31a	2.34±0.09a
One-way ANOVA (F-Statistic)	**23.622*** **	**23.583*** **
Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at ***: P≤0.001.		

Table 4.3: Effect of different combinations of mycorrhiza and nutrient supplementation on wet and dry weights of *A. afra* roots in grams.		
Treatment	**Wet weight**	**Dry weight**
1 No mycorrhiza, no supplementation	11.04±0.18f	2.87±0.05f
2 No mycorrhiza +Zn	11.26±0.40f	2.93±0.10f
3 No mycorrhiza +Cu	11.51±0.43ef	2.99±0.11ef
4 No mycorrhiza +P	12.37±0.35e	3.22±0.09e
5 No mycorrhiza +Zn+Cu	11.63±0.21ef	3.02±0.05ef
6 No mycorrhiza +Zn+P	13.36±0.28d	3.48±0.07d
7 No mycorrhiza +Cu+P	13.69±0.29d	3.56±0.08d
8 No mycorrhiza +Zn+P+Cu	14.24±0.48d	3.70±0.13d
9 Mycorrhiza, no supplementation	18.09±0.41c	4.71±0.11c
10 Mycorrhiza +Zn	18.50±0.35bc	4.81±0.09bc
11 Mycorrhiza +Cu	18.29±0.38bc	4.76±0.10bc
12 Mycorrhiza +P	18.97±0.26abc	4.93±0.07abc
13 Mycorrhiza +Zn+Cu	18.52±0.26bc	4.81±0.07bc
14 Mycorrhiza +Zn+P	19.03±0.33ab	4.95±0.09abc
15 Mycorrhiza +Cu+P	19.07±0.26ab	4.96±0.07ab
16 Mycorrhiza +Zn+P+Cu	19.46±0.33a	5.06±0.08a
One-way ANOVA (F-Statistic)	**102.58*** **	**102.71*** **
Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at ***: P≤0.001.		

Table 4.4: Effect of different combinations of mycorrhiza and nutrient supplementation on *A. afra* root lengths.	
Treatment	**Root length in centimetres**
1 No mycorrhiza, no supplementation	19.9±0.13e
2 No mycorrhiza +Zn	20.7±0.25de
3 No mycorrhiza +Cu	20.0±0.25e
4 No mycorrhiza +P	21.0±0.45d
5 No mycorrhiza +Zn+Cu	20.8±0.26de
6 No mycorrhiza +Zn+P	20.7±0.40de
7 No mycorrhiza +Cu+P	21.1±0.43d
8 No mycorrhiza +Zn+P+Cu	20.6±0.38de
9 Mycorrhiza, no supplementation	31.0±0.50a
10 Mycorrhiza +Zn	30.8±0.23a
11 Mycorrhiza +Cu	30.2±0.43a
12 Mycorrhiza +P	27.5±0.23c
13 Mycorrhiza +Zn+Cu	30.2±0.31a
14 Mycorrhiza +Zn+P	29.0±0.39b
15 Mycorrhiza +Cu+P	27.7±0.35c
16 Mycorrhiza +Zn+P+Cu	27.4±0.35c
One-way ANOVA (F-Statistic)	**173.78*****
Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at ***: P≤0.001.	

Table 4.5: Effect of different combinations of mycorrhiza and nutrient supplementation on total wet and dry weights of *A. afra.*		
Treatment	**Total wet weight**	**Total dry weight**
1 No mycorrhiza, no supplementation	15.21g	4.12±0.10i
2 No mycorrhiza +Zn	15.86g	4.31±0.16i
3 No mycorrhiza +Cu	16.03g	4.35±0.16i
4 No mycorrhiza +P	17.43ef	4.73±0.09gh
5 No mycorrhiza +Zn+Cu	16.31fg	4.43±0.06hi
6 No mycorrhiza +Zn+P	18.45de	5.00±0.09fg
7 No mycorrhiza +Cu+P	18.72d	5.07±0.11f
8 No mycorrhiza +Zn+P+Cu	19.53d	5.29±0.18f
9 Mycorrhiza, no supplementation	24.27	6.56±0.14e
10 Mycorrhiza +Zn	25.14c	6.80±0.11de
11 Mycorrhiza +Cu	24.55c	6.63±0.13e
12 Mycorrhiza +P	26.00b	7.04±0.08bcd
13 Mycorrhiza +Zn+Cu	25.37bc	6.87±0.09cde
14 Mycorrhiza +Zn+P	26.56ab	7.21±0.13ab
15Mycorrhiza +Cu+P	26.46ab	7.18±0.11abc
16 Mycorrhiza +Zn+P+Cu	27.26a	7.40±0.11a
One-way ANOVA (F-Statistic)	**110.08*** **	**106.23*** **
Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at ***: P≤0.001.		

Table 4.6: Effect of different combinations of mycorrhiza and nutrient supplementation on SPAD-502 levels of *A. afra.*			
Treatment	**Week 2**	**Week 6**	**Week 12**
1 No mycorrhiza, no supplementation	19.6±0.43ab	11.7±0.39ef	11.9±0.38ef
2 No mycorrhiza +Zn	19.4±0.40ab	12.0±0.52ef	12.1±0.31ef
3 No mycorrhiza +Cu	19.5±0.27ab	11.8±0.45ef	12.6±0.31ef
4 No mycorrhiza +P	19.4±0.37ab	12.4±0.42ef	13.8±0.47ef
5 No mycorrhiza +Zn+Cu	19.1±0.31b	11.1±0.34f	11.5±0.37f
6 No mycorrhiza +Zn+P	19.3±0.33ab	12.8±0.28e	15.9±0.50e
7 No mycorrhiza +Cu+P	19.8±0.36ab	11.6±0.47ef	12.3±0.37ef
8 No mycorrhiza +Zn+P+Cu	19.0±0.37b	16.6±0.31d	16.1±0.35d
9 Mycorrhiza, no supplementation	19.2±0.39ab	17.1±1.03d	17.3±0.36d
10 Mycorrhiza +Zn	19.0±0.39b	18.9±0.86c	19.4±0.60c
11 Mycorrhiza +Cu	19.6±0.45ab	19.3±0.56bc	19.2±0.53bc
12 Mycorrhiza +P	19.7±0.37ab	20.3±0.45abc	21.4±1.09abc
13 Mycorrhiza +Zn+Cu	19.4±0.31ab	19.1±0.35c	19.4±0.73c
14 Mycorrhiza +Zn+P	20.0±0.30ab	20.3±0.42abc	20.1±1.32abc
15 Mycorrhiza +Cu+P	20.2±0.42a	20.6±0.45ab	20.5±0.99ab
16 Mycorrhiza +Zn+P+Cu	19.5±0.34ab	21.2±0.32a	22.0±1.02a
One-way ANOVA (F-Statistic)	**0.85ns**	**58.64*** **	**58.64*** **
Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at ***: P≤0.001, ns: not significant			

CHAPTER FIVE

EFFECTS OF DIFFERENT COMBINATIONS OF ARBUSCULAR MYCORRHIZA AND SUPPLEMENTARY FERTILIZATION ON PHOTOSYNTHETIC PROCESSES AND ANTHOCYANIN LEVELS OF *ARTEMISIA AFRA* JACQ. GROWN IN A SIMULATED SOIL.

EFFECTS OF DIFFERENT COMBINATIONS OF ARBUSCULAR MYCORRHIZA AND SUPPLEMENTARY FERTILIZATION ON PHOTOSYNTHETIC PROCESSES AND ANTHOCYANIN LEVELS OF *ARTEMISIA AFRA* GROWN IN A SIMULATED SOIL.

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5.1 ABSTRACT

Artemisia afra is one of the most widely wild harvested plants in Africa. Traditional users do not chose to cultivate the plant due to costs involved combined with higher yields achieved from wild harvesting. As a plant that forms mycorrhizal relationship with symbiotic soil fungus there is potential for the cultivation of the plant using arbuscular mycorrhiza and supplementary fertilization to increase yields. This study investigated the effects of arbuscular mycorrhizal inoculation alone and in conjunction with supplementary nutrient applications by assessing photosynthetic processes and chlorophyll content throughout a 3 month growing period. It was found that the inoculations of arbuscular mycorrhiza had a significant effect upon both photosynthetic processes and chlorophyll content, whether alone or in conjunction with nutrient supplementation.

Keywords: Medicinal plant, plant production, low input, plant growth.

Abbreviations: mg = milligrams; mg / L= milligram per liter;

5.2 INTRODUCTION

The use of traditional medicine is one of the largest industries in Africa. More than 100 million people rely on traditional healing as their primary source of health care in Africa (Rabe & van Staden 1997). The source of traditional medicine in Africa is predominantly the wild harvesting of specific crops (Diederichs, 2006). The high costs involved in the cultivation, due to fertilizer input, combined with poor performance of cultivated crops has resulted in a situation of threat for many indigenous medicinal plants (Canter et al., 2005). Africa is experiencing a growth in marginal land- land that is lacking the prerequisite nutrients for suitable plant growth (Gupta et al., 2002). The use of symbiotic relationships to ameliorate poor soil conditions is an area that is receiving growing amounts of attention.

Arbuscular mycorrhiza is a symbiotic relationship between specific soil borne funguses and the roots of plants. There are many species fungus that can create an arbuscular mycorrhizal relationship, with most species belonging to the *Glomus* genus. The various species tend to have a cumulative effect upon nutrient uptake, and are usually found to be working in concert with each other (Cardoso & Kuyper, 2006). Although there is some speculation into the exact mechanics of the assistance provided to the host plant, it is generally accepted that the hyphae of the fungus invades the host plant roots, and in doing so assists with the uptake of nutrients by the increased surface area provided by the hyphal strands (Azcon-Aguilar & Barea, 1997).

Arbuscular mycorrhiza have been shown to assist with the uptake of nutrients that have low soil mobility; such as zinc, copper, and phosphorus (Azcon-Aguilar & Barea, 1997; Clark & Zeto, 1996; Feddermann et al., 2010; Khaosaad et al., 2006; Zak et al., 1998). These micro and macro nutrients play a large part in plant growth and metabolic processes and so increasing the amounts available to the plants tends to have an impact upon plant health as measured by various photosynthetic processes (Feldmann et al., 1989). Chlorophyll content, photosynthetic rate, substomotal CO_2, stomotal conductance and transpiration rates are all affected by levels of soil nutrients, and as such are good indicators of the overall health of the plants. Anthocyanin levels can indicate plant health and are indicative of overall plant health, as lower levels tend to be found in less healthy plants.

Artemisia afra is one of the most popular of traditional African medicinal plants, and it is utilized from Kenya to South Africa (Liu et al., 2008; Thring & Weitz, 2006). It is used in a wide range of traditional medicines, and is used to treat a large variety of health issues (Diallo et al., 1996). The documented uses include a nasal decongestant, a tea for lung infections, a poultice for open wounds and ringworm, as well as many other uses (Bohlmann & Zdero 1972). The main parts of the plant that are used are the above ground parts such as leaves and stems (Wyk, 2008). *A. afra* is usually collected from wild populations by traditional healers. This stems from two main causes. The first is that the cultivation of a wild growing plant tends to be costly due to required fertilizer inputs (Zak et al., 1998)., and the second is that the traditional healers are sceptical that cultivated plants are as effective as wild harvested plants (Van Andel & Havinga, 2008; Zobayed et al., 2007). This study was undertaken to assess the potential of growing this sought after plant utilizing beneficial arbuscular mycorrhizal relationships to reduce the dependence on nutrient supplementation.

5.3 MATERIAL AND METHODS

5.3.1 Glasshouse experiment

The experiment was conducted from August to October 2012. It was located in the research greenhouse of the Cape Peninsula University of Technology in the Western Cape of South Africa. The latitude and longitude are S33°55' 58 E18°25' 57. The climate controlled greenhouse had temperatures ranging from 20 - 29°C during the days, and 14 - 18°C at night. The relative humidity of the glasshouse averaged 35%. There is a 40% Alunet shade cloth suspeded 2 m above the ground of the glasshouse. The light intensities ranged from 8 kLux to 20 kLux, as measured by a Toptronic T630 light meter. Irrigation water was supplied from a Hager IP65 Water Filtration Plant de-ioniser, and had an average temperature of 16°C.

5.3.2 Plant selection and planting process

Two month old *A. afra* Jacq. plants were obtained from Good Hope Nursery. They all originated from one mother stock plant identified as a suitable phenotype for medicinal use by a group of local traditional healers (Grey, 2009).

Plants for the experiment were propagated by cuttings taken from an individual. 6cm tip cuttings were rooted in a perlite medium on a hotbed with intermittent misting for 3 weeks. After being placed in cutting trays the cuttings were sprayed with the fungicide 'Funginex'. This was to ensure that there was no contamination of the cuttings with any fungus. A second application of 'Funginex' was given at 2 weeks. During the rooting period there was no application of fertilizers. When the plants were rooted they were removed from their rooting medium and were washed with reverse osmosis filtered water to remove all traces of the perlite.

The rooted cuttings were planted into 23cm round plastic pots which had been each filled with 1.4kg of sterilized medium. The medium consisted of 2 parts washed perlite, 1 part polystyrene balls, 2 parts coco coir, 2 parts sifted bark, and 1 part silica sand. This medium was chosen to represent a mineral deficient soil. The medium was sterilized using a steam sterilizer at 100°C for 2 hours to destroy any spores and pathogens.

5.3.3 Treatment preparation

16 treatments were tested. The treatments consisted of a randomised factorial design, made up of two groups. Group A had no mycorrhizal inoculation and 1) no supplementary fertilization, 2) supplementary zinc application, 3) supplementary copper 4) supplementary phosphorus 5) supplementary zinc and copper, 6) supplementary zinc and phosphorus, 7) supplementary copper and phosphorus, 8) supplementary zinc, copper, and phosphorus.

Group B had mycorrhizal inoculation in combination with 9) no supplementary fertilization, 10) supplementary zinc application, 11) supplementary copper 12) supplementary phosphorus 13) supplementary zinc and copper, 14) supplementary zinc and phosphorus, 15) supplementary copper and phosphorus, 16) supplementary zinc, copper, and phosphorus.

There were 10 plants per treatment, and the treated plants were placed in mixed blocks on raised steel tables in the greenhouse. Nutrient applications consisted of phosphoric acid 85% to give 40mg/kg phosphorus, zinc chelate AAC 10% to give 2.5 mg/kg, and copper AAC chelate 10% to give 1.8 mg/kg. Each of the nutrient supplementations was applied weekly in the irrigation water. The plants were watered twice weekly, using 600ml of reverse osmosis filtered water.

Mycorrhizal inoculation was performed by the application of 30 g of the commercially available product Mycoroot™. The applications were made at planting, and a second application was made 2 weeks into the experiment.

5.3.4 Data collection

The photosynthetic parameters photosynthetic rate (A), transpiration rate (E) substomotal CO_2 concentration (Ci) and stomotal conductance (gs) were recorded using a LCpro+ portable infra-red gas analyzer supplied by ADCBioscientific (Hoddesdon, Herefordshire, UK). Measurements were performed on 3 leaves from each plant, and average values for each plant were calculated. The readings were taken between 8H00 and 13H00 on weeks 2, 6 and 12 of the experiment. During the readings leaves were allowed to acclimatize to the chamber's light conditions for 5 minutes, and readings were taken after values stabilized for a further 2 minutes. The environmental conditions in the leaf chamber were: photosynthetic photon flux density (PPFD) = 1100 µmol (quantum) $m^{-2}.s^{-1}$, relative humidity = 45%, leaf vapour deficit = 1.85kPa, flow rate = 400 $\mu mol.s^{-1}$, reference CO_2 = 400 ppm, and leaf chamber temperature = 25°C.

Chlorophyll content was measured non-destructively using a SPAD-502 meter supplied by Konica-Minolta. This instrument measures transmission of red light at 650 nm, (the frequency at which chlorophyll absorbs light) and transmission of infrared light at 940 nm (at which no absorption occurs). Using these two transmission values the instrument calculates a SPAD (Soil Plant Analysis Development) level which is indicative of chlorophyll content. The readings were taken at midday on weeks 2, 6 and 12 of the experiment with average daylight levels of 10 kLux.

Anthocyanin levels were recorded non-destructivly using a CCM200A Plus hand held anthocyanin meter (supplied by ADCBioscientific (Hoddesdon, Herefordshire, UK). This device measures the energy absorbed in the 530nm band and uses this to estimate of the amount of anthocyanin present in the tissue. Absorbance in the infrared band is used to quantify leaf thickness resulting in an accurate ACI value.

5.3.5 Statistical analysis

Data collected were analysed using a One-Way analysis of variance (ANOVA). The analysis was performed using STASTICA Software Programme 2010 (StatSoft Inc., Tulsa OK, USA). Where F-value was found to be significant, Fisher's least significant difference (LSD) was used to compare the means at P≤0.05 level of significance (Steel & Torrie, 1980).

5.4 RESULTS

5.4.1 Effect of different combinations of mycorrhiza and nutrient supplementation on photosynthetic rate of *A. afra.*

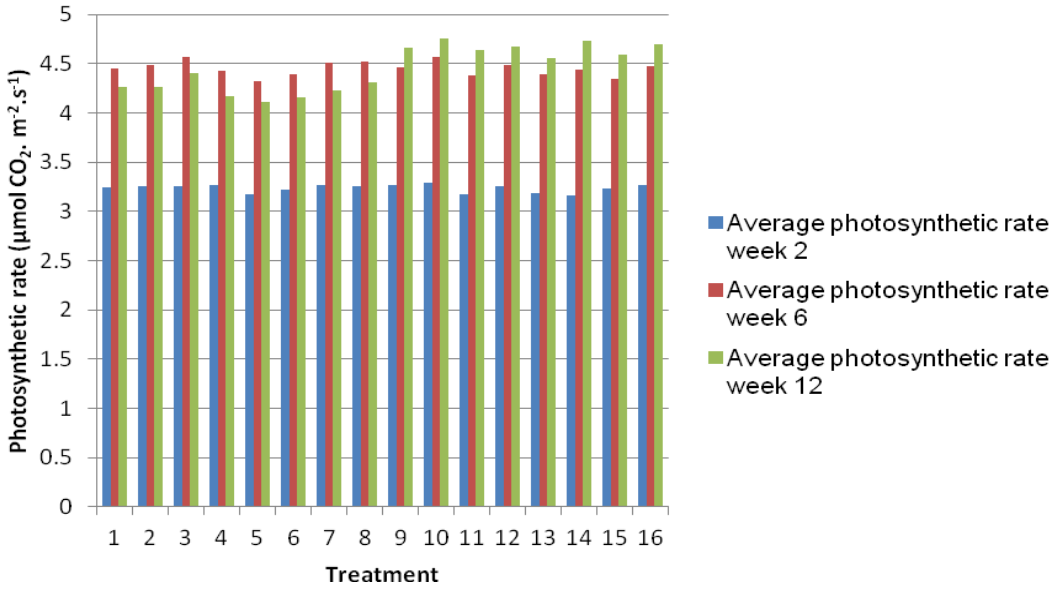

Figure 5.1: Average photosynthetic rate of *A. afra* (treatments 1-8 without mycorrhiza, treatments 9-16 with mycorrhiza).

At the end of the experiment the treatments that included mycorrhizal inoculation all had higher photosynthetic rates when compared to those that did not receive the inoculations (Figure 5.1). The treatments that contained both mycorrhizal inoculation and phosphorus supplementation all had generally higher photosynthetic rates. Those treatments lacking the inoculations had significantly lower photosynthetic rates when compared to those that received the inoculations. In week 6 the treatments lacking the inoculations had average photosynthetic rates close to those that did receive the mycorrhizal inoculations, but by week

12 the treatments lacking the mycorrhizal inoculations had lower photosynthetic rates than those in week 6, while the photosynthetic rates of those that did receive the mycorrhizal inoculations had higher rates than those in week 6 (Table 5.1).

5.4.2 Effect of different combinations of mycorrhiza and nutrient supplementation on substomotal CO_2 of *A. afra.*

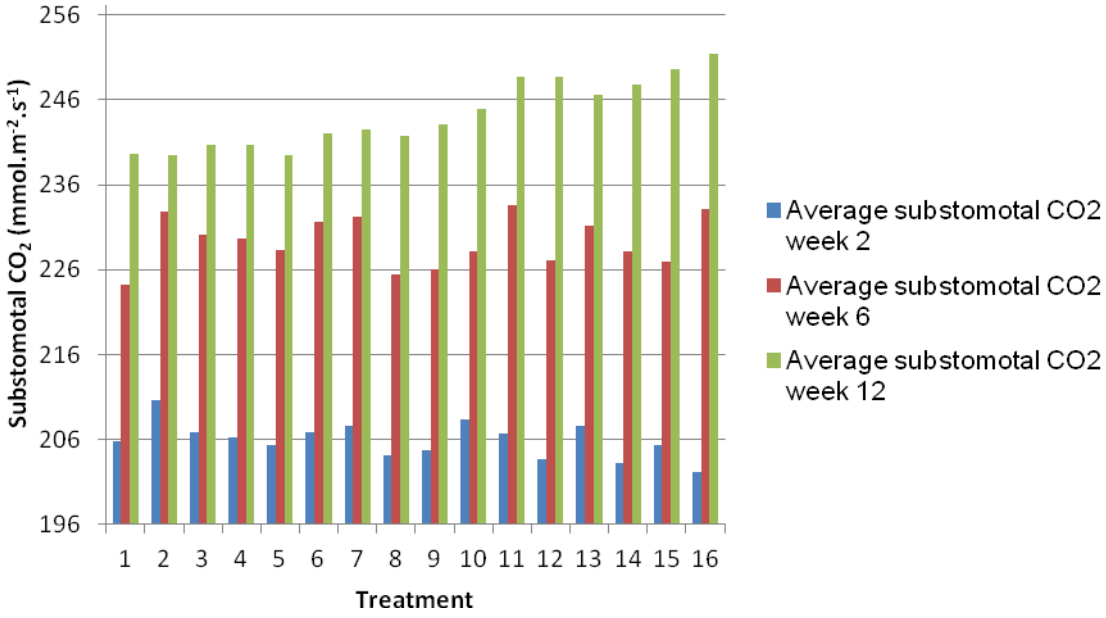

Figure 5.2: Average substomotal CO_2 of *A. afra* (treatments 1-8 without mycorrhiza, treatments 9-16 with mycorrhiza).

There was significant correlation between mycorrhizal inoculated treatments and those that did not receive the mycorrhizal inoculations (Figure 5.2). The highest levels of substomotal CO_2 were found in those plants that received the mycorrhizal inoculation combined with zinc, copper and phosphorus supplementation. After this were those plants that received the mycorrhizal inoculations in combination with phosphorus and copper. The treatments that did not received the mycorrhizal had the lowest levels of substomotal CO_2 when compared to those that did receive the mycorrhizal innoculations. The lowest levels in the non inoculated plants were found in those plants that received no nutrient supplementation (Table 5.2).

5.4.3 Effect of different combinations of mycorrhiza and nutrient supplementation on stomotal conductance of *A. afra.*

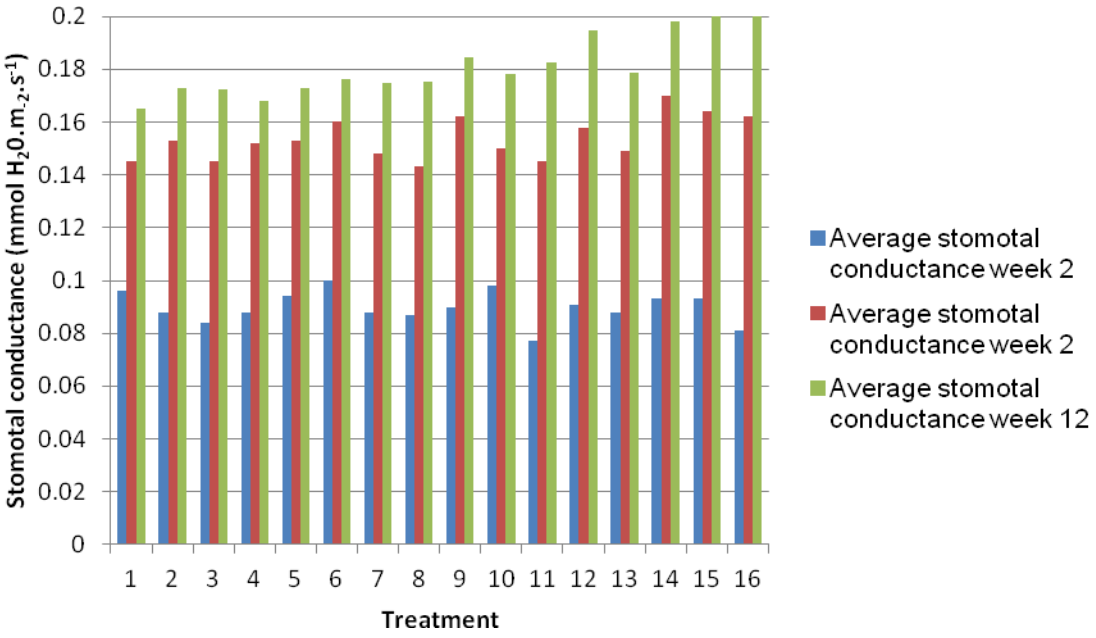

Figure 5.3: Average stomotal conductance of *A. afra* (treatments 1-8 without mycorrhiza, treatments 9-16 with mycorrhiza).

At weeks 2 and 6 there was little variation between the stomotal conductance of the treatments (Figure 5.3). By week 12 stomotal conductance was highest in those plants receiving the mycorrhizal inoculations. The plants that received both mycorrhizal inoculation and phosphorus supplementation had significantly higher stomotal conductance than those without. The plants that received no mycorrhizal inoculations all had low stomotal conductance levels, with those not receiving nutrient supplementation or inoculations having the lowest stomotal conductance of all treatments. Those plants that received phosphorus without mycorrhizal inoculations had higher levels of stomotal conductance than those that did not receive the supplementation (Table 5.3).

5.4.4 Effect of different combinations of mycorrhiza and nutrient supplementation on transpiration rates of *A. afra.*

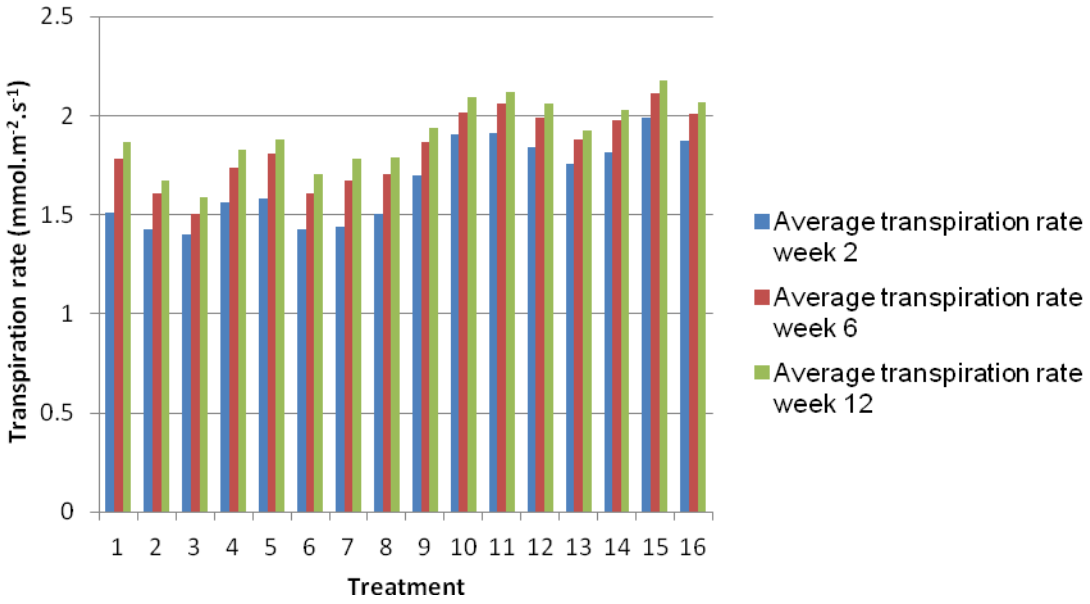

Figure 5.4: Average transpiration rate of *A. afra* (treatments 1-8 without mycorrhiza, treatments 9-16 with mycorrhiza).

The transpiration rates of *A. afra* were significantly affected by the applications of mycorrhiza (Figure 5.4). At week 2 there were already effects on the transpiration rates of those plants that received the mycorrhizal inoculations as they were higher than those that did not receive the inoculations. At week 6 the treatments containing mycorrhizal inoculations had significantly higher transpiration rates than those that did not receive the treatments, with those plants receiving mycorrhizal inoculations and supplementary phosphorus having significantly higher levels when compared to those that did not receive the inoculations or phosphorus. The plants with the lowest levels at weeks 2, 6 and 12 were those that did not revieve either mycorrhizal inoculations or supplementary phosphorus (Table 5.4).

5.4.5 Effect of different combinations of mycorrhiza and nutrient supplementation on anthocyanin levels of *A. afra.*

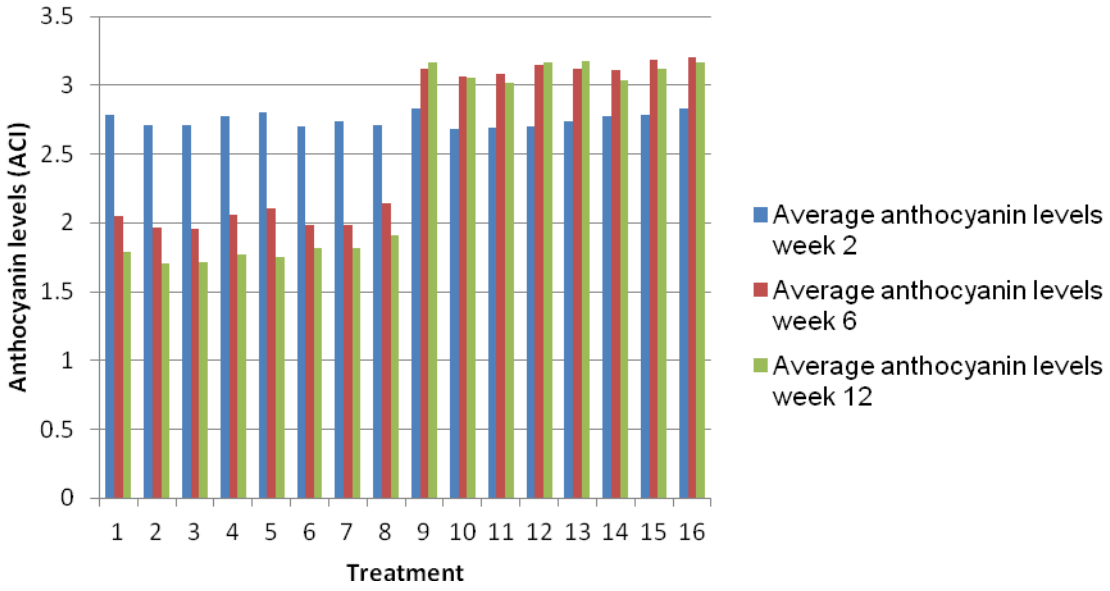

Figure 5.5: Average anthocyanin levels of *A. afra* (treatments 1-8 without mycorrhiza, treatments 9-16 with mycorrhiza).

During the first week there was little variation between the treatments with regards to anthocyanin levels (Figure 5.5). By week 6 there was a marked drop in anthocyanin levels of those plants that did not receive the mycorrhizal inoculations. At week 12 there was significantly higher levels of anthocyanins in the plants that received the inoculations of mycorrhiza when compared to those that did not receive the treatments. At weeks 6 and 12 there was little variation between the plants receiving the treatments of mycorrhiza (Table 5.5).

5.5 DISCUSSION

The applications of mycorrhiza had a strong influence on the photosynthetic processes of *A. afra*, whether alone or in conjunction with phosphorus supplementation (Azcon-Aguilar & Barea, 1997). This shows that the use of mycorrhizal inoculation has a positive influence upon plant growth, and indicates that there is potential for improving plant growth with its use. The fact that there was better growth in plants treated with both the inoculation and those that received phosphorus supplementation shows that, while the mycorrhizal inoculation will produce positive results, it works best in conjunction with nutrient supplementation. The plants with no nutrient supplementation that received the inoculation outperformed those that did not receive the inoculation, and this shows that the presence of the mycorrhiza alleviates some of the stresses caused by the lack of available nutrients (Feldmann et al., 1989).

The higher anthocyanin levels in those plants with mycorrhizal inoculations indicates that these treatments resulted in plants that are visibly healthier when compared to those that did not receive the inoculation. As visual health is a large factor in the selection of plant material by traditional healers (Canter et al., 2005) the use of the mycorrhizal inoculation has potential for influencing the traditional users, as those plants cultivated with the mycorrhizal inoculation will be more sought after than those that did not receive the mycorrhizal inoculation. This is especially relevant in an African setting, as the cost of nutrient supplementation can be prohibitive to the small scale cultivator (Diederichs, 2006).

5.6 CONCLUSION

The use of the mycorrhizal inoculation in the cultivation of the traditional medicinal plant *A. afra* shows potential due to it influencing the photosynthetic processes of the plant. The fact that plants with the mycorrhizal inoculation but lacking nutrient supplementation still outperformed those that received the nutrient supplementation without the mycorrhizal inoculation shows that there is the potential for small scale cultivators of medicinal plants to reduce spending costs on nutrient supplementation with the use of the mycorrhizal inoculation.

5.7 ACKNOWLEDGEMENTS

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Table 5.1: Effect of different combinations of mycorrhiza and nutrient supplementation on photosynthetic rate of *A. afra.*			
Treatment	**Week 2**	**Week 6**	**Week 12**
1 No mycorrhiza, no supplementation	3.24±0.04ab	4.45±0.04abcd	4.27±0.05def
2 No mycorrhiza +Zn	3.25±0.04ab	4.48±0.05abc	4.27±0.08def
3 No mycorrhiza +Cu	3.26±0.03ab	4.57±0.04a	4.41±0.08cd
4 No mycorrhiza +P	3.26±0.04ab	4.43±0.05abcd	4.17±0.05ef
5 No mycorrhiza +Zn+Cu	3.17±0.04b	4.33±0.05d	4.11±0.06f
6 No mycorrhiza +Zn+P	3.22±0.05ab	4.40±0.07bcd	4.16±0.07ef
7 No mycorrhiza +Cu+P	3.27±0.04ab	4.51±0.06ab	4.23±0.07def
8 No mycorrhiza +Zn+P+Cu	3.26±0.05ab	4.52±0.06ab	4.31±0.08de
9 Mycorrhiza, no supplementation	3.26±0.04ab	4.47±0.05abcd	4.66±0.07ab
10 Mycorrhiza +Zn	3.29±0.04a	4.57±0.04a	4.75±0.08a
11 Mycorrhiza +Cu	3.18±0.04b	4.38±0.04bcd	4.64±0.06ab
12 Mycorrhiza +P	3.25±0.04ab	4.49±0.06abc	4.68±0.06ab
13 Mycorrhiza +Zn+Cu	3.19±0.05ab	4.39±0.06bcd	4.56±0.07bc
14 Mycorrhiza +Zn+P	3.16±0.04b	4.44±0.03abcd	4.73±0.04ab
15 Mycorrhiza +Cu+P	3.24±0.04ab	4.35±0.06cd	4.59±0.07ab
16 Mycorrhiza +Zn+P+Cu	3.26±0.05ab	4.47±0.06abcd	4.70±0.06ab
One-way ANOVA (F-Statistic)	**0.92ns**	**1.9****	**12.34*****
Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at ***: P≤0.001,:P≤0.05, ns: not significant**			

Table 5.2: Effect of different combinations of mycorrhiza and nutrient supplementation on substomotal CO_2 of *A. afra.*			
Treatment	**Week 2**	**Week 6**	**Week 12**
1 No mycorrhiza, no supplementation	205.75±3.38ab	224.19±4.10b	239.59±4.04d
2 No mycorrhiza +Zn	210.57±3.25a	232.89±2.79ab	239.48±1.79d
3 No mycorrhiza +Cu	206.81±3.11ab	230.10±3.98ab	240.65±2.62cd
4 No mycorrhiza +P	206.30±2.88ab	229.67±2.61ab	240.66±3.11cd
5 No mycorrhiza +Zn+Cu	205.38±2.47ab	228.29±3.42ab	239.54±3.49d
6 No mycorrhiza +Zn+P	206.88±3.03ab	231.64±1.38ab	242.01±2.16bcd
7 No mycorrhiza +Cu+P	207.58±3.01ab	232.17±4.58ab	242.48±3.57bcd
8 No mycorrhiza +Zn+P+Cu	204.15±3.66ab	225.47±3.68ab	241.79±3.75bcd
9 Mycorrhiza, no supplementation	204.72±3.10ab	226.02±3.67ab	243.14±3.68bcd
10 Mycorrhiza +Zn	208.36±2.05ab	228.09±2.99ab	244.90±3.20abcd
11 Mycorrhiza +Cu	206.72±2.54ab	233.53±1.58a	248.76±1.90ab
12 Mycorrhiza +P	203.61±2.94ab	227.05±1.17ab	248.72±1.45abc
13 Mycorrhiza +Zn+Cu	207.59±2.84ab	231.13±3.56ab	246.56±3.72abcd
14 Mycorrhiza +Zn+P	203.29±2.80ab	228.20±2.51ab	247.85±1.76abc
15 Mycorrhiza +Cu+P	205.39±b3.49ab	226.92±4.19ab	249.58±1.65ab
16 Mycorrhiza +Zn+P+Cu	202.11±2.94b	233.07±1.56a	251.36±2.17a
One-way ANOVA (F-Statistic)	**0.50ns**	**0.86ns**	**2.0****
Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at **: P≤0.05, ns: not significant			

Table 5.3: Effect of different combinations of mycorrhiza and nutrient supplementation on stomotal conductance of *A. afra.*			
Treatment	**Week 2**	**Week 6**	**Week 12**
1 No mycorrhiza, no supplementation	0.10±0.007ab	0.15±0.008bc	0.17±0.004d
2 No mycorrhiza +Zn	0.09±0.006abc	0.15±0.008abc	0.17±0.006d
3 No mycorrhiza +Cu	0.08±0.005abc	0.15±0.005bc	0.17±0.004d
4 No mycorrhiza +P	0.09±0.005abc	0.15±0.008abc	0.17±0.005d
5 No mycorrhiza +Zn+Cu	0.09±0.008abc	0.15±0.006abc	0.17±0.008d
6 No mycorrhiza +Zn+P	0.10±0.007a	0.16±0.009abc	0.18±0.007cd
7 No mycorrhiza +Cu+P	0.09±0.007abc	0.15±0.009abc	0.18±0.009cd
8 No mycorrhiza +Zn+P+Cu	0.09±0.008abc	0.14±0.010c	0.18±0.009cd
9 Mycorrhiza, no supplementation	0.09±0.006abc	0.16±0.005abc	0.18±0.007abcd
10 Mycorrhiza +Zn	0.10±0.007ab	0.15±0.007abc	0.18±0.010bcd
11 Mycorrhiza +Cu	0.08±0.003c	0.15±0.005bc	0.18±0.006abcd
12 Mycorrhiza +P	0.09±0.006abc	0.16±0.006abc	0.19±0.007abc
13 Mycorrhiza +Zn+Cu	0.09±0.006abc	0.15±0.008bc	0.18±0.008bcd
14 Mycorrhiza +Zn+P	0.09±0.006abc	0.17±0.008a	0.20±0.007ab
15 Mycorrhiza +Cu+P	0.09±0.005abc	0.16±0.005ab	0.20±0.007a
16 Mycorrhiza +Zn+P+Cu	0.08±0.006bc	0.16±0.009abc	0.20±0.008a
One-way ANOVA (F-Statistic)	**0.935ns**	**1.200ns**	**2.72*** **
Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at ***: P≤0.001, ns: not significant			

Table 5.4: Effect of different combinations of mycorrhiza and nutrient supplementation on transpiration rates of *A. afra.*			
Treatment	**Week 2**	**Week 6**	**Week 12**
1 No mycorrhiza, no supplementation	1.51±0.11de	1.79±0.12cde	1.87±0.12cdg
2 No mycorrhiza +Zn	1.43±0.08e	1.61±0.09ef	1.67±0.09gh
3 No mycorrhiza +Cu	1.40±0.07e	1.51±0.08f	1.59±0.07h
4 No mycorrhiza +P	1.56±0.07cde	1.74±0.08def	1.83±0.08deg
5 No mycorrhiza +Zn+Cu	1.58±0.09cde	1.81±0.07cdef	1.88±0.07cdeg
6 No mycorrhiza +Zn+P	1.42±0.11e	1.61±0.09ef	1.70±0.09egh
7 No mycorrhiza +Cu+P	1.44±0.08e	1.67±0.09def	1.78±0.09egh
8 No mycorrhiza +Zn+P+Cu	1.50±0.09de	1.71±0.10de	1.79±0.11egh
9 Mycorrhiza, no supplementation	1.70±0.04bcd	1.87±0.05bcd	1.94±0.05bcde
10 Mycorrhiza +Zn	1.90±0.08ab	2.02±0.09abc	2.09±0.09abc
11 Mycorrhiza +Cu	1.91±0.05ab	2.06±0.05ab	2.12±0.05ab
12 Mycorrhiza +P	1.84±0.09ab	1.99±0.09abc	2.06±0.09abc
13 Mycorrhiza +Zn+Cu	1.76±0.07bc	1.88±0.07abcd	1.93±0.07bcde
14 Mycorrhiza +Zn+P	1.81±0.07ab	1.98±0.07abc	2.03±0.08abcd
15 Mycorrhiza +Cu+P	1.99±0.06a	2.11±0.08a	2.18±0.08ad
16 Mycorrhiza +Zn+P+Cu	1.87±0.09ab	2.01±0.09abc	2.06±0.08abcd
One-way ANOVA (F-Statistic)	**6.492*** **	**4.756*** **	**4.193*** **
Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at ***: P≤0.001			

Table 5.5: Effect of different combinations of mycorrhiza and nutrient supplementation on anthocyanin levels of *A. afra.*			
Treatment	**Week 2**	**Week 6**	**Week 12**
1 No mycorrhiza, no supplementation	2.79±0.055abcd	2.05±0.086b	1.79±0.055bc
2 No mycorrhiza +Zn	2.71±0.041cd	1.97±0.054b	1.71±0.041c
3 No mycorrhiza +Cu	2.71±0.042bcd	1.95±0.059b	1.71±0.042bc
4 No mycorrhiza +P	2.77±0.043abcd	2.06±0.087b	1.77±0.043bc
5 No mycorrhiza +Zn+Cu	2.80±0.035abc	2.10±0.044b	1.75±0.035bc
6 No mycorrhiza +Zn+P	2.71±0.058cd	1.98±0.059b	1.81±0.052bc
7 No mycorrhiza +Cu+P	2.74±0.044abcd	1.98±0.074b	1.82±0.044bc
8 No mycorrhiza +Zn+P+Cu	2.71±0.036cd	2.15±0.157b	1.91±0.125b
9 Mycorrhiza, no supplementation	2.83±0.039a	3.12±0.074a	3.17±0.091a
10 Mycorrhiza +Zn	2.68±0.031cd	3.06±0.062a	3.06±0.078a
11 Mycorrhiza +Cu	2.69±0.045cd	3.08±0.030a	3.02±0.047a
12 Mycorrhiza +P	2.70±0.043cd	3.15±0.074a	3.17±0.058a
13 Mycorrhiza +Zn+Cu	2.74±0.045abcd	3.12±0.111a	3.17±0.095a
14 Mycorrhiza +Zn+P	2.78±0.047abcd	3.11±0.049a	3.03±0.072a
15 Mycorrhiza +Cu+P	2.79±0.030abcd	3.18±0.049a	3.12±0.077a
16 Mycorrhiza +Zn+P+Cu	2.83±0.043ab	3.21±0.070a	3.17±0.098a
One-way ANOVA (F-Statistic)	**1.37ns**	**55.11*** **	**95.83*** **
Values (Mean ± SE, n = 10) followed by dissimilar letters in a column are significantly different at ***: P≤0.001, ns: not significant			

CHAPTER SIX

GENERAL DISCUSSION AND CONCLUSION

6.1 GENERAL DISCUSSION

The widespread wild harvesting of traditional medicines is leading to the loss of many plant species, and unless there is development of low input sustainable cultivation techniques that are suited to the African setting the trend will continue. To cultivate plants of a high quality requires various nutrient inputs, and this can result in the costs of cultivation being prohibitive to traditional healers. The desire of traditional healers for plants of a high quality is leading to the destruction of natural populations, and they will only begin to cultivate their medicinal plants if it can be demonstrated that the cultivation of these plants can result in products of a high quality with low cost inputs.

The investigation into the ideal pH for the production of *Artemisia afra* showed that there is highest growth at a pH of 6.5 in a hydroponic system. This could be due in part to the availability of nutrients at this pH level. Although there is a variation between the ideal pH in hydroponics when compared to growing in soil, the information in this study indicates that the cultivation of *A. afra* requires a specific pH range to achieve acceptable growth, with growth outside of this range being poor. This is useful information for the production of a quality crop, as hydroponic production is known to have higher growth rates and productivity when compared to traditional soil cultivation (Denisen, 1979). This was also apparent in the higher growth heights and weights of those plants grown in the hydroponic experiment when compared to those that were grown in the soil experiments.

The use of mycorrhizal inoculation in the cultivation of the medicinal plant *A. afra* has been shown to have dramatic results on both plant growth and marketability as well as photosynthetic processes, and this indicates that there is potential for the cultivation of this plant in a low input system.

Mycorrhizal inoculations resulted in plants that have higher yields of material with regards to both wet and dry weights of shoots, roots, total weights and plant heights. This could be attributed to those plants receiving the inoculations better utilizing the nutrients available to them. According to Azcon-Aguilar and Barea, (1997), this can be attributed to the increased surface area of the roots of inoculated plants, which allows the plant to access nutrients of low soil mobility such as zinc, copper and phosphorus.

Zinc is a micro nutrient required by plants in minute quantities, but its absence can lead to stunted and slow growth (Vallee et al., 1993). The developing leaves of plants deficient in zinc are smaller than normal, and internodes are shorter. Zinc is an integral component of enzyme structures and has three main aspects in plant growth: catalytic, coactive, or structural (Ohki, 1976). In the enzyme carbonic anhydrase which catalyzes the hydration of

carbon dioxide there is one zinc atom (Vallee & Auld, 1990). Carbonic anhydrase is found in chloroplasts and cytoplasm, and as the substrate for photosynthesis is carbon dioxide, zinc is necessary for photosynthesis to occur. In C3 plants there is no direct relationship reported between carbonic anhydrase activity and the photosynthetic carbon dioxide assimilation, however in C4 plants photosynthesis requires high carbonic anhydrase activity (Burnell & Hatch, 1988). The fact that the plants treated with mycorrhizal inoculations and zinc applications outperformed those that did not receive the inoculations in terms of photosynthetic processes shows that the uptake of zinc was positively affected by the mycorrhiza. Plants inoculated with the mycorrhiza that received supplementary zinc applications also outperformed those that received the zinc without the mycorrhiza in terms of wet weight, dry weight height and all other recorded growth variables. This indicates that the mycorrhiza had a positive impact on the plant's uptake of the available zinc.

Copper is a micronutrient required in low amounts for the proper functioning of plant processes such as photosynthetic and respiratory electron transport chains. Copper is necessary for many plant enzymes, such as phenolase, ascorbate oxidase, cytochrome oxidase, and superoxide dismutase, among others (Walker & Webb, 1981). Copper deficiencies can result in the reduction of the activity of these enzymes, leading to reduced plant growth and photosynthetic processes. The fact that plants treated with mycorrhizal inoculation and copper applications outperformed those that received the copper applications alone in terms of growth parameters and photosynthetic parameters shows that there was a significant effect upon the uptake and utilization of available copper for the plants. The higher levels of substomotal CO_2 in those plants that received combinations of copper supplementation and mycorrhizal inoculations shows that there was a higher uptake and therefore utilization in those plants that were inoculated with mycorrhiza.

Phosphorus is an essential macronutrient, needed in larger quantities by plants than zinc or copper (Edmond et al., 1975). Phosphorus has a low soil mobility, and as such it's uptake is greatly influenced by the use of mycorrhiza. Phosphorus is an essential component of Adenosine-5'-triphosphate (ATP), and as such it is needed for the conversion of light energy to chemical energy during photosynthesis (Marschner, 1995). Since ATP can be used for the biosynthesis of many plant bio-molecules, phosphorus is important for plant growth in terms of growth parameters and photosynthetic processes. The higher photosynthetic processes recorded in those plants that received the phosphorus treatments and mycorrhizal inoculations when compared to those that did not receive the inoculations indicates that there is a significant effect upon phosphorus uptake by the plants. The plants treated with phosphorus supplementation and mycorrhizal inoculation demonstrated higher wet and dry

weights and heights, which indicates that there was a positive influence on the uptake of phosphorus by the mycorrhizal inoculations.

The presence of nutrients in the soil can only increase the growth of plants if the nutrients can be accessed. The inoculations of mycorrhiza have been shown to have a large impact upon a plant's ability to access soil nutrients, and in this study it was shown that inoculations had positive effects upon growth variables such as plant height, wet and dry weights of roots and shoots, as well as higher levels of marketability. This indicates that the use of mycorrhizal inoculations is a potential concept for popularising the cultivation of medicinal plants, especially *A. afra.* The higher levels of marketability of plants inoculated with the mycorrhiza demonstrates that it is worthwhile to utilise mycorrhizal symbiosis in increasing profitability of cultivated medicinal plants.

The photosynthesis parameters of *A. afra* tended to be positively influenced with the applications of mycorrhizal inoculation, regardless of nutrient application. This demonstrates that the mycorrhizal relationship was assisting the plants in attaining the necessary nutrients for growth- the fact that the plants lacking nutrient supplementation but receiving mycorrhizal inoculation still performed well indicates the increased nutrient use efficiency of the inoculated plants.

6.2 CONCLUSION

The use of mycorrhizal inoculations had significant affects upon the growth and photosynthetic processes of *A. afra*. This indicates that there is potential for the low input cultivation of *A. afra* for production as a medicinal crop utilizing supplementary nutrition application and mycorrhizal inoculation. It is recommended that further investigations be made into the nutrient uptake of mycorrhizal inoculated plants, specifically focused on nutrient content of plant tissues. This could be a valuable direction of investigation as it would illustrate the effect upon uptake levels in inoculated plants. Another useful area of investigation for the future could be into the variation between ideal pH in soil cultivation vs. that of hydroponic cultivation, as the investigation into pH ranges had significant results.

CHAPTER SEVEN

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APPENDIX A: PHOTOGRAPHS

Figure 8.1: No mycorrhizal inoculation and no supplementary fertilization

Figure 8.2: No mycorrhizal inoculation combined with supplementary zinc application

Figure 8.3: No mycorrhizal inoculation combined with supplementary copper

Figure 8.4: No mycorrhizal inoculation combined with supplementary phosphorus

Figure 8.5: No mycorrhizal inoculation combined with supplementary zinc and copper

Figure 8.6: No mycorrhizal inoculation combined with supplementary zinc and phosphorus

Figure 8.7: No mycorrhizal inoculation combined with supplementary copper and phosphorus

Figure 8.8: No mycorrhizal inoculation combined with supplementary zinc, copper, and phosphorus

Figure 8.9: Mycorrhizal inoculation and no supplementary fertilization

Figure 8.10: Mycorrhizal inoculation combined with supplementary zinc application

Figure 8.11: Mycorrhizal inoculation combined with supplementary copper

Figure 8.12: Mycorrhizal inoculation combined with supplementary phosphorus

Figure 8.13: Mycorrhizal inoculation combined with supplementary zinc and copper

Figure 8.14: Mycorrhizal inoculation combined with supplementary zinc and phosphorus

Figure 8.15: Mycorrhizal inoculation combined with supplementary copper and phosphorus

Figure 8.16: Mycorrhizal inoculation combined with supplementary zinc, copper, and phosphorus

APPENDIX B: PAPER PUBLISHED IN *THE JOURNAL OF MEDICINAL PLANT RESEARCH*