Cape Peninsula University of Technology

EFFECTS OF COMPOST TEA EXTRACT ON GROWTH, NUTRITIONAL VALUE, SOIL QUALITY OF *HYPOXIS HEMEROCALLIDEA* AND *SIPHONOCHILUS AETHIOPICUS.*

by

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DECLARATION

I, Timothy Ivan Jasson, 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**

T.I. Jasson

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DEDICATION

I dedicate this thesis to my late
Parents, Ivan and Thelma Jasson, and
My wife, Joy-Ann Jasson,
who always inspired and encouraged me.

ABSTRACT

The exact responses to the concentration of compost tea extract and methods of irrigation application were not previously measured on *Hypoxis hemerocallidea* and *Siphonochilus aethiopicus*. Commercial exploitation, habitat loss and degradation, overharvesting, and enhancement of their medicinal properties, have led to this investigation and the need to replenish both these valuable plant species. This is crucial for plant survival, especially in the wild and for use of the traditional medicinal plants.

Hypoxis hemerocallidea and *Siphonochilus aethiopicus*, known as star flower and wild ginger respectively continue to decline, due to overharvesting from their natural habitat. Both these species have tremendous traditional medicinal value among localized African people. To enhance their commercial cultivation, compost tea extracts, in the following ratios (no catalyst added (control1); T 1000:1, T750:1, T500:1, and T250:1L) were applied in equal dosages to determine an optimal compost tea extract ratio. The experiment was conducted in a temperature controlled greenhouse. Mushroom compost (500 g, per brew) was used for all extracts. Brewing was done with no catalyst added (Control 1), and 24 hours later another brew was done with catalyst added, weekly for 20 weeks. The Control treatment received water only. Both species were slow growing and comparatively, the Hypoxis plants responded faster than the Siphonochilus plants. In this investigation, plant growth parameters such as plant height, number of leaves, leaf width, leaf length and leaf colour, were measured and evaluated. Despite the plants positive response to the mushroom compost tea extracts, across all the above plant growth parameters, no significant differences were noted between the treatments during the twenty-week application period.

Leaf chlorophyll content peaked in week 11 of the hypoxis plants and was the highest in week 14 of the Siphonochilus plants with no significant interaction between weeks vs. treatment over the twenty-week experiment. The chlorophyll readings indicate that both species increased their chlorophyll production over time.

Although the total wet leaf length, root length, corm diameter, leaf weight, corm weight and root weight of Hypoxis were non-significant between treatments, T500:1 total wet weight was significantly higher when compared to the rest of the treatments. The total dry weight analysis of hypoxis was non-significant. Control Calcium level was significantly lower between the control and the treatments of the Hypoxis total dry leaf nutrient analysis. The Hypoxis dried roots nutrient analysis was non-significant across treatments.

Siphonochilus total wet analysis revealed significant differences between treatments with regard to leaf length, height, total root length, leaf and stem weights. The total dry weight analysis revealed no significant difference between treatments. The total dry leaf nutrient analysis revealed significant differences in sodium, manganese and zinc levels, whilst the nitrogen, phosphorus, potassium, calcium, magnesium, iron, copper and boron were non-significant.

The antioxidant capacity and content yielded the following results; the polyphenol antioxidant content was not significant in both species. There was an increased flavonol content observed in the corms of *H. hemerocallidea*. In the rhizomes of *Siphonochilus* there were significant differences in flavonol content between CEO (Control 1), T250:1 and T1000:1 compost tea treatments. The corms of *H. hemerocallidea* showed higher flavonol antioxidant activity. No flavanol content was detected in *H. hemerocallidea*. Significant differences were detected in the flavanol content in *S. aethiopicus*. ORAC (oxygen radical absorbance capacity) was not significant in both species. Significant differences were detected in the FRAP (ferric reducing antioxidant power) of the *H. hemerocallidea* compost tea treatments, but not in *S. aethiopicus*. ABTS (2,2′-azino-di-3-ethylbenzthialozine sulphonate) values were non-significant in both species.

Both species responded differently in all the treatments. Mushroom compost tea extract is recommended to enhance growth of these two endangered species in an organic growth system.

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1.1 Research problem

1.1.1 Problem statement

The aim of this investigation was to analyse the effects of commercial compost tea catalyst added to mushroom compost tea extracts, on yield, nutritional value and soil quality for the cultivation of *Hypoxis hemerocallidea* and *Siphonochilus aethiopicus*. Known as star flower and wild ginger respectively, both species have been, and are still declining, due to overharvesting, because of their tremendous traditional medicinal value among localized African people (Cunningham, 1993; Williams et al., 2008).

1.1.2 Research question

Both species are slow growing therefore, research is needed to determine how quickly the species can grow and multiply, under controlled growing conditions. According to Ingham (2005), compost tea research has been done on a variety of plants and crops, such as asparagus, potato, corn, wheat, tomato, lettuce and radish, in orchards and vineyards, landscape trees, in greenhouses, and in nurseries. However, insufficient compost tea research has been done so far on *Hypoxis hemerocallidea* and *Siphonochilus aethiopicus*.

1.1.3 Hypotheses

Mushroom compost tea extract solutions may have a positive effect on the yield (the fresh weight) and quantity (the number of bulbs) of the *Hypoxis hemerocallidea* and *Siphonochilus aethiopicus* plant structures (roots, bulbs (corm, rhizome) and leaves).

It is hypothesised that:

- the compost tea extract will improve the growth of the plants, across all dosages grown under soil drench methods.

- The growth difference in the Hypoxis and Siphonochilus plant structures will differ between the species and the treatments due to, different growth patterns between the two species, the effect of soil drench applications of the compost tea extracts, thereby affecting nutrient uptake and location in the respective plant structures.

- The nutrient value in the Siphonochilus and Hypoxis plant structures differ between different soil drench applications.
- The effects of the compost tea extract treatments on wet and dry weights, soil nutrient levels, and the availability of soil mineral elements of the soil drench methods will not be significantly different when compared to the control treatment.

- The effect of various concentrations of compost tea extracts may enhance the antioxidant activity and content of both species. The use of compost tea extracts could possibly enhance the medicinal properties, specifically its antioxidant activity, of both the above mentioned species as well as promoting the future commercial cultivation to supply the growing demand for the species.

1.1.4 Objectives

Main objective

To increase growth and nutritional value of *Hypoxis hemerocallidea* and *Siphonochilus aethiopicus* through soil drench applications of mushroom compost tea extract.

Specific objectives

1. To determine the desired compost tea extract concentration on the physiological improvements/ growth response (total shoot growth, plant height, number of leaves, leaf length, photosynthesis and chlorophyll content).
2. To assess which compost tea extract concentration will exhibit the best antioxidant capacity and –content.
3. To assess the effects of the compost tea extracts on soil nutrient levels, and the availability of mineral elements (N, P, K, Ca, Mg, S, Fe, B, Mn, Cu, and Zn).
4. With the results, conclusions can be made on the use of compost tea, growth improvement, nutritional content, and antioxidant content and capacity.

1.2 Outline of the chapters

The dissertation consists of seven chapters. Chapter one describes and highlights the research problem, the hypotheses tested, the objectives, which guided the study and highlights the overview of the chapters. In chapter two, the potential effects of organic compost tea extracts on the growth and medicinal potential of *H. hemerocallidea* and *S. aethiopicus* were investigated. Chapter three evaluated the effects of mushroom compost tea extracts on the growth of *H. hemerocallidea* and *S. aethiopicus* under different soil drench applications to improve their cultivation as traditional medicinal species. In chapter four the

focus was on the effects of mushroom compost tea extract on chlorophyll content in *H. hemerocallidea* and *S. aethiopicus*. Chapter five evaluated the effects of mushroom compost tea extracts on weight, growth media nutrient and mineral elements on the growth of *H. hemerocallidea* and *S. aethiopicus.* Chapter six tested the effect of compost tea extracts on the antioxidant capacity and content of *H. hemerocallidea* and *S. aethiopicus*. In chapter seven the general conclusion and recommendations of the dissertation covering all the chapters are discussed.

Chapter Two

LITERATURE REVIEW AND GENERAL INTRODUCTION

THE EFFECTS OF ORGANIC COMPOST TEA EXTRACTS ON THE GROWTH AND MEDICINAL POTENTIAL OF *HYPOXIS HEMEROCALLIDEA* AND *SIPHONOCHILUS AETHIOPICUS:* A REVIEW

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2.1 ABSTRACT

Compost tea extracts are gaining popularity over conventional fertilizers as the demand for organic production increases for crops to be healthier and less susceptibility to disease. Further advances are the improvement of soil conditions, cost saving on pesticides and reduced fertilizer degraded ecosystems. The increased demand for African medicinal species such as *Hypoxis hemerocallidea* and *Siphonochilus aethiopicus* have led to their commercial exploitation with overharvesting, degradation and habitat loss. The use of compost tea extracts in their cultivation could lead to the enhancement of their medicinal properties with the improvement of nutrient content of leaves, corms, rhizomes and roots. The investigation of improved cultivation methods designed to replenish both these valuable plant species could be crucial for their survival in the wild and future traditional and commercial medicinal plant utilization. This review aimed to highlight the potential effects of organic compost tea extracts on the growth of *H. hemerocallidea* and *S. aethiopicus* medicinal species.

Keywords: compost tea extract, compost, leachate, fertilizer, plant nutrition, NTC

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2.2 INTRODUCTION

Compost teas are widely used as an alternative nutrient supplement to improve the quality of plant production in field grown and nursery crops in substituting/replacing chemical fertilizer programmes (Ingham, 2005; Khalid *et al*., 2006; Naidu *et al*., 2010). The increase in inorganic fertilizer and fungicide costs have also led to the increase in popularity of compost tea as an economical alternative to supply nutrients for plant growth (Touart, 2000; Ingham, 2005; Sanwal *et al*., 2006; Hargreaves *et al*., 2008; Siddiqui *et al*., 2011). Various studies have proved the effects of beneficial organisms assisting in the prevention of diseases when applying compost tea to plant leaves (Scheuerell, 2004; Ingham, 2005; Souleymane *et al*., 2009). Internationally compost tea has gained extreme popularity for turf applications due to cost and the environmental restrictions in the use of traditional chemicals (Ingham, 2005; van Zwieten *et al*., 2007; Anonymous, 2011; Kelloway, 2012; Mullaivannan, 2013). While several studies have highlighted the beneficial organism and micronutrient potential of compost tea which support a reduction in the use of fungicides, the benefits of compost tea are also seen in weed control and the improvement of root penetration of sport turf grasses (Scheuerell *et al.*, 2002; Bellows, 2003; Sachs, 2004; Ingham, 2005; Arumugam, 2012; Islam *et al.*, 2013, Modal *et al*., 2013; Garrett, 2014). Compost tea has also gained popularity in conventional agriculture crops with current trend towards organic agriculture production (Theunissen *et al*., 2010). These crops, mostly field vegetables such as potatoes tend to be healthier and less susceptibility to diseases after compost tea application (Scheuerell *et al.*, 2002; Ghorbani, 2004; Litterick *et al*., 2004; Larkin, 2008).

The use of compost tea has also gaining considerable interest internationally in wheat production, mainly due to the rising cost of fertilizers. The organic component amendments to the soil allow low cost growth stimulation and building up of a sustainable soil structure (Ingham, 2005; Anonymous, 2011). Additionally, in the international landscaping industry, compost tea has become the preferred organic fertilizer to use, as contractors' report that plants exhibit a 'compost tea glow', on turf, ornamentals and trees while this has also led to a reduction in standard fertilizer and pesticide usage without compromising plant vitality and performance (Ingham, 2005; Anonymous, 2011). Studies on commercial vine cultivation showed an increase in grapes size where compost tea are used as a sustainable fertilizer alternative in cultivation (Ingham, 2005; Dufour, 2006; Anonymous, 2011). Fungal diseases such as powdery mildew and botrytis have also shown a decline with an increase in soil and vine health and ultimately resulting in better fruit quality and harvests (Anonymous, 2011). There is little evidence however on the growth responses of medicinal species to compost

tea applications. The use of compost tea on medicinal species would be in line with current organic global growing trends as commercial cultivation of medicinal species requires optimum growth and plant vitality with the least amount of chemical substance use and the cultivation of many species needs to be expanded (Koehorst *et al*., 2010). This review aimed to report on the potential of compost tea extracts on plant growth of two medicinal species, *Hypoxis hemerocallidea* and *Siphonochilus aethiopicus*.

2.2.1 Soil improvements both inorganic and organic

Commercial farming practices and crop variation are constantly evolving, as soils need to be improved with the addition of chemical sources, such as fertilizers and individual elements such as nitrogen, phosphorous and potassium (Li *et al.*, 2003; Cordell *et al.*, 2009, Dawson *et al.*, 2011). Agricultural harvests also deplete soil health of essential nutrients with a resulted loss of tilth which needs to be compensated with replacement nutrients for future reuse (Sanchez, 2002; Denning *et al*., 2009). While the use of inorganic fertilizers is also seen as a temporary measure to replace nutrients until it leaches from the soil again (Weaver *et al*., 1988; Eriksen, 2001), chemical fertilizers are associated with water quality control during cultivation (Shaviv, 1993; Foley *et al*., 2005). Where excess nutrients are no longer held in the soil or washed out during heavy irrigation, leaching continues into underground streams and rivers (Hillel, 1991; Plaster, 2013; Stauffer, 2013). This leads to extensive degrading of the environment and a continual need for replenishment of excess amounts of inorganic nutrients in soils (Theunissen *et al*., 2010). Healthy soils should contain sufficient naturally decomposed materials where excess nutrients are more easily contained and prevented from leaching from the earth (Ingham, 2005). As a preventative measure, and an important natural resource, soil needs to be preserved at all times to retain its ability to provide sufficient nutrients to sustain improved plant growth (Lal, 1997; Thien, 1998; Doran & Zeiss, 2000). According to Doran and Parkin (1994) soil quality is defined as “the capacity to function within an ecosystem and sustain biological productivity, maintain environmental quality and promote plant, animal and human health”. Key to the survival of all plant life is the addition of compost, as well as other organic materials, in order to boost the soil fertility and crop yield (Magdoff & Van Es, 2000; Pettit, 2004; Bot & Benites, 2005). In organic agriculture, adding organics, in the form of compost is fundamental and by enriching the soil organically, its soil fertility can increase to optimize crop production capacity (Theunissen *et al*., 2010). Additionally, land should be utilized optimally where water management can be enforced and chemical pollution of the environment be prevented (Amberger, 2006; White *et al.*, 2012). All forms of inorganic substances such as pesticides, fertilisers could be excluded or drastically reduced, while organics, replenished regularly will maintain and sustain long term soil fertility thereby providing optimal soil biological activity (Killham, 1994; Bardgett,

2005; Diacono & Montemurro, 2010). Organic agriculture therefore recognises that everything in the environment affects everything else, and that one component cannot be taken out of the system, without positively or negatively affecting other things (Sharma, 2001). A good practice of organic composting in the making of compost tea will depend on how it is made and what compost material can be sourced (Haug, 1993; Scheuerell & Mahaffee, 2002; Litterick *et al*., 2004). For this reason, on-going trials remain necessary to evaluate organic improvement of soils to support healthy plant growth for medical plant crops.

2.2.2 The making of compost

The decomposition of organic material such as garden waste, plant leaves, manure, straw and other organic based materials are derivatives for making compost (Chen *et al*., 1997; Veeken & Hamelers, 2002; Manios, 2004). Compost making is processes of organic material breakdown through an aerobic reaction whereby microorganisms convert a mixed organic substrate into carbon dioxide (CO_2) and water with essential minerals and stabilized organic matter (Zucconi & Bertoldi, 1987). Under controlled conditions, moisture and aeration is required to produce temperatures of 50-140 °C for microorganisms to enhance the composting process (Chen & Inbar, 1993). The extent of organic matter decomposition at any particular time depends on the temperature at which decomposition takes place, as well as the chemical composition of the organic substrate undergoing decomposition (Levi-Minzi *et al*., 1990). Good conditions are created for oxygen supply for microbial respiration by ensuring a pore space of approximately 5% in the material, a moisture content of approximately 40-65 %, particle sizes of approximately 0.3175 to 5.08 cm in diameter, and a C/N ratio of between 20:1 and 40:1 (Rynk *et al*.,1992). During the first stage of composting, which lasts 1-2 days and at temperatures between 30-40° C, there are mesophilic strains of microorganisms that start the decomposition of the material (Rynk, 2003; Schuchardt, 2005; Kutzner, 2008). These microorganisms rapidly consume proteins, fats, sugars and starches, thereby increasing the heat, resulting in a rise in temperature of the substrate. Typically, pH drops as organic acids are made (de Bertoldi *et al.*, 1983; Rynk, 1992; Haug, 1993; Epstein, 1996). The second phase, also known as the thermophilic stage, involves microbial activity to increase temperatures between 50-60° C (de Bertoldi *et al*., 1983; Rynk, 1992; Haug, 1993; Epstein, 1996). Around these temperatures specific heat loving thermophilic bacteria actively degrade the organic material (de Bertoldi *et al*., 1983; Rynk, 1992; Haug, 1993; Epstein, 1996). As long as the temperatures remain at this range, with adequate material available and a sufficient oxygen supply, microbial activity will continue to be active in the process (Chen & Inbar, 1993). Organic matter further degrades as particle size reduces. While above temperatures of 55 °C pathogens are destroyed, fly larvae and most weed seeds are

destroyed above 60 °C. As the protein degrades, the pH rises above 7 and ammonia is released (Rynk *et al*., 1992). Active composting is completed when the temperature gradually drops to 40 °C, and the volume of the original material shrinks by 25 to 50% (de Bertoldi *et al*., 1983; Rynk, 1992; Haug, 1993; Epstein, 1996). Slowly as the breakdown of the organic material continues, no more heat is generated, the temperature drops to that of ambient air, only then is the compost ready for curing (Rynk *et al*., 1992). Curing can last up to 30 days and helps to insure against negative consequences of applying immature compost to crops, which could result in the inhibition of seed germination (de Bertoldi *et al*., 1983; Rynk, 1992; Haug, 1993; Epstein, 1996; Chen *et al.*, 1997). While compost is curing, less heat is generated, and the medium pH turns slightly alkaline (Rynk, 1992; Chen & Inbar, 1993). Common microorganisms, both beneficial and pathenogenic, and microfauna recolonize the compost and intense microbial competitions for food occur in the compost, through both direct antagonism and production of antibiotics (Chen *et al.,* 1991; Chen & Inbar, 1993; Litterick *et al*., 2004). Pathenogenic competition from fungi such as *Pythium de baryanum*, *Rhizoctonia solani* and *Phytophthora infestans*, is often suppressed by beneficial microbial species (Hoitink & Fahy, 1986; Whipps, 1992). Reports also suggest that compost induced protection against *Fusarium oxysporum* in sweet basil plants (Reuveni *et al*., 2002; Hashem *et al*., 2010). The composting materials, bacteria, oxygen and moisture determine the quality of the compost used for making compost tea extracts, while experimentation with various composts such as mushroom compost can determine the quality of the compost tea extract.

2.2.3 Mushroom compost: a source of compost tea

There are several mushroom compost recipes to be found worldwide. As mushroom compost is initially used to grow mushrooms, it typically consists of the following: wheat straw, horse manure, ground chalk, poultry litter, peat moss, gypsum, as well as other organic substances (Chen *et al*., 2000; Fidanza, 2011). During commercial mushroom compost production, straw is first moistened for several days, after which manure, gypsum and nitrogen supplements are added (Martin *et al.*, 1992; Davis *et al*., 2005). The compost is then stacked on average 2 m high x 2 m wide, in the open, or under roof, with the stacks being turned mechanically every three days (Haug, 1993; Fletcher & Gaze, 2007). The temperature should not exceed 70° C during the composting process, which can last for up to three weeks (Hoitink & Fahy, 1986; Hoitink *et al*., 1997). The compost is then pasteurized at 60° C for 6-8 hours, conditioned at 48-52° C for 5-7 days, and cooled down to 25° C (Chen *et al*., 2000; Uzun, 2004). As a result of this, many microbes that would compete with the mushrooms for food, and restrict mushroom growth are eliminated. Toxic ammonium levels are converted into protein, which the mushroom can use for growing (Chen *et al*., 2000; Uzun, 2004). The compost is then transferred onto shelves, with spawn added, and placed in the growing

rooms. Two weeks later, once colonized by mycelium, it is covered with peat, and three weeks later the first mushrooms are harvested. (Chen *et al*., 2000; Uzun, 2004). There are normally three flushes of mushroom harvesting, after which productivity declines. It is then called spent mushroom compost (SMC). While the properties of this compost vary greatly, it is mainly dependant on the composting processes, cultivation techniques and weather conditions. Furthermore, SMC also contains no pest and weed seeds, due to the high composting and pasteurization temperatures (Uzun, 2004). SMC is sourced from mushroom growers for further decomposition, after which it is bagged and sold through garden centres and hardware stores or used in the landscaping and production nursery sector (Chen *et al*., 2000; Uzun, 2004; Fidanza 2011). Additional testing of compost samples for the nutrient makeup before their use is vital, as this will provide information on the nutrient quantities, thereby justifying their use (Theunissen *et al*., 2010). As most compost are used for gardening purposes very little is known on it effects in commercial cultivation. Future studies are necessary to determine the effect of compost on commercial plant cultivation. Research efforts should focus on the influence of compost from different sources on nutrient availability in medicinal plants.

2.2.4 The brewing of compost tea

The brewing of compost tea is not a new research field as over centuries, since the Roman Empire it has evolved into a complex formula of different mixes and doses to be used as a compost tea (Ingham, 2005; Hargreaves *et al*., 2008; Litterick & Wood, 2009). Recipes and machinery can be found worldwide, with many compost tea companies each promoting their products and ‘solutions’ (Ingham, 2003). Literature abounds of testimonial observations that prove that compost teas have some sort of benefit for growing plants (Scheuerell & Mahaffee, 2002; Litterick *et al*., 2004; Ingham, 2005). Some scientifically based studies found that organic slurries not only dissolve nutrients, but over time and with oxygen, they can extract humic acids, organic nutrients, vital enzymes and beneficial microbes, all of which enhance more vigorous plant growth (Merrill *et al*., 1998). The advantages of compost tea are reported to be 100% natural and safe in improving soil and plant health, reduce the risk of plant disease, aid in nutrient recycling, improve root growth and penetration, and reduces pesticide application (Ingham, 2005; Anonymous 2011).

There are clear differences between, manure teas, compost leachate and compost extract, with the former often being called or referred to as being compost tea (Scheuerell & Mahaffee, 2002; Ingham 2005). Compost tea is a water extract of compost that is brewed so that the beneficial organisms, i.e. the beneficial bacteria, fungi, protozoa and nematodes, are extracted from the compost and given the right environment to increase in number and

activity by providing soluble food sources and the nutrients present in the water (Scheuerell & Mahaffee, 2002; Ingham 2005). The quality of the compost will determine how large a diversity of these beneficial organisms will be present (Ingham, 2003). Manure tea is a water extract of manure. The result can contain soluble nutrients, high nitrates, salts, phosphorous and potassium. Manure tea can also contain high numbers of harmful bacteria unless there has been an antibiotic used in the animal feed (Gershuny, 2004; Ingham 2005). Manure tea often contains root feeding nematodes and almost always contains human and animal pathogens (Gershuny, 2004; Ingham 2005). Manure tea typically contains *Escherichia coli* since manure comes from mammalian digestive systems and can also harbour *Salmonella* and *Shigella* species (Ingham, 2003). Compost leachate involves the draining of water through over saturated compost. It contains soluble nutrients but very few organisms unless it is cycled through the compost many times (El Hanafi Sebti, 2005; Ingham 2005). If it is cycled enough times, adequate numbers of organisms may be leached from the compost to protect leaf and roots surfaces (El Hanafi Sebti, 2005; Ingham 2005). It has been argued that most leachate contains only soluble nutrients and few organisms (Ingham, 2003). Compost extract is obtained when adding water to compost and allow excess water to drain from the compost. Typically, this means the compost is over saturated (Scheuerell & Mahaffee, 2002; Ingham 2005).

2.3 THE POSSIBLE EFFECTS OF COMPOST TEA ON SOIL MEDIUM IMPROVEMENT AND PLANT GROWTH

Research evidence has shown that, compost tea extract can improve soil characteristics, plant growth and the quality of the final products (Bess, 2000; Touart, 2000; El Hanafi Sebti, 2005; Zalle, 2006). The use of a commercial compost tea extract as part of an organic, environmentally sound approach, in crop growing has slowly gained momentum (Bess, 2000; Touart, 2000, Ingham, 2005; Anonymous 2011). There are various approaches to compost tea extraction and its utilization, in the horticultural, turf care, agriculture, landscaping, and viticulture sectors. It is good practice to start with good, high quality compost (El Hanafi Sebti, 2005). The following considerations are to be borne in mind: (1) aeration and circulation: where oxygen can be depleted when the compost tea is not adequately agitated, resulting in a reduction of aerobic microbial growth, thereby favouring anaerobic conditions and ultimately resulting in the poor extraction of minerals from the compost and (2) The brew time: whereby more soluble materials will be extracted from the compost, the longer it remains suspended in the water (Merril *et al.*, 1998; Ingham, 2005). Under more basic conditions it may be necessary to allow the compost too steep for a few days to a few weeks to improve its quality. It remains vital in brewing a high quality compost tea to source high quality compost with a good compost to water ratio. Too much water, results in insufficient

nutrients from the compost, while too much compost, possibly can result in an excess of nutrients for bacteria to grow which can lead to oxygen depletion and anaerobic conditions (Merril *et al*., 1998; Ingham, 2005). During the mid-1980s, compost tea research started to develop, with some researchers following a non-aerated method which resulted in anaerobic conditions during compost tea production while others followed the aerated method that aerates the water/ media (Merril *et al*., 1998; Ingham, 2005).

During non-aerated extraction of compost, the compost/manure/ or any reasonable organic matter is simply left to soak in water and agitated occasionally. After a few days the aerobic microbes have pulled out all the oxygen out of the water (Merrill *et al*., 1998; Ingham, 2005). The anaerobic microbes (which do not require oxygen) increase, resulting in a poor tea that has little nutrients, few organic acids for example acetic, butyric, and proprionic acids, with odours of sulphur and nitrogen that can harm plant growth (Merrill *et al*., 1998). In contrast, with the use of an open container, aerated compost extraction occurs. The mixture is stirred to circulate the matter, introducing air into the water. This leads to the maximum amount of nutrients being extracted, retaining the oxygen, thereby producing a tea high in aerobic fungi and bacteria, as well as protozoa and nematodes (Diver, 2002). Sufficient oxygen must be pumped into the water so that all the beneficial organisms can be extracted and sustained (Diver, 2002; Ingham, 2005). The easiest, is to mix compost to water at 1:5 ratio, and allow the mixture to rest and ferment (Greene, 1999). Growing Solutions system 10, recommends a ratio of 1:8 which translates into 500 g of compost: 40 litres of water (Anonymous, 2011). Another recommended ratio is 1:10 (Wickland *et al*., 2001). Environmental conditions such as evaporation, temperature and humidity can affect the quality of the compost tea extract. Cold water will reduce extraction and microbial growth, whilst warm water inhibits microorganisms' or excessive evaporation can occur (Martens, 2001; Ingham, 2005). The ambient temperature can be changed by placing a lid over during hot weather thereby helping to control evaporation. Using a fine mesh bag, will draw out only tiny soluble ingredients into the water. This is vital should the compost tea extract be applied via a sprayer or via irrigation systems. Chemical properties of the water can influence the quality of the compost tea extract produced (Martens, 2001; Ingham, 2005). Water that contains high levels of heavy metals, pesticides, nitrates, salts, pathogens and chlorine should not be used, as they will affect the production, reproduction and survival of beneficial organisms from the compost. These compounds in any combination may affect the plants on which the compost tea extract is applied (Martens, 2001).

These so called 'failures' vary tremendously depending of the type of plant species, fungus species, compost mixture/s, aerated vs. non aerated teas, vs. anaerobic tea vs. manure tea vs. compost extract vs. compost leachate and the research interest. Often there are mixed

results. In a study conducted on non-aerated compost tea (NCT), the extract failed to control powdery mildew, but was able to control tomato grey mould while the NCT made from sheep manure compost consistently provided the highest inhibition of mycelial growth and disease suppression of grey mould (Souleymane *et al*., 2009). Compost water extracts (CWE) suppressed anthracnose on the roots of peppers and cucumbers, but failed to reduce the diseases on the upper leaves of the plants (Sang & Kim, 2011). There are many variables that can influence the results of research, such as the source of the compost, temperature of decomposition and time of decomposition (Rynk, 1992; Haug, 1993; Epstein 1996). It is well known that compost tea extract improves the growth of crops (Scheuerell & Mahaffee, 2002; Ingham 2005), but the exact response to the volume of compost tea extract and methods of irrigation has not been previously measured and tested locally and more importantly on medicinal plant species such as *H. hemerocallidea* and *S. aethiopicus*. Mainly due to these reasons, further investigations are necessary to explore the future potential and 'failures' of compost teas especially on important medicinal species such *H. hemerocallidea* and *S. aethiopicus*.

2.3.1 Compost teas brewing systems and machinery

There are a variety of compost tea brewers commercially available, which vary according to its capacity, from commercial to homemade versions, dependant on the volume of compost tea to be sprayed and utilized on the farms as well as how often compost tea will be utilized (Figures 2.1, 2.2 and 2.3).

Figure 2.1 The Growing solutions compost tea brewer system 10™, front and rear view with lid and pump. (Picture: http://www.growingsolutions.com/compost-tea-systems/compost-tea-system10/)

Figure 2.2 The Growing solutions compost tea brewer range for commercial and domestic use. (Picture: http://www.growingsolutions.com/compost-tea-systems/compost-tea-system10/)

Figure 2.3 Commerical "Green Pro solutions" compost tea makers show indicater level and free standing pumps. (Picture: http://www.greenprosolutions.com/compost_tea_brewers.htm)

2.3.2 Importance of growing *Hypoxis hemerocallidea* organically

H. hemerocallidea (Hypoxidaceae) is a cormous perennial with long, hairy, strap-like leaves and yellow star-shaped flowers borne on 5 to 6 long inflorescences during spring (SANBI 2009). The name hypoxis is derived from the Greek words, hypo (below) and oxy (sharp), with reference to the ovary which is pointed at the base. The specific epithet is derived from the Greek hemera (a day) and kallos (beauty) presumably referring to the flowers that are short lived and bears resemblance to the day lily, *Hemerocallis* sp. Formerly known as *H.*

rooperii, the broad leaves are arranged one above the other, resulting in three distinct sections spreading outwards from the centre of the plant (Van Wyk *et al*., 2009). The genus Hypoxis consists of approximately 90 species, of which 30 species are found mostly in eastern southern Africa (Singh, 2004; Singh, 2007). The name 'African Potato' was given to the species after the Afrikaans, 'Afrika patat'. The plant is however a compressed underground corm which grows vertically and is commonly known as 'Inkomfe' among African Zulu speaking people (Van Wyk *et al*., 2009). Tubers are dark brown in colour, large and covered with bristly hairs (Figure 2.4). When freshly cut, the tubers are bright yellow in colour, with a bitter taste (SANBI, 2009).

The species are valued by traditional healers for treating tuberculosis (TB), cancer, urinary tract infections, anxiety, palpitations, and rheumatoid arthritis, amongst others. Some studies reported that the aqueous extract has anticonvulsant activity, thereby lending credence to traditional herbal use to treat childhood convulsions and epilepsy (Ojewole, 2008). It is also used to restore immune systems of patients recovering from HIV and cancer. Hypoxis phytosterols, used to treat benign prostrate hypertrophy was successfully marketed, and later phytosterols (based on industrial sources) claiming to have immune- stimulating effects, became and still is a huge marketing success (Drewes *et al*., 2008). The phytochemical, hypoxoside, is an inactive compound that is converted to rooperol. The species has thus potent pharmacological properties to treat relevant inflammations as well as anti-infective, anti-diabetic, antioxidant, and antineoplastic conditions in patients (Owira & Ojewole, 2009). Using the corms raw could be toxic and any extract must be used with caution (SANBI, 2009). While earlier taxonomic studies have been conducted (Singh, 2007), morphological, chemical and genetic diversity studies in *Hypoxis sp.* needs attention (Van Wyk, 2008) as the focus has been mainly on the quantitative and qualitative analysis of sterols found in *Hypoxis* sp. (Boukes *et al*., 2008). *H. hemerocallidea* has other versatile uses and can be made into rope, while the leaves and corms are used as a dye to give a black colour that is used to stain floors (SANBI, 2009).

The market and trade usage by traditional healers of *Hypoxis sp*. that has been investigated, found that it is one of the most frequently traded plant species in KwaZulu-Natal (Dold & Cocks, 2002). *H. hemerocallidea* corms continue to decline in South Africa, mainly due to overharvesting of this miracle muthi and wonder potato for its prized medicinal value (Cunningham, 1993; Williams *et al*., 2008). As found in studies of *Gethyllis* sp., urban development also contributes to its decline (Daniels *et al.,* 2013). *H. hemerocallidea* usually occurs in open grasslands and woodland areas, and was widespread in the eastern summer rainfall provinces such as the Eastern Cape, Free State, KwaZulu-Natal, Mpumalanga, Limpopo and Gauteng. There are also plant populations recorded in Botswana, Swaziland

and Lesotho. Reluctance among South African traditional healers to utilise medicinal plants from cultivated stocks (Dold & Cocks, 2002), is a further cause of plundering of naturally occurring populations. The species is easy to grow, drought tolerant, frost resistant, hardy, and grows well in full sun, provided the soil is well drained. Corms are dormant in winter, and should be kept as dry as possible. Leaves die down after summer and re-appear in late winter. *H. hemerocallidea*, which is fire tolerant, as it is dormant during the fire season and resprouts after fire. Fire stimulates the growth of new leaves, while the fibres protect the corm against fire damage. Fire also stimulates the seed to germinate. The star flower is not easily propagated from seed, with seed remaining dormant for up to one year and longer. Germination studies found that the complete mechanical removal of the hard seed coat is necessary, but only lead to partial germination. This seed dormancy can be the main reason why few seedlings are found naturally, while corm division is a more rapid method of propagation and more successful (Figure 2.4). Cross pollination is encouraged when 1-3 flowers are open at the same time. The Agricultural Research Council (ARC) has focused on gene bank maintenance and propagation methods of *Hypoxis sp.* (Coetzee *et al*., 1999) while several tissue culture and seed germination studies have been conducted (Page & Van Staden, 1984: 1986: 1987; Hammerton & van Staden, 1988: Hammerton *et al*., 1989. The use of compost tea extracts could possibly improve the medicinal properties of *H. hemerocallidea* as well as encourages the future commercial cultivation to supply the growing demand for the species.

Figure 2.4 The flowers, corm and new plant growth of *Hypoxis hemerocallidea* (Pictures: Jasson).

2.3.3 Importance of growing *Siphonochilus aethiopicus* organically

S. aethiopicus (Schweinf.) B.L. Burtt, (Zingerberaceae) is a rhizome with a restricted distribution to Mpumalanga and Northern Province while becoming extinct in Kwa-Zulu Natal province of South Africa (Van Wyk *et al*., 2009). Siphonochilus is derived from the Greek ‘siphono’ meaning tube and ‘chilus’ meaning lip, referring to the shape of the flower and ‘aethiopicus’ meaning from southern Africa (Gordon-Gray *et al*., 1989). Known as wild ginger,

the deciduous, small, aromatic cone-shaped rhizomes and leaves (Figure 2.5) when crushed smell similar to that of real ginger, *Zingiber officinale* (Van Wyk *et al*., 2009). The species was originally found on forest floors where leaves sprout annually from the deciduous underground stems in spring. Pink and white short-lived flowers, delicately scented, with a small yellow blotch in the centre are borne annually (Gordon-Gray *et al*., 1989). Flowers often appear before the leaves, thus perhaps allowing them to be more visible to pollinators (Nicols, 1989). After flowering, small berry-like fruits are produced at or near ground level. Most rhizomes are bisexual and have larger flowers than female plants (Gordon-Gray *et al*., 1989).

Used traditionally by the Zulu people for a variety of medicinal ailments such as asthma, hysteria, colds, coughs and flu and the protection against lightning and snakes. The Swati people use wild ginger to treat malaria and for menstruation when the fresh rhizomes and roots are chewed for medicinal use. It is also known in treatment to prevent horse sickness. Wild ginger has similar culinary uses as true ginger and the plant family has a number of valuable spice plants such as cardamom and turmeric. In an anti- inflammatory and antibacterial study, it was suggested that fresh rhizomes of wild ginger exhibited better efficacy than leaves, for medicinal use, despite the fact that aqueous rhizome extracts displayed moderately high levels of cytotoxicity (Light *et al*., 2002). Anti-inflammatory research of wild ginger for the treatment of pain and inflammation has been confirmed (Fennell *et al*., 2004). Research at the ARC has focused on gene bank maintenance and propagation methods of wild ginger (Coetzee *et al*., 1999). The market and trade usage by traditional healers of wild ginger found that it was one of the most frequently traded plant species in KwaZulu- Natal (Dold & Cocks, 2002; Institute of Natural Resources, 2003). During earlier studies wild ginger exhibited antiplasmodial compounds present in dried rhizomes that may play a role in the traditional use of wild ginger to treat malaria (Lategan *et al*., 2008). With these advances new medicinal products have been developed, where wild ginger is commercially grown, harvested using freeze dried roots and rhizomes, then marketed and sold in the form of tablets to treat asthma, candida, coughs, colds, headaches and malaria (Van Wyk, 2011a). Both antibacterial and antifungal activities were exhibited in the rhizomes and leaves of the species (Coopoosamy *et al*., 2010). Further studies of wild ginger as a potential source of new flavours and spice have also renewed its future value to the food industry (Van Wyk, 2011b) while no comprehensive taxonomic studies have been documented (Van Wyk, 2008).

Overharvesting of this sought-after medicinal plant, has led to the plant almost being lost to total extinction as the cone shaped rhizomes and fleshy roots are dug up and sold at muthi markets throughout South Africa (Raimondo *et al.*, 2009). Provided with a well-drained,

compost enriched soil and planted in a shady position, wild ginger is easy to cultivate as the rhizomes are dormant through winter and resume growing in spring. Plants respond well to high levels of organic matter especially during the growing season (Nichols, 1989). The germination of seed can take up to one year; however, an easier way to propagate the plant is by division, while the rhizomes are dormant in winter. Root damage must be minimised when handling during the division process (Nichols, 1989). While micro propagation has saved the species from extinction where wild populations were thought to be almost totally depleted the species remain on the listed data for Red Listed species of South African plants (SANBI, 2002). For these and several other reasons the cultivation of *S. aethiopicus* using compost tea extracts would have potential in improving its medicinal properties, ensuring sustainable production and relieve the harvesting threat on the wild populations.

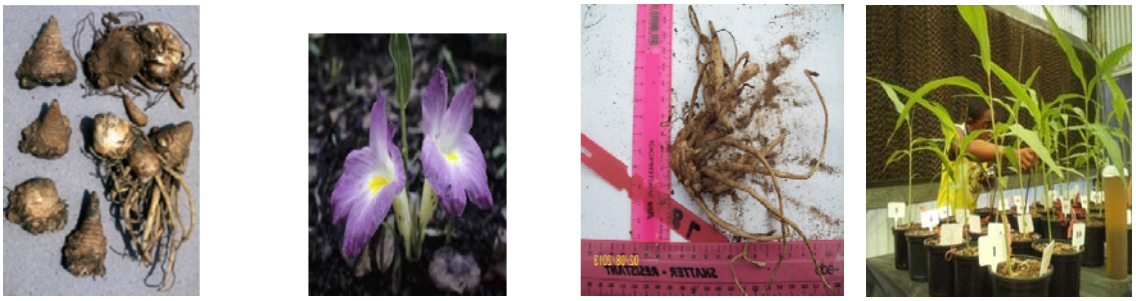

Figure 2.5 The rhizomes, flowers and plants of *Siphonochilus aethiopicus* (Pictures: Jasson).

2.4 CONCLUSION

It is clear that both *H. hemerocallidea* and *S. aethiopicus* continue to decline due to overharvesting from their natural habitat and that the demand for their medicinal value exceeds the supply especially amongst African people. While both species are slow growing it becomes important to increase production with improved cultivation techniques. Similarly, growing both medicinal species organically could enhance their product quality for medicinal commercialisation and reduce the amount of chemical fertilizers being used.

Organic cultivation of these medicinal species could possibly lead to increase their yield, nutritional content and antioxidant values as these responses are controlled by essential growth promoting substances in the growth media. With the current trends toward growing plants organically, many studies have focussed on the cultivation of a variety of ornamental and edible crops however, documentation on the application of compost tea for medicinal species remain limited. It is therefore worthwhile to investigate the effect and rate of application of mushroom compost tea extracts for future cultivation of important medicinal species such as *H. hemerocallidea* and *S. aethiopicus.* This research will not only gain a

better understanding on the effect of compost teas to enhance their future cultivation potential as commercial products, but also greatly contribute to the survival of these species in their natural habitat.

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Chapter Three

EFFECTS OF MUSHROOM COMPOST TEA EXTRACTS ON GROWTH OF *HYPOXIS HEMEROCALLIDEA* AND *SIPHONOCHILUS AETHIOPICUS* UNDER DIFFERENT SOIL DRENCH APPLICATIONS

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3.1 ABSTRACT

This study aimed to investigate the effects of commercial compost tea catalyst added to mushroom compost tea extracts, on growth, during the growing of *Hypoxis hemerocallidea* and *Siphonochilus aethiopicus*. As both species are slow growing, the research conducted, aimed to determine growth responses of the species under controlled growing conditions in response to mushroom compost tea extracts (CTE). Average height, leaf length, leaf colour, leaf width, and leaf number, varied in each parameter, across all treatments. The plants of both species, from the start of the experimental period, received the same CTE dosages, as required per treatment. The Hypoxis corms responded from the start of the experiment, as the CTE was applied weekly, and began growing first, resulting in treatment 500:1 with the best height, leaf length and leaf width. Treatment 750:1 had the highest number of leaves. The Siphonochilus rhizomes responded to the compost tea treatments', only seven weeks later, and resulted in the control 1 (CEO) with the best height, the control with the longest and widest leaves, while treatment 1000:1L had the highest leaf number. There was a significant interaction between weeks vs. treatment over the twenty weeks and period of growth; the different dosages of CTE did influence the growth significantly over time, irrespective of the strength of the compost tea extract. Mushroom compost tea is recommended as an organic alternative to chemically based fertilizers'. For Hypoxis and Siphonochilus, mushroom compost tea can be applied to these species during the growing period, although further investigation is required to quantify responses.

Keywords: compost tea extract, *Hypoxis hemerocallidea, Siphonochilus aethiopicus*

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3.2 INTRODUCTION

Compost tea is widely used as an alternative to improve quality plant production of field grown and nursery crops in substituting chemical fertilizer programmes (Ingham, 2005;

Khalid *et al*., 2006; Naidu *et al*., 2010). Various studies have proved that beneficial organisms assist in the prevention of diseases when applying compost tea to plant leaves (Scheuerell, 2004; Ingham, 2005; Souleymane *et al*., 2009; Marin *et al*., 2013). Internationally compost teas have gained extreme popularity for turf applications due to low cost and environmental restrictions in the use of traditional chemicals (Ingham, 2005; van Zwieten *et al*., 2007; Anonymous, 2011; Kelloway, 2012; Mullaivannan, 2013). Several studies have highlighted the beneficial organisms and micronutrients potential of compost tea to support the reduction in the use of fungicides, to assist in weed control and the improvement of root penetration of sport turf grasses (Scheuerell *et al.*, 2002; Bellows, 2003; Sachs, 2004 Ingham, 2005; Arumugam, 2012; Islam *et al.*, 2013; Garrett, 2014). Compost tea has also gained popularity in conventional agriculture with current trends of organic agriculture production (Theunissen *et al*., 2010). According to Ingham (2005), compost tea research has been conducted on a variety of plants and crops, such as asparagus, potato, corn, wheat, tomato, lettuce and radish, in orchards and vineyards, landscape trees, in greenhouse crop production, and agricultural crops. The source and type of compost materials used; the brewing method and duration; the additives added and plant species selected, will determine the effect of the compost tea on the plant, and how the plant growth improves. To date, no compost tea research has been conducted considering the above, using *Hypoxis hemerocallidea* and *Siphonochilus aethiopicus*.

3.2.1 *Hypoxis hemerocallidea*

H. hemerocallidea (Hypoxidaceae) is a cormous perennial with long, hairy, strap-like leaves and yellow star-shaped flowers borne on 5 to 6 long inflorescences during spring (SANBI, 2009). The name hypoxis is derived from the Greek words, hypo (below) and oxy (sharp), with reference to the ovary which is pointed at the base. The specific epithet is derived from the Greek hemera (a day) and kallos (beauty) presumably referring to the flowers that are short lived and bears resemblance to the day lily, *Hemerocallis* sp. Formerly known as *H. rooperii*, the broad leaves are arranged one above the other, resulting in three distinct sections spreading outwards from the centre of the plant (Van Wyk *et al*., 2009). The species are valued by traditional healers for treating tuberculosis (TB), cancer, urinary tract infections, anxiety, palpitations, and rheumatoid arthritis, amongst others. Some studies reported that the aqueous extract has anticonvulsant activity, thereby lending credence to traditional herbal use to treat childhood convulsions and epilepsy (Ojewole, 2008). It is also used to restore immune systems of patients recovering from HIV and cancer. Hypoxis phytosterols, used to treat benign prostrate hypertrophy was successfully marketed, and later phytosterols (based on industrial sources) claiming to have immune- stimulating effects, became and still is a huge marketing success (Drewes *et al*., 2004).

3.2.2 *Siphonochilus aethiopicus*

S. aethiopicus (Schweinf.) B.L. Burtt, (Zingerberaceae) is a rhizome with a restricted distribution to Mpumalanga and Northern Province while becoming extinct in Kwa-Zulu Natal province of South Africa (Van Wyk, *et al*., 2009). The name Siphonochilus is derived from the Greek 'siphono' meaning tube and 'chilus' meaning lip, referring to the shape of the flower and 'aethiopicus' meaning from southern Africa (Gordon-Gray *et al.,* 1989). Known as wild ginger, the deciduous, small, aromatic cone-shaped rhizomes and leaves when crushed smell similar to that of real ginger, *Zingiber officinale* (Van Wyk *et al*., 2009). Used traditionally by the Zulu people for a variety of medicinal ailments such as asthma, hysteria, colds, coughs and flu and the protection against lightning and snakes. The Swati people use wild ginger to treat malaria and for menstruation when the fresh rhizomes and roots are chewed for medicinal use. It is also known in treatment to prevent horse sickness. Wild ginger has similar culinary uses as true ginger and the plant family has a number of valuable spice plants such as cardamom and turmeric. In an anti- inflammatory and antibacterial study, it was suggested that fresh rhizomes of wild ginger exhibited better efficacy than leaves, for medicinal use, despite the fact that aqueous rhizome extracts displayed moderately high levels of cytotoxicity (Light *et al*., 2002).

This study aimed to investigate the effects of commercial compost tea catalyst added to mushroom compost tea extracts, on growth of *H. hemerocallidea* and *S. aethiopicus*. As both species being are slow growing, the research conducted, aimed to determine growth responses under controlled growing conditions in response to mushroom compost tea extracts (CTE).

3.3 MATERIALS AND METHODS

3.3.1 Plant material

The plant sample consisted of 60 *S. aethiopicus* and 60 *H. hemerocallidea* specimens. The plants were purchased from Afro Indigenous Nursery, KwaZulu-Natal, and couriered down to the Bellville campus, CPUT. The trial was conducted in the greenhouse at the nursery complex, Department of Horticultural Sciences, Cape Peninsula University of Technology (CPUT), Bellville - 33°55'56" S, 18°38'25" E, under controlled environmental conditions. The tropical greenhouse had humidity and temperature controls, with an average of 70% RH and 26° C. All the plant stock, once received was weighed, for initial record keeping and plant identification. The plants were planted in a mixture of 1 part of pine bark chips, 1 part of vermiculite and 1 part of perlite in 15 cm plastic pots, and watered three times a week to allow them to become established. The trial plants received no feeding or any other plant

nutrition for two months, after which they were moved into the tropical greenhouse where the experiment commenced. The experiment was laid out in a randomized complete block design in a factorial arrangement. Each experiment comprised 10 (n=10) pots per plant type per treatment. Treatments comprised of ten, weekly treatments, consisting of 100 ml per treatment per pot. The pots were soil drenched, with the required amount of compost tea extract. The treatments included the following application rates of the compost tea extracts:

Control = no added compost tea catalyst, no added compost extract, only water.
Control 1 = no added compost tea catalyst with compost extract only.
T250:1 = 250 ml compost tea extract with compost tea catalyst to 1L water.
T500:1 = 500 ml compost tea extract with compost tea catalyst to 1L water.
T750:1 = 750 ml compost tea extract with compost tea catalyst to 1L water.
T1000:1 = 1000 ml compost tea extract with compost tea catalyst to 1L water.

3.3.2 Preparation of the compost tea extract

The compost tea catalyst and compost tea brewing was prepared according to manufacturer's instructions in using the Growing Solutions® system 10 recommendations with 1:8 ratios, which was converted to 500 g of compost: 40 litres of water (Anonymous, 2011). Municipal water was used, and rested to allow all chemical additions to evaporate over 24 hours. 500g mushroom compost was added to the filter and brewed aerobically for 24 hours for the first brew. After each brew the brewer was cleaned and rinsed. The second aerobic brew was with 100 ml of compost tea catalyst added to the 500 g of mushroom compost: 40 litres of water. After brewing the above mentioned formulations were mixed separately using measuring cylinders and stored in lidded flasks until the specific formulation was ready to be applied to the plant samples (Figure 3.1). The plants were assessed weekly over 20 weeks (September 2013 to mid-January 2014), for various growth responses.

Figure 3.1 Compost tea brewing and extracts during the experiment. From the left the compost tea brewer, concentrations of 25%; 50%; 75%, 100% and compost tea extract no catalyst. (Photos by the author: Jasson)

3.3.3 Data collection and statistical analysis.

Data on the growth rates, included plant height (leaf length in the case of Hypoxis), leaf width were collected and recorded at weekly intervals for both plant species. The length of leaf samples measured (mm) from the base to the tip of the leave with all values recorded to the nearest 1 mm (Uzun & Celik, 1999). Data collected of all leaf parameters were analysed statistically using the repeated analysis of variance, general linear model, across all treatments. The interaction between the effect of growth time and the effect of the compost tea extract on both species were also analysed. The post hoc multiple comparisons using the Bonferroni technique were used to compare treatment means at $P \leq 0.05$ level of significance. These computations were done using the software of the SPSS version 23 programme (Urdan, 2005).

3.4 RESULTS

3.4.1 The growth results of *Hypoxis hemerocallidea*

The growing of *H. hemerocallidea* using various applications of compost tea extracts showed interesting results throughout the experiment. The species was found to be slow growing throughout the experimental period.

3.4.1.1 The effects of compost tea extract on plant height (mm) of *Hypoxis hemerocallidea*

Plant heights were recorded on a weekly basis over the twenty week growing period. At the start of the experiment plant heights were low as the plants started to initiate new growth from the corms. The average plant height (mm) which were recorded per treatment ranged as follows: Control = 204 mm; Control 1 = 200 mm, T1000:1 = 165 mm, T750:1 = 104 mm, T500:1 = 108 mm, and T250:1 = 201 mm. After ten weeks of CTE applications, the plants were measured and the following readings were recorded: Control = 304 mm, Control 1 = 349 mm, T1000:1 = 305 mm, T750:1 = 258 mm, T500:1 = 305 mm, and T250:1 = 421 mm. At the end of the experiment, at twenty weeks the average heights ranged from highest to lowest as follows; treatment T500:1 = 491 mm followed by T250:1 = 454 mm, Control 1 = 433 mm, T1000:1= 418 mm, T750:1 = 357 mm and the lowest was Control = 328 mm respectively (Table 3.1). All treatments were found to be not significant across all weeks. All measurements were compared to the Control to see the effect of CTE treatments. The T250:1 treatment

showed the highest plant height average (359 mm) across the 20-week growth period. This was followed by the T500:1 (301 mm) and the Control 1 (327 mm) (Table 3.1).

Table 3.1 The effect of compost tea extracts (CTE) on average plant height (mm) of *Hypoxis hemerocallidea* during the twenty week growing period.

Treatments	Week 1	Week 10	Week 20	Height increase	Total average height
Control	204 ± 3.29a	304 ± 5.02a	328 ± 5.20a	124	279
Control 1	200 ± 3.79a	349 ± 5.32a	433 ± 5.52a	233	327
T250:1	201 ± 3.73a	421 ± 5.7a	454 ± 5.90a	253	359
T500:1	108 ± 3.29a	305 ± 5.02a	491 ± 5.20a	383	301
T750:1	104 ± 3.73a	258 ± 5.7a	357 ± 5.90a	253	239
T1000:1	165 ± 3.18a	305 ± 4.77a	418 ± 4.94a	253	296
F-Statistic	1.8 ns	0.966ns	1.298ns		

Values presented are means ± SE. ns = not significant. Means with the same letter(s) are not significantly different at P ≤ 0.05.

Plant heights and leaf lengths were similar as the leaves develop from the base of the central growth point. Plant height for all treatments increased over time (weeks) as the leaf length increased. There was however a significant interaction between weeks vs. treatment over the twenty weeks. The T500: 1 had a better increase when compared to the other treatments at twenty weeks and had the highest average total although not significantly, when compared to the other treatments (control; control 1; T250: 1; T750: 1 and T1000: 1). These treatments began to slowdown in growth from weeks 10 to 20. The increase over the growth period did show a plant height increase over time while the different dosages of CTE did influence the growth over the twenty-week growth period (Figure 3.2).

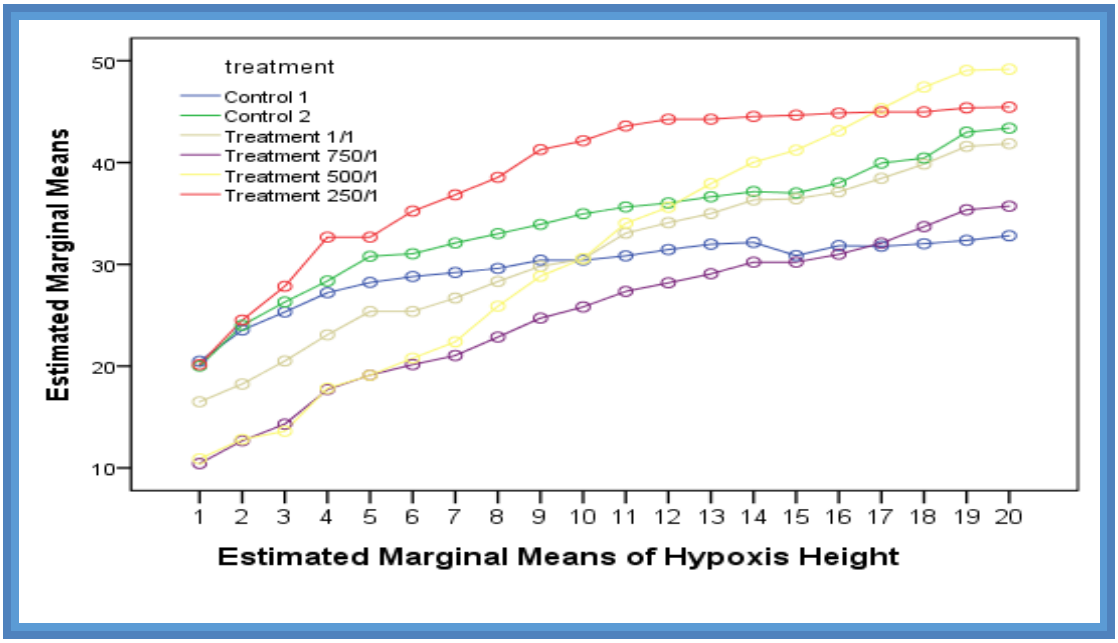

Figure 3.2 Plant height (cm) of *Hypoxis hemerocallidea* as affected by compost tea extracts (CTE) treatments. Values represent the means ± SD for the different treatments (n=10) per week. Different treatments are compared to each other per plant species. Estimated marginal means = height. Estimated marginal means of Hypoxis height = weeks.

3.4.1.2 The effects of compost tea extract on leaf count (n) of *Hypoxis hemerocallidea*.

The leaves were counted and recorded individually throughout the experiment. At the start of the experiment the average leaf count per treatment ranged as follows: Control = 10.11, Control 1 = 9.14, T1000:1 = 7.9, T750:1 = 8.57, T500:1 = 8.57, and T250:1 = 11. After ten weeks of CTE applications, the average leaf count increased per treatment to the following: Control = 13.22, Control 1 = 12.29, T1000:1 = 12.5, T750:1 = 16, T500:1 = 15.14, and T250:1 = 14.57. At the end of the experiment, at twenty weeks the average leaf count increased and ranged from highest to lowest as follows: T750:1 = 15.14 followed by T500:1 = 14.71, T250:1 = 14.29, Control 1 = 12.71, T1000:1 = 12.3, and the lowest was the Control at 11.89 (Table 3.2). All treatments showed slight increases but were not significant against the control across all weeks. Treatments T750:1 and T500:1 had the highest leaf number overall (Figure 3.2).

Table 3.2 The effects of compost tea extract (CTE) on average leaf count (n) of *Hypoxis hemerocallidea*.

Treatments	Week 1	Week 10	Week 20	Week 20-week 1 difference	Total average leaf number
Control	10.11 ± 1.1a	13.22 ± 1.06a	11.89 ± 0.84a	1.78	11.74
Control 1	9.14 ± 1.24a	12.29 ± 1.2a	12.71 ± 0.95a	3.57	11.38
T250:1	11.0 ± 1.24a	14.57 ± 1.2a	14.29 ± 0.95a	3.29	13.25
T500:1	8.57 ± 1.24a	15.14 ± 1.2a	14.71 ± 0.95a	6.14	12.80
T750:1	8.57 ± 1.24a	16.1 ± 1.2a	15.14 ±0.95a	6.57	13.27
T1000:1	7.9 ± 1.04a	12.5 ± 1.0a	12.3 ± 0.79a	4.4	10.9
F-Statistic	0.98ns	1.71ns	2.37ns		

Values presented are means ± SE. ns = not significant. Means with the same letter(s) are not significantly different at P ≤ 0.05.

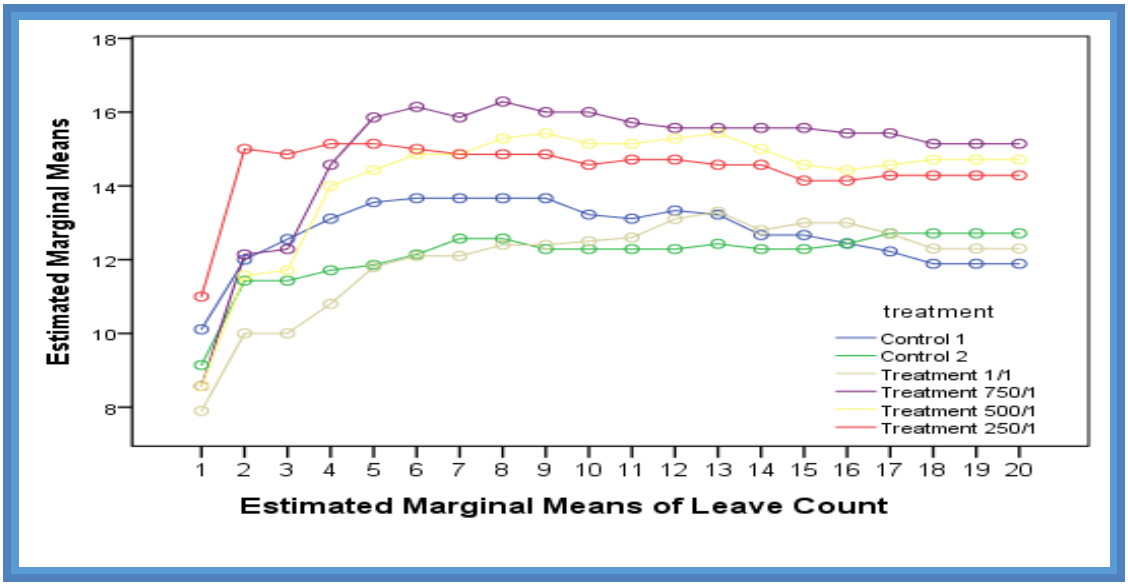

Figure 3.3 Leaf count (n) of *Hypoxis hemerocallidea* as affected by compost tea extract (CTE) treatments. Values represent the means ± SD for the different treatments (n=10). Different treatments are compared to each other per plant species. Estimated marginal means = leaf count. Estimated marginal means of leaf count = weeks.

3.4.1.3 The effects of compost tea extract on leaf length (mm) of *Hypoxis hemerocallidea*

The length of leaf samples were measured (mm) from the base to the tip of the leaves. In the Hypoxis example, plant height and leaf length were the same parameters as the leaves emerge from the centre of the corm annually. The measurements are recorded above under paragraph 3.4.1.1 and Table 3.1 and Figure 3.2.

3.4.1.4 The effects of compost tea extract on leaf width (mm) of *Hypoxis hemerocallidea*

The leaf width was measured and recorded individually per plant throughout the experiment. At the start of the experiment the average leaf width per treatment ranged, as follows: Control = 14 mm, Control 1 = 15.4 mm, T1000:1 = 16.2 mm, T750: = 13 mm, T500:1 = 15.4 mm and T250:1 = 18.1 mm. After ten weeks of CTE applications, the average leaf width per treatment was as follows: Control = 21.7 mm, Control 1 = 20.9 mm, T1000:1 = 22.3 mm, T750:1 = 21mm, T500:1 = 27mm and T250:1 = 26.4 mm. At the end of the experiment, at twenty weeks the average leaf width increased, ranging from highest to lowest treatments as follows; T500:1 = 32.9 mm, followed by T250:1 = 31.4 mm, T750:1 = 26.1 mm, Control 1 = 26 mm, Control at 24.9 mm and lastly T1000:1 = 24 mm. The statistics showed no significant differences of the leaf width over the twenty weeks and between the treatments despite treatment T500:1 having the widest leaves on average (Figure 3.3).

Table 3.3 The effects of compost tea extract (CTE) on average leaf width (mm) of *Hypoxis hemerocallidea*.

Treatments	Week 1	Week 10	Week 20	increase	Total average leaf width
Control	14 ± 0.15a	21.7 ± 0.19a	24.9 ± 0.22a	10.9	20.2
Control 1	15.4 ± 0.16a	20.9 ± 0.2a	26 ± 0.23a	10.6	20.7
T250:1	18.1 ± 0.17a	26.4 ± 0.21a	31.4 ± 0.25a	13.3	25.3
T500:1	15.4 ± 0.17a	27 ± 0.21a	32.9 ± 0.25a	17.5	25.1
T750:1	13 ± 0.17a	21 ± 0.21a	26.1 ±0.25a	13.1	20
T1000:1	16.2 ± 0.14a	22.3 ± 0.18a	24 ± 0.21a	7.8	20.8
F-Statistic	1.1ns	1.81ns	2.45ns		

Values presented are means ± SE. ns = not significant. Means with the same letter(s) are not significantly different at P ≤ 0.05.

From Table 3.3 it is clear that the best total average leaf width was measure in the T250:1 and T500:1 treatment. These measurements are above all other treatments including the Control (Table 3.3).

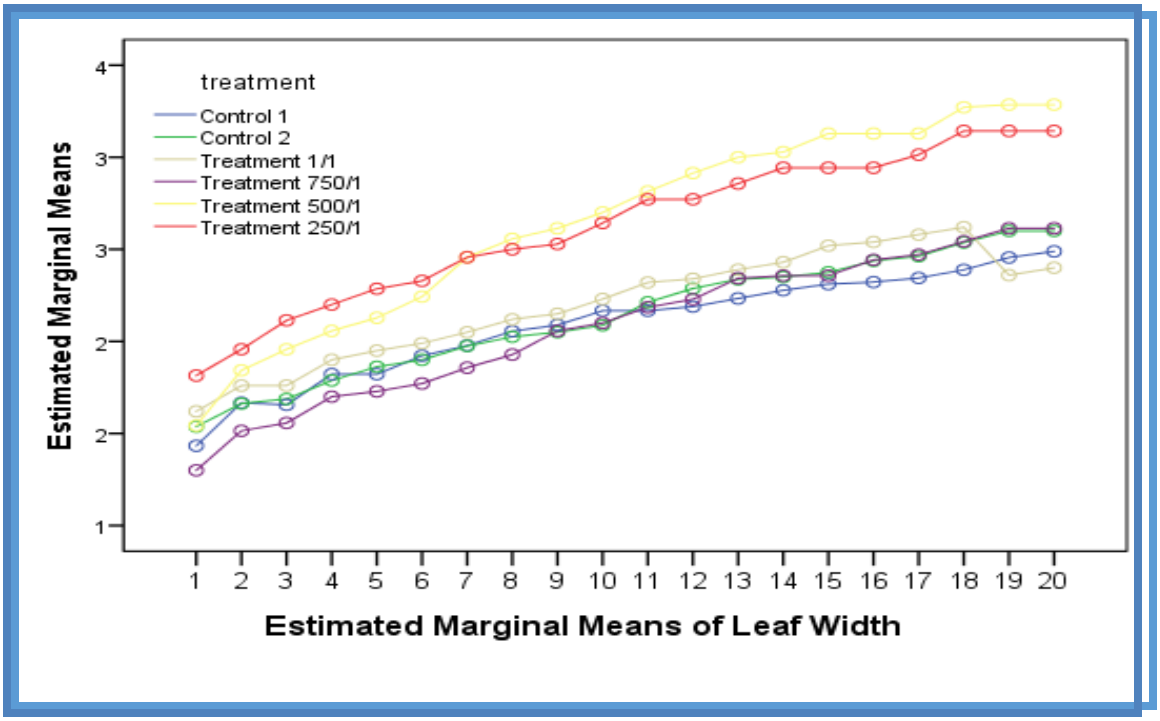

Figure 3.4 Effects of compost tea extract on leaf width (1cm = 10 mm) of *Hypoxis hemerocallidea*. Values represent the means ± SD for the different treatments (n=10). Different treatments are compared to each other per plant species. Estimated marginal means = leaf width. Estimated marginal means of leaf width = weeks.

3.4.2 Growth results of *Siphonochilus aethiopicus*

3.4.2.1 The effects of compost tea extract (CTE) on plant height (mm) of *Siphonochilus aethiopicus*.

During the experimental stage, the first seven weeks, the rhizomes of *S. aethiopicus* showed no growth response above ground. Compost tea extracts in their various dosages were applied weekly as per with the Hypoxis experiment. Plant heights were recorded individually throughout the experiment. In week one, the average plant height response ranged between treatments as follows: Control = 13.6 cm; Control 1 = 12.1 mm, T1000:1 = 2.3 mm, T750:1 = 7.1 mm, T500:1 = 2.7 mm, and T250:1 = 2.9 mm. After ten weeks of CTE applications, the average plant height per treatment was as follows: Control = 230.8 mm; Control 1 = 247.1 mm; T1000:1 = 133.6 mm, T750:1 = 151.8 mm, T500:1 = 145.4 mm and T250:1 = 146.7 mm.

At the end of the experiment at week 13, the average heights ranging from highest to lowest as follows; Control 1 = 351.7 cm; Control = 318.5 mm; T1000:1 = 231.8 mm, T750:1 = 229.2 mm, T500:1 = 228 mm, and the lowest was T250:1 at 226 mm (Figure 3.4). The *S. aethiopicus* rhizomes responded, after 7 weeks of compost tea applications and then observations were made in week 8, of above ground emergence of new shoot tips emerging slowly from the rhizomes. There were no significant differences between the treatment groups over the weeks compared to the Control. Table 3.4 shows that Control was far more successful in plant height. This average was followed by Control 1 and then the CTE treatments.

Table 3.4 The effects of compost tea extract (CTE) on average plant height (mm) of *Siphonochilus aethiopicus*.

Treatments	Week 1 (wk 11)	Week 5 (wk15)	Week 9 (wk17)	Week 13 (wk20)	Increase	Total average plant height
Control	13.6 ± 0.29a	23.1 ± 1.82a	211.6 ± 2.31a	818.5 ± 2.64a	804.9	266.7
Control 1	12.1 ± 0.28a	139 ± 1.81a	215.3 ± 2.30a	351.7 ± 2.62a	339.6	179.5
T250:1	2.9 ± 0.30a	46.8 ± 1.92a	116.3 ± 2.44a	226.0 ± 2.78a	223.1	98.0
T500:1	2.7 ± 0.28a	43.4 ± 1.81a	111.3 ± 2.30a	228.0 ± 2.62a	225.3	96.35
T750:1	7.1 ± 0.28a	58.8 ± 1.80a	117.9 ± 2.30a	229.2 ± 2.62a	222.1	103.25
T1000:1	2.3 ± 0.28a	28.6 ± 1.80a	106.7 ± 2.30a	231.8 ± 2.62a	229.5	92.35
F-Statistic	3.03ns	6.33ns	4.97ns	4.42ns		

Values presented are means ± SE. ns = not significant. Means with the same letter(s) are not significantly different at P ≤ 0.05.

There was a significant interaction between weeks vs. treatment over the twenty weeks, and as the plant height increased throughout the twenty weeks, the different dosages of CTE did influence the growth significantly over time (Figure 3.5). The graph shows that there was a linear increase in the plant height but a clear separation between Control, Control 1 and the CTE treatments.

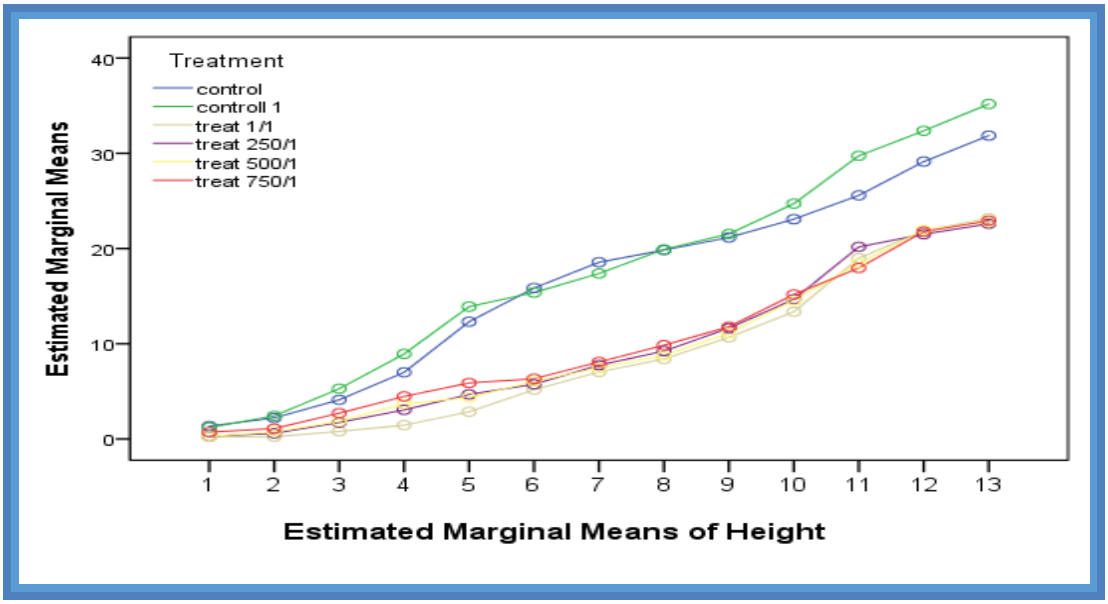

Figure 3.5 The effects of compost tea extract (CTE) on plant height (10mm = 1cm) of *Siphonochilus aethiopicus*. Values represent the means ± SD for the different treatments (n=10). Different treatments are compared to each other per plant species. Estimated marginal means = height. Estimated marginal means of Height = weeks.

3.4.2.2 The effects of compost tea extract (CTE) on leaf count (n) of *Siphonochilus aethiopicus*.

The leaf count was dependent on the new shoot emerging from the rhizomes and new leaves on the stems from the bottom up of the stem. At week 11 the average leaf count per treatment ranged between treatments as follows: Control = 0.3, Control 1 = 0.4, T1000:1 = 0, T750:1 = 0.2, T500:1 = 0.11 and T250:1 = 0. After 5 weeks of CTE applications, the average leaf numbers' increased, per treatment were as follows: Control = 2.6, Control 1 = 2.8, T1000:1 = 1.9, T750: = 1.8, T500:1 = 1.44, and T250:1 = 1.2. Towards the end of the experiment, at week 10, the average leaf count per treatment ranged from highest to lowest as follows; T1000:1 = 5.9, Control = 5.2, Control 1 = 4.9, T750:1 = 4.6, T500:1 = 4.67 and T250:1 = 4.28 (Table 3.5). There was a significant interaction between weeks vs. treatment over the weeks, and as the leaf count increased throughout the ten weeks, the different dosages of CTE did influence the growth significantly over time (Figure 3.6). There was no significant difference between treatments even though T1000:1 exhibited a different pattern of increase in the number of leaves.

Table 3.5 The effects of compost tea extract (CTE) treatments on leaf count (n) of *Siphonochilus aethiopicus*.

Treatments (Fig. 3.6)	Week 11 (wk1)	Week 15 (wk5)	Week 17 (wk7)	Week 20 (wk10)	Increase	Total average leaf number
Control	0.3 ± 0.12a	2.6 ± 0.48a	3.1 ± 0.54a	5.2 ± 0.58a	4.9	2.8
Control 1	0.4 ± 0.12a	2.8 ± 0.48a	3.3 ± 0.54a	4.9 ± 0.58a	4.5	2.8
T250:1	0 ± 0.12a	1.2 ± 0.48a	2 ± 0.54a	4.28 ± 0.58a	4.28	1.8
T500:1	0.11 ± 0.12a	1.44 ± 0.51a	2.56 ± 0.57a	4.67 ± 0.61a	4.56	2.1
T750:1	0.2 ± 0.12a	1.8 ± 0.48a	2.77 ± 0.54a	4.6 ± 0.58a	4.4	2.3
T1000:1	0 ± 0.12a	1.9 ± 0.48a	3.8 ± 0.54a	5.9 ± 0.58a	5.9	2.9
F-Statistic	2ns	1.7ns	1.33ns	1.02ns		

Values presented are means ± SE. ns = not significant. Means with the same letter(s) are not significantly different at P ≤ 0.05.

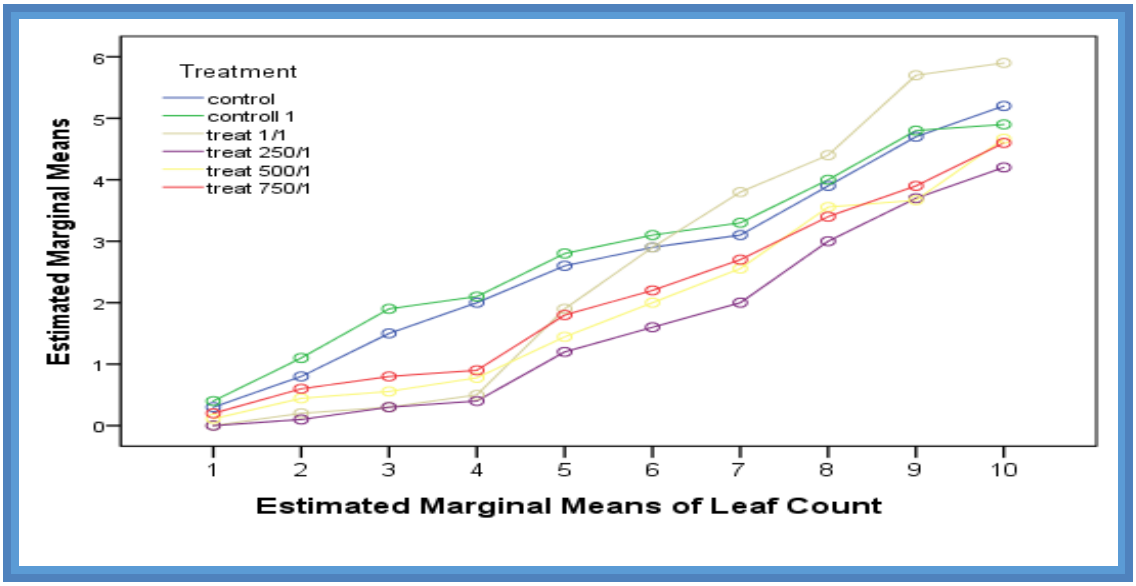

Figure 3.6 The effects of compost tea extract (CTE) on leaf count (n) of *Siphonochilus aethiopicus.* Values represent the means ± SD for the different treatments (n=10). Different treatments are compared to each other per plant species Estimated marginal means = leaf count. Estimated marginal means of leaf count = weeks. Week 1= week 11 on Table 3.5.

3.4.2.3 The effects of compost tea extract on leaf length (mm) of *Siphonochilus aethiopicus*.

Leaf length measurements were delayed during the experiment by 9 weeks because of slow new shoot emergence from underground rhizomes. Leaf lengths however, were recorded individually throughout the remainder of the experiment. At week 11 of the experiment the average leaf lengths per treatment were ranged as follows: Control = 6.2 mm, Control 1 = 17.9 mm, T1000:1 = 0 mm, T750:1 = 5.9 mm, T500: 1 = 2.9 mm and T250:1 = 0 mm. After a further 5 weeks of CTE applications, the average leaf lengths per treatment increased and were as follows: Control = 156.5 cm, Control 1 = 100 mm, T1000:1 = 41.5 mm, T750:1 = 71.5 mm, T500:1 = 50 mm and T250:1 = 51 mm. Towards the end of the experiment, at week 10, the average leaf lengths ranged from highest to lowest as follows; Control = 210.5 mm; T1000:1 = 143.5 mm followed by Control 1 = 130 mm, T750:1 = 122.1 mm, T250:1 = 114.3 mm and the lowest was Treatment 500:1 = 108.1 mm (Table 3.6). Leaf length changed between the groups with the exception of the control which remained the highest. There were no significant differences between the treatments. There was a significant interaction between weeks vs. treatment over the weeks, and as the leaf length increased throughout the thirteen weeks, the different dosages of CTE did influence the growth significantly over time (Figure 3.7).

Table 3.6 the effects of compost tea extract (CTE) treatments on leaf length (mm) of *Siphonochilus aethiopicus*.

Treatments (Fig 3.7)	Week 11 (wk1)	Week 14 (wk4)	Week 17 (wk7)	Week 20 (wk10)	Increase	Total average leaf length
Control	6.2 ± 0.59a	132.5 ± 1.92a	159.7 ± 1.74a	210.5 ± 1.15a	204.3	127.2
Control 1	17.9 ± 0.59a	99.8 ± 1.92a	102.4 ± 1.74a	130 ± 1.15a	112.1	87.5
T250:1	0 ± 0.59a	35.9 ± 1.92a	100.5 ± 1.74a	114.3 ± 1.15a	114.3	62.6
T500:1	2.9 ± 0.59a	31.8 ± 1.92a	86.5 ± 1.74a	108.1 ± 1.15a	105.2	57.3
T750:1	5.9 ± 0.59a	58.8 ± 1.92a	97.4 ± 1.74a	122.1 ± 1.15a	116.2	71.0
T1000:1	0 ± 0.59a	30.8 ± 1.92a	100.3 ± 1.74a	143.5 ± 1.15a	143.5	92.35
F-Statistic	1.29ns	5.09ns	2.25ns	10.62ns		

Values presented are means ± SE. ns = not significant. Means with the same letter(s) are not significantly different at P ≤ 0.05.

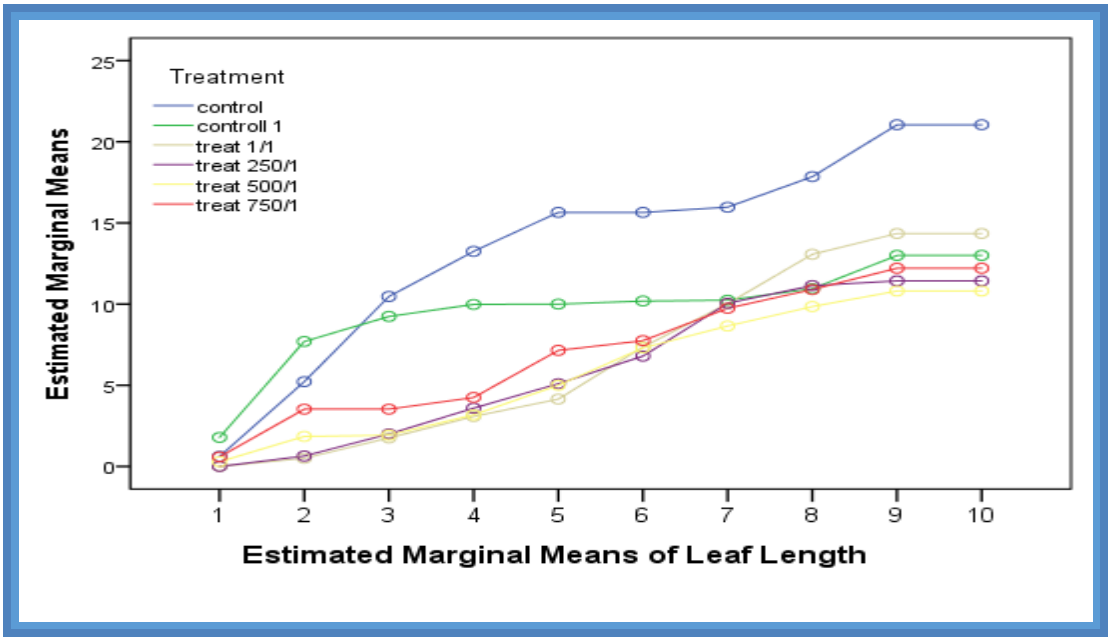

Figure 3.7 The effects of compost tea extract (CTE) on leaf length (cm) of *Siphonochilus aethiopicus*. Values represent the means ± SD for the different treatments (n=10). Different treatments are compared to the control. Estimated marginal means = leaf length. Estimated marginal means of Leaf Length = weeks. Week 1= week 11 in Table 3.6.

3.4.2.4 The effects of compost tea on leaf width (mm) of *Siphonochilus aethiopicus*.

Leaf width measurements were dependent on slow new shoot emergence from underground and leaf emergence on the shoot. At week 11, the average leaf widths per treatment ranged between treatments as follows: Control = 1.1 mm, Control 1 = 4 mm, T1000:1 = 1mm, T750:1 = 2.4 mm, T500:1 = 1.7 mm and T250:1 = 0.9 mm (Table 3.7). After a further 5 weeks at week 15 of CTE applications, the average leaf widths per treatment increased and were as follows: Control = 29 mm, Control 1 = 21 mm; T1000:1 = 13 mm, T750:1 = 16 mm, T500:1 = 15 mm and T250:1 = 19 mm. Towards the end of the experiment, at week 20 the average leaf widths ranged from highest to lowest as follows; Control = 36 mm, T1000:1 = 27 mm followed by Control 1 = 29 mm, T750:1 = 29 mm, T500:1 = 26 mm, and T250:1 = 28 mm. All the other growth measurements were dependant on new shoots emerging from the rhizomes which were delayed for eight weeks. Two weeks later new leaves on the stems began to emerge and unfurl slowly. This slow response affected the leaf width as can be seen below in Table 3.7. Total average leaf width peaked only towards the end of the experiment. There was a significant interaction between weeks vs. treatment over the weeks, and as the leaf length

increased throughout the ten weeks, the different dosages of CTE did influence the growth significantly over time (Figure 3.8).

Table 3.7 The effects of compost tea extract (CTE) treatments on leaf width (mm) of *Siphonochilus aethiopicus*.

Treatments	Week 11 (wk1)	Week 15 (wk 5)	Week 17 (wk 8)	Week 20 (wk 10)	Increase	Total average leaf width
Control	1.1 ± 0.14a	29 ± 0.33a	31 ± 0.33a	36 ± 0.17a	34.9	24.2
Control 1	4.0 ± 0.14a	21 ± 0.33a	22 ± 0.33a	29 ± 0.17a	25	19
T250:1	0.0 ± 0.15a	19 ± 0.34a	20 ± 0.33a	28 ± 0.17a	28	15.4
T500:1	1.7 ± 0.14a	15 ± 0.33a	19 ± 0.33a	26 ± 0.17a	24.3	15.4
T750:1	2.4 ± 0.14a	16 ± 0.33a	22 ± 0.33a	29 ± 0.17a	26.6	17.3
T1000:1	1.0 ± 0.14a	13 ± 0.33a	22 ± 0.33a	27 ± 0.17a	26	15.7
F-Statistic	107.489ns	107.489ns	107.489ns	107.489ns		

Values presented are means ± SE. ns = not significant. Means with the same letter(s) are not significantly different at P ≤ 0.05.

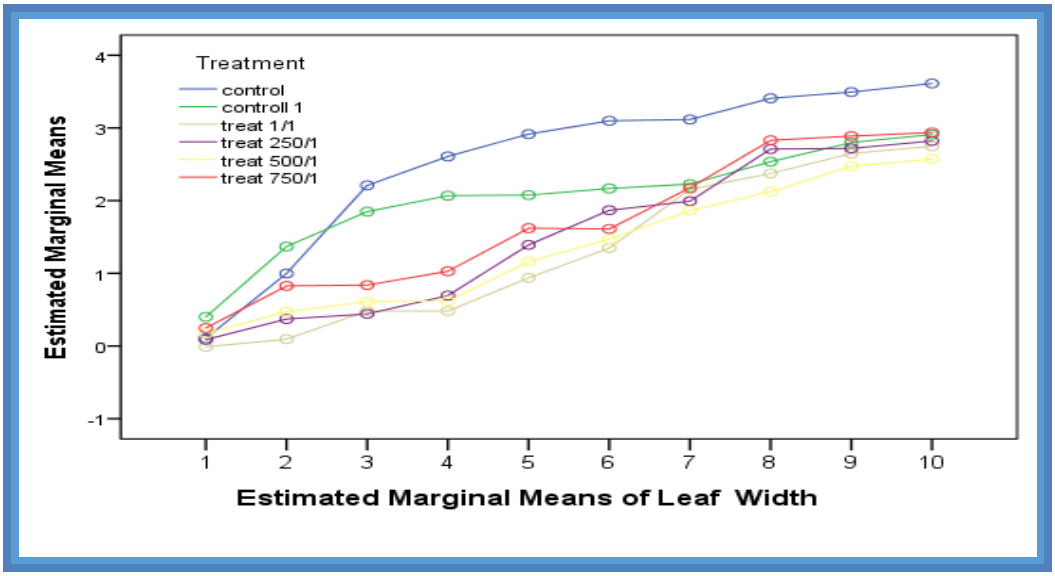

Figure 3.8 The effects of compost tea extract (CTE) on leaf width (10 mm = 1 cm) of *Siphonochilus aethiopicus.* Values represent the means ± SD for the different treatments (n=10). Different treatments are compared to each other per plant species. Estimated marginal means = leaf width. Estimated marginal means of leaf width = weeks. Week 1 = Week 11 in Table 3.7.

3.5 DISCUSSION

H. hemerocallidea grows in full sun in its natural environment while *S. aethiopicus* prefers more shaded areas of subtropical to tropical regions. The CTE in its different dosages contributed to increase the height, leaf number, leaf length and leaf width across all treatments and both species had to adapt to the greenhouse environment. Differences in the geographical environment from a Subtropical to a Mediterranean climate could contribute to different growth responses. The plants of both species, from the start of the experimental period in the greenhouse, received the same weekly CTE dosages which resulted in different growth responses between the two species. The Hypoxis corms showed an increase in growth responses from the start of the experiment, while the Siphonochilus rhizomes responded to the compost tea treatments only seven weeks later. The delayed response of Siphonochilus could be attributed to the size of the rhizomes, the time adjustment to the new climate in the green house and the fact that the wild gingers have a unique physiological underground rhizome structure. The initial ten-week experiment developed into a twenty-week experiment from September 2013 to January 2014 due to the slow response of the Siphonochilus rhizomes.

3.5.1 *Hypoxis hemerocallidea*

It is well known fact that compost tea extract improves the growth of many crops (Scheuerell & Mahaffee, 2002; Ingham 2005). The results of this study are in line with these earlier reports. Although the study showed no significance in the growth variables, the average plant height increase for *H. hemerocallidea* was found to be most successful in the T250:1 treatment followed by the Control 1 and T500:1 treatment over the week intervals. The same results were also reported in the leaf width in the same treatments. Similar recordings were reported in the leaf number, however a higher concentration (T750:1 treatment) was found to be more beneficial in this variable. From the results it is clear that the plants responded positively to the CTE applications (towards the end this response declined) and an overall increase in plant growth, over the 20-week period, advancing plant growth. These positive growth effects of compost tea are in agreement with reports in other studies by Gomez-Brandon *et al*. (2015). Hypoxis was found to be slow growing during the experimental weeks and even though the treatments were found not significant to the control there were clear differences in growth responses measured between treatments, over the weeks. The fact that growth may be inhibited by the corm physiological structure and only activated in the growing season, and factors such as environmental conditions (temperature, light, humidity) amongst others also may an impact. It is also important to note that the corms had different sizes and weights before the experiment started which could have also resulted in difference in growth responses measured.

3.5.2 *Siphonochilus aethiopicus*

S. aethiopicus, rhizomatous species were slow growing during the experimental period possibly due to the dormant rhizomes planted at the beginning of the experiment. It is also important to note that the rhizomes had different sizes and weights before the experiment started which could have resulted in difference in growth responses measured. The averages measured for plant heights, leaf lengths and leaf widths were higher in the Control but not significant, whereas leaf number averages were higher in the T1000:1 treatment. Overall these results show that CTE had little/negative effect, on average growth responses of the plants. These results are in contrast with reports that CTE yielded increased results of quality of ginger rhizomes (Sanwal *et al*., 2007). The positive manifold effects of compost tea were also reported by Gomez-Brandon *et al*. (2015) while an increase in strawberry yield was documented by Welke, (2005). Naidu *et al*. (2013) reported that a weekly foliar application of microbial-enriched CTE could be used successfully as a bio-fertiliser and bio-protectant on muskmelon crop without compromising fruit quality. It is speculated that due to the slow growth of the species, the fact that few leaves were recorded, that the CTE were possibly absorbed into the rhizomes of Siphonochilus. The plants could have shown better growth response averages over a longer trial period (at least another season) to assess and gain a better insight of the effect of the compost tea extract.

Compost tea has gained popularity in conventional agriculture with current trends of organic agriculture production (Theunissen *et al*., 2010). It is well reported that mushroom CTE can be recommended as an organic alternative to chemically based fertilizers (Ingham, 2005; Welke, 2005; Khalid *et al*., 2006; Gomez-Brandon *et al*., 2015; Naidu *et al*., 2013), and that specifically for *H. hemerocallidea* and *S. aethiopicus*, mushroom CTE can be applied to these species during the growing period. The compost tea extract will be dependant of the source of the compost, the brewing method, and additives added to enrich the compost tea (Ingham 2005).

3.6 CONCLUSION

The importance to commercialise southern African medicinal species should not be ignored. There is significant evidence that both *H. hemerocallidea* and *S. aethiopicus* have important value for traditional healers in the preparation of homeopathic remedies. However, both species remain threatened by overharvesting in the wild. This study shows a trend that there was a good interaction of plant growth between weeks' vs treatment over the twenty weeks due to the CTE applications. Both species have underground storage organs, with initial growth

dependent on the reserves in their underground storage organs. The growth delay of Siphonochilus affected the results even more. The use of CTE as an organic growth stimulate continues to gain popularity and can be beneficial for Hypoxis and Siphonochilus medicinal species. While this study aimed to investigate the effects of CTE, on the growth of *H. hemerocallidea* and *S. aethiopicus* to develop a protocol for commercial cultivation, there is a need to improve the cultivation of these and other medicinal species. It appears however, that because both species are slow growing, (with growth starting from the storage organs underground), Hypoxis being a corm and Siphonochilus a rhizome, further research is recommended, over two growth seasons, to evaluate the effect of CTE for these species over a longer period of time.

3.7 ACKNOWLEDGEMENTS

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Chapter Four

CHLOROPHYLL CONTENT AND LEAF COLOUR RESPONSES OF *HYPOXIS HEMEROCALLIDEA* AND *SIPHONOCHILUS AETHIOPICUS* TO DIFFERENT MUSHROOM COMPOST TEA EXTRACTS.

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4.1 ABSTRACT

Chlorophyll content and leaf colour was affected by compost tea extracts (CTE) on two medicinal species *Hypoxis hemerocallidea* and *Siphonochilus aethiopicus.* The species grow from corms and rhizomes respectively and remain endangered in South Africa. Chlorophyll readings were measured over a growing period of twenty weeks where both species received CT extracts in the following ratios (T1000:1, T750:1, T500:1, T250:1 = ml/L); and a compost tea extract only (Control 1) and the control (no extract). The CTE treatments did not have a significant effect on the Hypoxis leaves, possibly due to the bulk of the compost tea extract being absorbed into the Hypoxis corms. Chlorophyll levels were not constant over the twenty weeks as each of the treatments responded with different chlorophyll readings. In the Siphonochilus rhizomes only the T500:1 showed a response from week 8, while the rest showed a response later. All chlorophyll readings were found to be non-significant compared to the control. There was a significant interaction between weeks' vs treatment over the twenty weeks. Siphonochilus chlorophyll content was below 100 Chl/mg in the beginning. The different dosages of CTE did influence the growth in the rhizomes and new leaves over time, with treatments' averaging between 237-263 Chl/mg at week twenty. The leaf colour varied with increase in CTE concentrations, especially in Siphonochilus, but were not significant. The study served as a basis for future studies on these important medicinal species.

Keywords: Compost tea, chlorophyll fluorescence ratio (CFR),

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4.2 INTRODUCTION

4.2.1 Chlorophyll production in plants

The increase use of organic fertilizers, including compost tea has led to an interest in how plants receiving organic fertilizers are able to convert these in and during photosynthesis and chlorophyll production. Chlorophyll pigments are found in the chloroplast of plants as it absorbs light wavelengths required to convert water and carbon dioxide into chemical energy during photosynthesis. Chlorophyll molecules are arranged around various photosystems found in the chloroplasts' thylakoid membranes, of which there are several hundred molecules per photosystem. Chlorophyll is further divided into 4 different pigments as discovered in 1906 by Willstatter and Stoll (Dutta, 2005). In all living plants, chlorophyll production plays a vital role in and during the primary photosynthetic process, as light energy is captured and converted from the sun. The popularity of the use of non-destructive chlorophyll analysis by using fluorimeters' such as the CCM- 300 (OPTI-SCIENCES, Inc.) and the SPAD-502 (Konica Minolta, Illinois) has gained momentum over the past years. Numerous forms of chlorophyll studies have been under taken and found the following amongst others: The chlorophyll content of ten leafy vegetables was analysed via HPLC (High Performance Liquid Chromatography) measurement and SPAD (Soil Plant Analysis Development) values of 52.4 of Chinese pak-choi where the SPAD value was proportional to the relative chlorophyll amounts (Limantara *et al.,* 2015). Various readings taken via 3 portable non-destructive meters measuring chlorophyll and nitrogen estimates in *Citrus* sp. leaves were overestimated due to thicker leaves, and readings were influenced by various environmental factors such as temperature, light and humidity amongst others (Jifon *et al*., 2005). Leaf chlorophyll content in Gladiolus revealed levels ranging from 68.73 to 80.20, after applications of nutrients applied singly and in combination, resulting in an increase in chlorophyll content when all micronutrients were applied to the foliage (Fahad *et al*., 2014). The chlorophyll content was lower in *Populus balsamifera* (Castro & Sanchez-Azofeifa, 2008). Root vegetables showed more chlorophyll per leaf in the shade in comparison to the same crops that were exposed to sun (Johnston & Onwueme, 1998). The use of a leaf chlorophyll meter to predict nitrogen status and yield of grape fruit trees found better correlations in spring than summer (Li *et al*., 1996) and chlorophyll continent of muskmelon was lower at ten days but higher after seventeen days, using the SPAD-502 chlorophyll meter (Azia *et al*., 2001). Nxawe *et al.* (2011) study on measuring the effects of temperature regimes on *Ornithogalum longibracteatum* L. in a hydroponic solution showed improved chlorophyll formation and photosynthesis during winter months. Considering the value of these studies and the importance of the medicinal species such as Hypoxis and Siphonochilus, there is definitely a need to measure the chlorophyll content effect when CTE is applied on these species, as this has not been done before.

4.2.2 *Hypoxis hemerocallidea*

H. hemerocallidea (Hypoxidaceae) is a cormous perennial with long, hairy, strap-like leaves and yellow star-shaped flowers borne on 5 to 6 long inflorescences during spring (SANBI 2009). The name Hypoxis is derived from the Greek words, hypo (below) and oxy (sharp), with reference to the ovary which is pointed at the base. The specific epithet is derived from the Greek hemera (a day) and kallos (beauty) presumably referring to the flowers that are short lived and bears resemblance to the day lily, *Hemerocallis* sp. Formerly known as *H. rooperii*, the broad leaves are arranged one above the other, resulting in three distinct sections spreading outwards from the centre of the plant (Van Wyk *et al*., 2009). The genus Hypoxis consists of approximately 90 species, of which 30 species are found mostly in eastern southern Africa (Singh, 2007). The name 'African Potato' was given to the species after the Afrikaans, 'Afrika patat'. The plant is however a compressed underground corm which grows vertically and is commonly known as 'Inkomfe' among African Zulu speaking people (Van Wyk *et al*., 2009). Corms are dark brown in colour, large and covered with bristly hairs. When freshly cut, the corms are bright yellow in colour, with a bitter taste (SANBI, 2009).

4.2.3 *Siphonochilus aethiopicus*

S. aethiopicus (Schweinf.) B.L. Burtt, (Zingerberaceae) is a rhizome with a restricted distribution to Mpumalanga and Northern Province while becoming extinct in Kwa-Zulu Natal province of South Africa (Van Wyk, *et al*., 2009). Siphonochilus is derived from the Greek 'siphono' meaning tube and 'chilus' meaning lip, referring to the shape of the flower and 'aethiopicus' meaning from southern Africa (Gordon-Gray *et al.,* 1989). Known as wild ginger, the deciduous, small, aromatic cone-shaped rhizomes and leaves when crushed smell similar to that of real ginger, *Zingiber officinale* (Van Wyk *et al*., 2009). The species was originally found on forest floors where leaves sprout annually from the deciduous underground stems in spring. Pink and white short-lived flowers, delicately scented, with a small yellow blotch in the centre are borne annually (Gordon-Gray *et al*., 1989). Flowers often appear before the leaves, thus perhaps allowing them to be more visible to pollinators (Nicols, 1989). After flowering, small berry-like fruits are produced at or near ground level. Most rhizomes are bisexual and have larger flowers than female plants (Gordon-Gray *et al.*, 1989).

The objective of this study was to establish the chlorophyll content levels and determine the leaf colour in *H. hemerocallidea* and *S. aethiopicus*. Little or no information is available on chlorophyll levels, and ascertaining these levels may lead to an improvement in

determination and thereby assisting in the cultivation of these important species for the growing medicinal plant trade in South Africa.

4.3 Materials and methods

4.3.1 Experimental setup

S. aethiopicus and *H. hemerocallidea* plants were purchased from Afro Indigenous Nursery, KwaZulu-Natal, and couriered down to the Bellville campus, CPUT. The trial was conducted in the greenhouse at the nursery complex, Department of Horticultural Sciences, Cape Peninsula University of Technology (CPUT), Bellville - 33°55'56" S, 18°38'25" E, under controlled environmental conditions. The tropical greenhouse humidity and temperature controls averaged 70% RH and 26° C during the experimental phase. All the plants, once received were weighed, for initial record keeping and plant identification. The plants were then planted into a mixture of 1 part of pine bark chips, 1 part of vermiculite and 1 part of perlite in 15 cm plastic pots, and watered three times a week to allow them to become established. The trial plants received no feeding or any other plant nutrition for two months, after which they were moved into the tropical greenhouse where the experiment commenced. The experiment was laid out in a randomized complete block design in a factorial arrangement. Each experiment comprised 10 (n=10) pots per plant type per treatment = 60 plants per species. Treatments comprised of ten, weekly treatments, consisting of 100 ml per treatment per pot. The pots were soil drenched, with the required amount of compost tea extract. The treatments included the following application rates of the compost tea extracts:

Control = no added compost tea catalyst, no added compost extract.
Control 1 = no added compost tea catalyst with compost extract only.
T250:1 = 250 ml compost tea extract with compost tea catalyst to 1L water.
T500:1 = 500 ml compost tea extract with compost tea catalyst to 1L water.
T750:1 = 750 ml compost tea extract with compost tea catalyst to 1L water.
T1000:1 = 1000 ml compost tea extract with compost tea catalyst to 1L water.

4.3.2 Preparation of the compost tea extract

The compost tea catalyst and compost tea brewing was prepared according to manufacturer's instructions in using the Growing Solutions® system 10 recommendations with 1:8 ratios, which was converted to 500 g of compost: 40 litres of water (Anonymous, 2011). Municipal water was used, and rested to allow all chemical additions to evaporate over 24 hours. Only mushroom compost was added to the filter and brewed aerobically for

24 hours for the first brew. After each brew the brewer was cleaned and rinsed. The second aerobic brew was with 100 ml of compost tea catalyst added to the 500 g of mushroom compost: 40 litres of water. After brewing the abovementioned formulations were mixed separately using measuring cylinders and stored in lidded flasks until the specific formulation was ready to be applied to the plant samples. The plants were assessed weekly over a 20-week period (September 2013 to mid-January 2014), for various growth responses.

4.3.3 Chlorophyll content determination

Chlorophyll content of healthy leaves of individual plants was determined using non-destructive fluorometer analysis (Manetas *et al*., 1998). The greenest visible leaf was selected. The CCM-300 chlorophyll meter (a modulated ratio fluorescence chlorophyll fluorometer), made by OPTI-SCIENCES, Inc. (8 Winn Ave., Hudson, NH 03051, USA), was used to take the readings. Using a fibre-optic probe connected to an LED diode with two detectors allowed the device to direct light at the leaf surface at one wavelength and measures the re-emitted light (fluorescence) at another wavelength. It used an emission ratio of red chlorophyll fluorescence from 700 to 710 nm and far red emission fluorescence value from 730 to 740 nm with a peak at 735nm (Gitelson *et al*., 1999). The Gitelson equation is Chlorophyll in mg/m^2 = 634*F735/F700+391. Readings were obtained by keeping the probe against the adaxial side of the leaves. Another non-destructive chlorophyll study using the SPAD-502 (Konica Minolta, Spectrum technologies, Planinfiled, Illinois) showed such an instrument was accurate when used on new leaves (Manetas *et al*., 1998).

4.3.4 Measuring leaf colour

Leaf colour was recorded on a weekly basis and measured using a Likert scale of 1-5 as described by Ndakidemi & Makoi (2009), where 5 represented healthy green leaves and 1 represented severely chlorotic and/ or necrotic leaves.

4.3.5 Statistical analysis

Data collected from the leaves were analysed statistically using the repeated analysis of variance, general linear model, across all treatments. The interaction between the effect of growth time and the effect of the compost tea extract on both species were also analysed. The post hoc multiple comparisons using the Bonferroni technique were used to compare treatment means at $P \leq 0.05$, level of significance. These computations were done using the software of the SPSS version 23 programme (Urdan, 2005).

4.4 RESULTS

Both *Hypoxis hemerocallidea* corms and *Siphonochilus aethiopicus* rhizomes were slow growing from the commencement of the experiment resulting in inconsistent growth in both leaves and stems. While it was possible to measure chlorophyll content for Hypoxis from week 3, Siphonochilus was only readable from week 8 (Tables 4.1 and 4.2).

4.4.1 Leaf chlorophyll content (Chl/mg²) of *Hypoxis hemerocallidea*

Leaf chlorophyll content was recorded individually throughout the experiment for Hypoxis. At week 3 the average leaf chlorophyll content per treatment ranged as follows: Control = 250, Control 1 = 220, T250:1 = 167, T500:1 = 187, T750:1 = 194 and T1000:1 = 237 (Table 4.1). After ten weeks (W10) of CTE applications, the average leaf chlorophyll content per treatment increased as follows: Control = 309, Control 1 = 325, T250:1 = 356, T500:1 = 323, T750:1 = 302 and T1000:1 = 305 (Table 4.1). Towards the end of the experiment, at twenty weeks the average chlorophyll content ranged as follows; T250:1 = 264 followed by Control 1 = 255, T500:1 = 249, T1000:1 = 238, T750:1 = 229, and the lowest was the Control at 206 (Table 4.1). All treatments were found to be not significant across all weeks. Overall the average readings measured: Control = 231.6, Control 1 = 243, T250:1 = 262, T500:1 = 237.2, T750:1 = 215 and T1000:1 = 241 (Table 3.1). There was no significant interaction between weeks vs. treatment over the twenty weeks even as the plants grew throughout the twenty weeks. Chlorophyll content readings varied weekly with most treatments peaking at week 11 accept for the control (Figure 4.1).

Table 4.1 The effects of compost tea extract (CTE) treatments on leaf chlorophyll content (Chl/mg²) of *Hypoxis hemerocallidea*.

Treatments	Week 3	Week 5	Week 10	Week16	Week 20	Total average leaf chlorophyll
Control	250 ± 37a	200 ± 28a	309 ± 24a	193 ± 38a	206 ± 25a	231.6
Control 1	220 ± 37a	128 ± 28a	325 ± 24a	287 ± 28a	255 ± 25a	243
T250:1	237 ± 37a	221± 28a	356 ± 24a	242 ± 28a	254 ± 25a	262
T500:1	187 ± 37a	160 ± 28a	323 ± 24a	267 ± 28a	249 ± 25a	237.2
T750:1	194 ± 37a	122 ± 28a	302 ± 24a	229 ± 28a	229 ± 25a	215
T1000:1	237 ± 37a	101 ± 28a	305 ± 24a	319 ± 28a	238 ± 25a	241
F-Statistic	0.75ns	2.19ns	0.67ns	2.61ns	0.7ns	

Values presented are means ± SE. ns = not significant. (N=10). Means with the same letter(s) are not significantly different at P = ≤ 0.05.

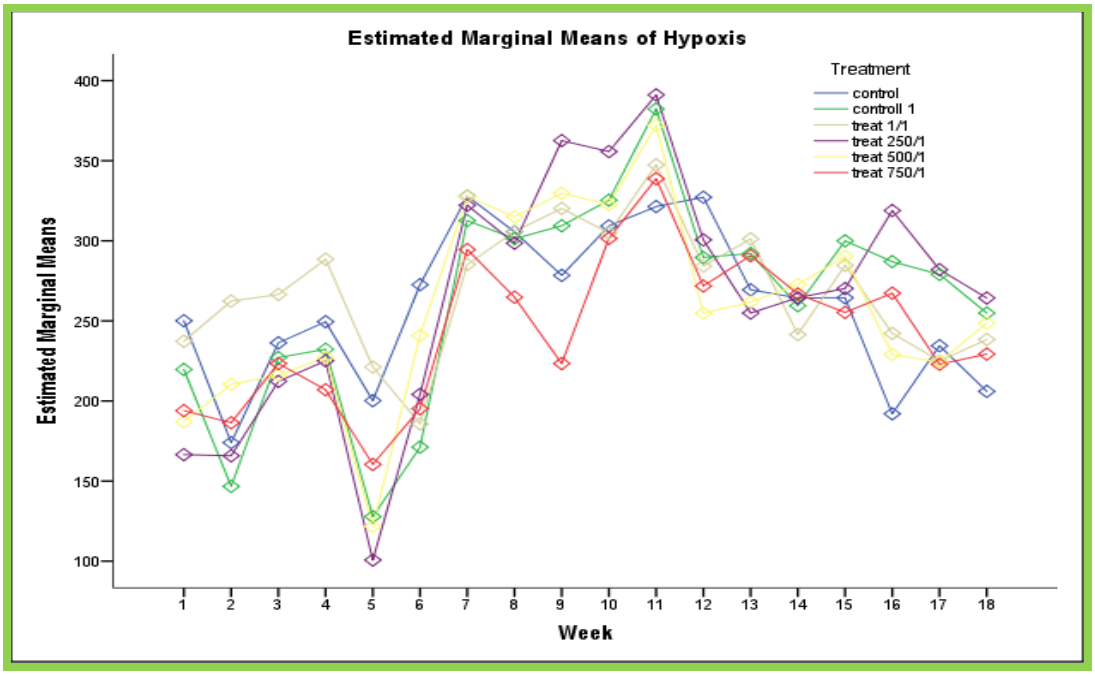

Figure 4.1: Leaf chlorophyll content (Chl/mg²) of *Hypoxis hemerocallidea.* Values represent the means ± SD for the different treatments (n=10). Different treatments are compared to each other per plant species. (Week 1 represents week 3 and Week 18 represents Week 20 respectively).

4.4.2 Leaf chlorophyll content (Chl/mg²) of *Siphonochilus aethiopicus*

Leaf chlorophyll content measurement was delayed due to delayed growth responses, and was recorded individually throughout the experiment. At the eighth week of the experiment the average leaf chlorophyll content per treatment ranged as follows: Control = 74; Control 1 = 76; T250:1 = 0, T500:1 = 7, T750:1 = 21 and T1000:1 = 21 (Table 4.2). The average leaf chlorophyll content in week fourteen, per treatment increased as follows: Control = 428, Control 1 = 358, T250:1 = 245, T500:1 = 223, T750:1 = 225 and T1000:1 = 200 (Table 4.2). Towards the end of the experiment, at twenty weeks the average chlorophyll content ranged as follows; Control 1 = 278 followed by Control and T250:1 = 268, T500:1 and T1000:1 = 243, and the lowest was T750:1 at 237 (Table 4.2). All treatments were found to be not significant across all weeks. The overall chlorophyll content averages for Siphonochilus measured: Control = 256.6, Control 1 = 237.3, T1000:1 = 254.6, T750:1 = 161, T500: L = 151.6 and T250:1 = 171. The total average leaf chlorophyll was the highest (256.6) in the Control followed by the Control 1 (237.3) in Table 4.2. There was no significant interaction between weeks vs. treatment over the twenty weeks even as the plants grew throughout the twenty weeks (Figure 4.2).

Table 4.2 The effects of compost tea extract (CTE) treatments on Leaf chlorophyll content (Chl/mg²) of *Siphonochilus aethiopicus*

Treatments	Week 8	Week 14	Week 20	Total average leaf chlorophyll
Control	74 ± 15.5a	428 ± 56a	268 ± 17.4a	256.6
Control 1	76 ± 15.5a	358 ± 28a	278 ± 17.4a	237.3
T250:1	0 ± 15.5a	245 ± 56a	268 ± 17.4a	171
T500:1	7 ± 16.4a	223 ± 59a	243 ± 18a	157.6
T750:1	21 ± 15.5a	225 ± 56a	237 ± 17.4a	161
T1000:1	21 ± 15.5a	200 ± 56a	243 ± 17.4a	154.6
F-Statistic	4.53ns	2.66ns	0.96ns	

Values presented are means ± SE ns = not significant. Means with the same letter(s) are not significantly different at P = 0 ≤ 05.

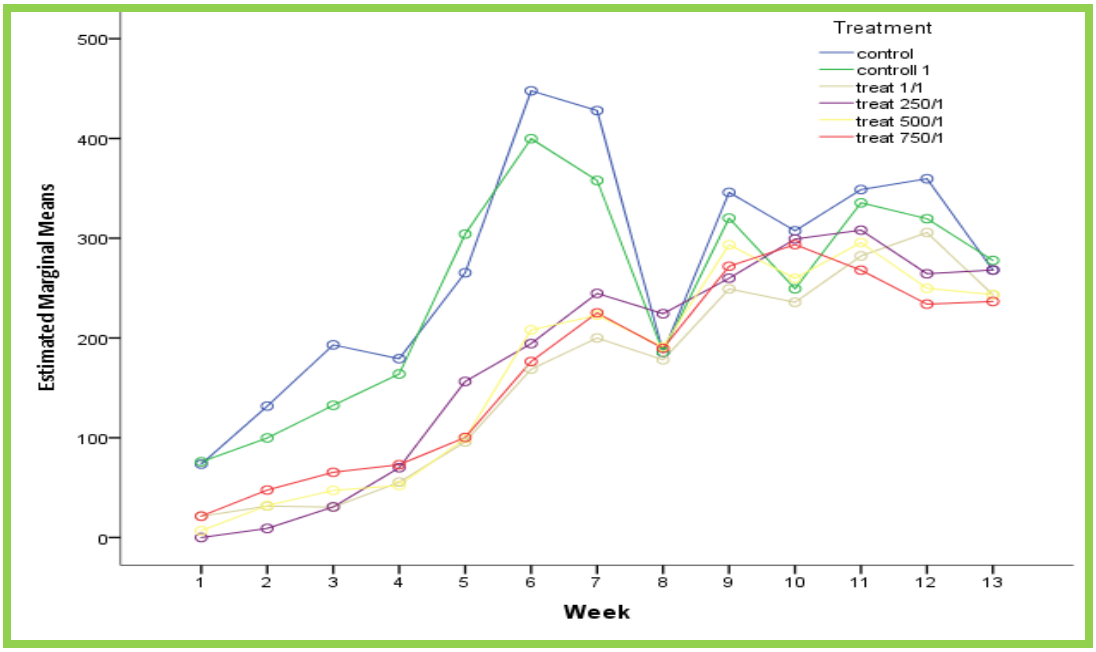

Figure 4.2 Leaf chlorophyll content (Chl/mg²) of *Siphonochilus aethiopicus.* Values represent the means ± SD for the different treatments (n=10). Different treatments are compared to each other per plant species. (Week 1 represents week 8 and Week 13 represents Week 20 respectively).

4.4.3 The effects of CTE on leaf colour of *Hypoxis hemerocallidea*

A Linkert scale of 1-5 was used to record the data on leaf colour on weekly basis. The number 5 represented healthy leaves and 1 represented severely chlorotic and/ or necrosis in leaves. There was no significant difference of the effect of the treatments on leaf colour over the twenty weeks. There was however, an effect of time on leaf colour of Hypoxis plants, with new leaves emerging, becoming longer and by week 20, with some of the older outer leaves slowly dying off, towards the end of the experiment. Both the T750:1 and T1000:1 showed an improvement from 1 – 3 and 1 – 5 respectively from week 10 to 20 (Table 4.3).

4.4.4 The effects of CTE on leaf colour of *Siphonochilus aethiopicus*

There was no significant difference of the treatments on leaf colour over the twenty weeks. At week 20 the Control 1 had the highest leaf colour, followed by the Control and the T250:1 treatment (Table 4.3). However, there was a significant effect of time on leaf colour of Siphonochilus plants, as the leaf colour improved over time, and towards the end of the experiment the leaves began to fade and die off. Only treatment T250 showed an improvement

in leaf colour from 1 – 4 between weeks 10 to 20. No other treatments or Control showed an improvement (Table 4.3).

Table 4.3 The effects of compost tea extracts on leaf colour of *Hypoxis hemerocallidea* and *Siphonochilus aethiopicus* at Weeks 10 and 20 of the experiment.

Treatments		Control	Control 1	T250:1	T500:1	T750:1	T1000:1	p-value
Hypoxis hemerocallidea	Week 10	2	4	5	3	1	1	Treatment = 0.462 Week = 0.000 Week*Treatment = .000
	Week 20	1	4	2	1	3	5	
Siphonochilus aethiopicus	Week 10	5	4	1	2	3	1	Treatment = 0.00 Week = 0.000 Week*Treatment = .000
	Week 20	4	5	4	3	2	3	

Values presented on a scale of 1-5, where 5 represent healthy leaves and 1 represents severely chlorosis and/ or necrosis of leaves.

4.5 DISCUSSION

In Hypoxis, samples were taken from the greenest visible leaf, across the 20 weeks, based on new shoot, subsequent leaf emergence. These samples varied from plant to plant within the treatments despite each plant receiving similar weekly dosages of the same compost tea extract for various treatments (Control 1, T250:1, T5000:1, T750:1 and T1000:1) and the control. As new leaf growth emerged, in the first two weeks, it was not sufficient to take a chlorophyll reading. In some cases, leaves were none existent as the new apical tip was just emerging from the centre of the corm, above ground. The greenest leaf was selected at all times, as leaves began their slow emergence. From the results it is clear that the Control 1 (255), T250:1 (254) and the T500:1 (249) treatments performed better than the control and all other treatments. These readings were in particular high, when compared to a study where leaf chlorophyll content was measured in Gladiolus corms revealing levels that ranged from 68.73 to 80.20, but after applications of iron, boron and zinc were applied, led to an increase in chlorophyll content when all three micronutrients were applied to the foliage (Fahad *et al*., 2014). Even though this current study showed no statistical differences, the treatments did show different response in the leaf colouring where higher concentrations in treatments (T1000:1 and T750:1) showed a higher scale of improvement on the Linkert scale varying from 1 – 3 and 1 – 5 respectively from week 10 to 20. In a study on muskmelon, the chlorophyll continent was lower at ten days but higher after seventeen days (Azia *et al*., 2001).

The chlorophyll and leaf colour readings were taken over a short growing period from October to January. The possibility of seasonal conditions cannot be excluded which could have affected the readings at that time of the year. Nxawe *et al*. (2011) reported that the effects of temperature regimes ranging from 26-30°C on *Ornithogalum longibracteatum* L. contributed to improved chlorophyll formation and photosynthesis during the winter months. In comparison, use of a leaf chlorophyll meter to predict nitrogen status and yield of grape fruit trees found better correlations in spring than summer (Li *et al*., 1996) while Johnston and Onwueme (1998) reported that yam, taro, tannia, cassava and sweet potato had more chlorophyll per leaf in the shade in comparison to the same crops that were exposed to sun. Even though this current study was only performed during summer months the chlorophyll and leaf colouring for Hypoxis could have been affected by the season and the compost tea extract nutrient content. Environmental factors can play a big role during the growth periods of plants. This was also recorded by Jifon *et al.* (2015) who reported the chlorophyll readings in *Citrus* species. As the CTE treatments did not have a significant effect on the chlorophyll production and leaf greening of Hypoxis leaves it is speculated that the bulk of the CTE was absorbed into the Hypoxis corms. Chlorophyll levels were thus not constant over the weeks due the plant characteristics and physiology at the time of the experiment. Corm development is controlled by the seasons as well. According to Hartmann *et al.* (2014) for corms such as Gladiolus, corm development is controlled by photoperiods while the plant shows competition between flower assimilation for the current year and corm development for the following year. Longer day growth in summer will assimilate corm growth in size.

For Siphonochilus the initial seven-week growth period measured 0 in chlorophyll readings across all the treatments of the experiment. As new apical tips emerged, chlorophyll readings could only be taken as from week 8 whereby leaves were more mature. As for Hypoxis a better understanding of the growth in this case, the Siphonochilus rhizome structure and season became relevant. Even though two different species were used in this study, different chlorophyll and leaf colouring was observed in each species. A similar result was reported in two Populus species, being lower chlorophyll content measured in *Populus balsamifera* in comparison to *Populus tremuloides* (Castro & Sanchez-Azofeifa, 2008). By weeks 8 and 14 the control took over with the highest reading. Towards week twenty the control, control 1 and T250:1 were head to head with the highest readings in chlorophyll. The plants were slow in growth and the greenest leaf was selected at all times for chlorophyll readings. All chlorophyll readings were found to be not significant between treatments. However, depending on the chlorophyll concentration there was a slow but definite increase in the chlorophyll levels towards the end of the experiment period, across all treatments. There was thus a significant interaction between weeks vs. treatment over the twenty weeks. Wild ginger chlorophyll content was below 100 Chl/mg in the beginning and as the plants grew throughout the twelve weeks, the different

dosages of CTE did influence the growth in the rhizomes and subsequently the new shoot formation, significantly over time, with all treatments' averaging between 237-263 Chl/mg in week twenty. A study on leafy vegetable chlorophyll content also showed measurements in Chinese cabbage (52.4) which had darker green foliage proportional to SPAD values and consistent with that particular vegetable colour (Limantara *et al.,* 2015). In comparison other root crops such as yam, taro, tannia, cassava and sweet potato showed higher levels of chlorophyll measurements per leaf according to Johnston and Onwueme (1998). Despite low chlorophyll readings in Siphonochilus, the leaf colour measurements in the Control 1, T250:1, T500:1 and T1000:1 treatment all showed an increase between weeks 10 and 20. This indicates that an increase over the weeks indicating that the compost tea applications might have had an effect over time, thereby indicating that chlorophyll content increased over time. Even though the CTE treatments had no significant effect on the chlorophyll and greening of the leaves, it was possible that the bulk of the compost tea extract being absorbed into the Siphonochilus rhizomes. A clear description of the Siphonochilus growth structure could support a better understanding of its growth habit. Hartmann *et al.* (2014) described the ginger plant as a pachymorph rhizome with a thick fleshy and short stems where each section ends in a flowering stalk and growth continues from lateral branching. The rhizome stems grow in summer and autumn, flowering in spring. For some rhizomes such as blue berry, higher temperatures and longer days' increase growth variables (Hartmann *et al.,* 2014). In most flowering rhizomes, stems grow several leaves to feed the plant for the following flowering season. This short growth pattern could have caused a delay in the growth period of the Siphonochilus and could have resulted in a lack/slowdown of producing better chlorophyll and leaf colour readings from the species, thereby influencing any significant differences between treatments.

4.6 CONCLUSION AND RECOMMENDATIONS

This study found it necessary to establish chlorophyll levels after CTE application in both Hypoxis and Siphonochilus species to support their future commercial cultivation. Both these species remain endangered in their habitat and their commercial cultivation remains limited. The medicinal importance of these species cannot be ignored and it is important to refrain from using chemical fertilizers during their cultivation. This study therefore aimed to measure their performance using organic growth regulators such as CTE at various concentrations. Although a significant effect of CTE could not be seen over the short growth period, it is recommended that future studies concentrate on longer (2-3) growing periods and also test variations in seasons as seen in other crops. While this study is evaluated at a starting point in their cultivation, more research is necessary about their specialised corm and rhizome growth

structures in reaction of compost tea extracts. Future studies can also place more emphasis on these factors to advance commercial organic cultivation of Hypoxis and Siphonochilus species.

4.7 ACKNOWLEDGEMENTS

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Chapter Five

NUTRIENT AVAILABILITY IN THE SOIL MEDIUM AND THE WET AND DRY WEIGHTS OF *HYPOXIS HEMEROCALLIDEA* AND *SIPHONOCHILUS AETHIOPICUS* EXPOSED TO VARIOUS MUSHROOM COMPOST TEA EXTRACTS.

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5.1 ABSTRACT

Hypoxis hemerocallidea and *Siphonochilus aethiopicus* are summer growing, summer/spring flowering, deciduous and bulbous geophytes that grow naturally in the subtropical regions of southern Africa. Both species are threatened in their natural habitat and listed on the 'Red Data list of southern Africa Plants'. The use of various compost tea extract formulations has gained popularity. The aim of this study was to determine the weight of these two endangered plant species, and growth media nutrient readings and the availability of nutrients using mushroom compost tea extracts and what influence it had on the growth and anti-oxidant content and capacity. Mushroom compost tea extracts (T1000:1; T750:1; T500:1; T250:1), and Control 1 (Compost tea extract no catalyst) were applied to both species over 20 weeks. Significant differences in total wet weight were found for *H. hemerocallidea* between T500:1 and Control. T750: 1, T1000:1, Control 1, and T250: 1 were significantly higher than the Control. The total dry weights of *H. hemerocallidea* were not significantly different. The analysis of soil sample used for growing *H. hemerocallidea* revealed that nutrient levels differed across all treatments, except magnesium(Mg). Dry leaf nutrient analysis revealed significant difference in the calcium contents of the different *H. hemerocallidea* treatments, ranging from 170 mg - 290 mg.

No significant differences were found in the dried root analysis of *H. hemerocallidea*.

The total wet weights of *S. aethiopicus* in the different treatments were significant different. Control and Control 1 had significantly longer leaf lengths compared to the rest of the treatments. Control 1 and control had significantly better heights compared to the rest of the treatments. Control 1, Control, T750: 1, T1000: 1 had significantly longer root lengths than T500: 1 and T250:1. Control 1 and Control had significantly higher leaf and stem weights than treatments T1000:1, T750:1, T500: 1, T250: 1. There were no significances in the dry weight analysis. The dry soil analysis of *S. aethiopicus* revealed nutrient levels differed

between treatments. The dry leaf analysis revealed that sodium (Na) levels in treatments T500:1 and T250:1 respectively were significantly lower than the Control 1. The manganese (Mn) levels in the Control was significantly lower than treatments T750:1, T500:1, T250:1. The Zinc (Zn) levels in the Control treatment, was significantly lower than treatments T1000:1 and T500:1. Based on the results in this study mushroom compost tea is recommended as an organic fertilizer for *H. hemerocallidea* and *S. aethiopicus*.

Keywords: nutrient levels, *Hypoxis hemerocallidea, Siphonochilus aethiopicus*

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5.2 INTRODUCTION

The increasing use of organic fertilizers, including compost tea has led to an interest in how plants receiving organic fertilizers are able to use nutrients to increase growth, subsequently weight and its availability within the plants' structure. Over the past few decades the medicinal value and importance of various plants have showed a heightened interest. Phytochemicals including antioxidants and nutrients are found in many plant foods such as vegetables, seed, beans, nuts, fruits, etc. High yield of these crops could translate to yield of medicinal and food materials. However, the quantity of these materials can vary as indicated by a study done by Herrmann (1988), and Chan *et al.* (2008). Using mushroom compost tea extracts, the aim of this investigation was to determine the nutrient levels in the leaves and underground structures of two endangered species, which could also influence antioxidant capacity and content.

5.2.1 *Hypoxis hemerocallidea*

H. hemerocallidea (Family: Hypoxidaceae) is a cormous perennial with long, hairy, strap-like leaves and yellow star-shaped flowers borne on long stalks during spring (Van Wyk *et al*., 1997). The name Hypoxis is derived from the Greek words, hypo (below) and oxy (sharp), with reference to the ovary which is pointed at the base. The specific epithet is derived from the Greek hemera (a day) and kallos (beauty) presumably referring to the flowers that are short-lived and bears resemblance to the day lily, *Hemerocallis* sp. Formerly known as *H. rooperii*, the broad leaves are arranged one above the other, resulting in three distinct sections spreading outwards from the centre of the plant (Van Wyk *et al*., 1997). The genus Hypoxis consists of approximately 90 species, distributed on all continents, except Europe. Approximately 30 species are found in southern Africa (Singh, 2007). The name 'African Potato' was given to the species after the Afrikaans, 'Afrika patat'. The plant is however a compressed underground corm which grows vertically and is commonly known as 'Inkomfe'

among the African Zulu speaking people (Van Wyk *et al.,* 1997). Tubers are dark brown in colour, large and covered with bristly hairs. When freshly cut, the tubers are bright yellow in colour, with a bitter taste (SANBI, 2009).

The species is valued by traditional healers for treating tuberculosis (TB), cancer, urinary tract infections, anxiety, palpitations, and rheumatoid arthritis, amongst others.

5.2.2. *Siphonochilus aethiopicus*

S. aethiopicus (Schweinf.) B.L. Burtt, (Family: Zingerberaceae) is a rhizome with a restricted distribution to Mpumalanga and Northern Province while becoming extinct in Kwa-Zulu Natal province of South Africa (Van Wyk *et al.,* 1997). Siphonochilus is derived from the Greek 'siphono' meaning tube and 'chilus' meaning lip, referring to the shape of the flower and 'aethiopicus' meaning from southern Africa (Gordon-Gray *et al.,* 1989). Also known as wild ginger, the deciduous, small, aromatic cone-shaped rhizomes and leaves give off a smell when crushed similar to that of real ginger, *Zingiber officinale* (Van Wyk *et al*., 1997). This species was used traditionally by the Zulu people for a variety of medicinal ailments such as asthma, hysteria, colds, coughs, flu, dyspepsia, and travel sickness, amongst others (Cumes *et al*., 2009). Besides its unique and distinctive morphology, the Swati people use wild ginger to treat malaria and menstruation by chewing the fresh rhizomes and roots (Van Wyk *et al*., 1997).

5.2.3 Compost tea extracts

Compost tea is widely used as an alternative to improve quality plant production of field grown and nursery crops in substituting chemical fertilizer programmes (Ingham, 2005; Khalid *et al*., 2006; Naidu *et al*., 2010). Various studies have proved that beneficial organisms assist in the prevention of diseases when applying compost tea to plant leaves (Scheuerell, 2004; Ingham, 2005; Souleymane *et al*., 2009).

Internationally, compost teas have gained extreme popularity for turf applications due to low cost and environmental restrictions in the use of traditional chemicals (Ingham, 2005; Van Zwieten *et al*., 2007; Anonymous, 2011; Kelloway, 2012; Mullaivannan, 2013). While several studies have highlighted the beneficial organisms and micronutrient potential of compost tea which support reduction in use of fungicides, to assist in weed control and the improvement of root penetration of sport turf grasses (Scheuerell *et al.*, 2002; Bellows, 2003; Sachs, 2004 Ingham, 2005; Arumugam, 2012; Islam *et al.*, 2013; Garrett, 2014). Compost tea has also gained popularity in conventional agriculture with current trends of organic agriculture production (Theunissen *et al*., 2010). According to Ingham (2005), compost tea research has been conducted on a variety of plant crops, such as asparagus, potato, corn, wheat, tomato,

lettuce and radish, in orchards and vineyards, landscape trees, in greenhouse crop production, and agricultural crops. To our knowledge, no compost tea research has been done to date on *H. hemerocallidea* and *S. aethiopicus*. Compost tea is a water extract of compost that is brewed so that the beneficial organisms, i.e. the beneficial bacteria, fungi, protozoa and nematodes, are extracted from the compost and given the right environment to increase in number and activity by providing soluble food sources and the nutrients present in the water (Scheuerell & Mahaffee, 2002; Ingham 2005). The quality of the compost will determine how large a diversity of these beneficial organisms will be present (Ingham, 2003). Compost extract is obtained when adding water to compost and allow excess water to drain from the compost. Typically, this means the compost is over saturated (Scheuerell & Mahaffee, 2002; Ingham 2005). A typical extract contains only soluble nutrients and few organisms. More organisms may be pulled from the compost surfaces by cycling the water through the compost a number of times; thereby allowing more organisms to protect leaf and root surfaces (Ingham, 2003). The use of a commercial compost tea extract as part of an organic, environmentally sound approach, in crop cultivation is gaining popularity (Bess, 2000; Touart, 2000, Ingham, 2005).

5.2.4 Nutrient concentrations in indigenous corms and rhizomes, plants, fruit, and vegetables.

Numerous forms of nutrient studies have been under taken and found the following amongst others. The effects of non-aerated compost tea consisting of ruminant and municipal solid waste (MSW) on strawberries resulted in varied nutrient content in the fruits (Hargreaves *et al*., 2008). In a similar study in 2005, the non-aerated compost teas supplied equivalent levels of nutrients to strawberries, whilst the soil potassium (K) levels decreased with the applications of compost tea (Hargreaves *et al*., 2009). Non-aerated compost tea applied to raspberry plants resulted in lower K levels in the fruit and leaves, with a year to year variation observed in nutrient levels (Hargreaves *et al*., 2008- raspberries).

Vermicompost tea had a positive effect on pak choi (*Brassia rapa* cv. Bonsai) largely caused by nitrogen uptake by the plants. However, no significant differences were found between the different extraction methods on plant growth. Nutrient uptake in plant tissues of pak choi (*Brassia rapa* cv. Bonsai) varied across treatments (Pant *et al*., 2009). Aerated chicken manure tea, applied as a soil drench, resulted in better plant height, leaf number and flowers, shoot fresh and dry weight, and leaf chlorophyll in lemon basil (*Ocimum x citriodorum* Vis.) in comparison to vermicompost tea, with varied nutrient availability between the teas (Javanmardi & Ghorbani, 2012). Aerated compost tea extracts made from fennel and

artichoke composts, and applied to lettuce and kohlrabi plants resulted in improved crop yields, physiological and nutritional status (Pane *et al*., 2014).

5.3. MATERIALS AND METHODS

5.3.1 Plant material

The plant samples (n=60) for both *H. hemerocallidea* corms and *S. aethiopicus* rhizomes were purchased from Afro Indigenous Nursery, KwaZulu-Natal. The experiments were conducted at the nursery of the Department of Horticultural Sciences, Cape Peninsula University of Technology (CPUT), Bellville - 33°55'56" S, 18°38'25" E. The plant samples were grown in the tropical greenhouse under controlled environmental conditions with an average relative humidity of 70% and an average temperature of 26 °C daily. The plant soil media consisted of equal parts of fine pine bark, perlite and vermiculite in 15 cm plastic pots.

The experiment was laid out in a randomized complete block design in a factorial arrangement. Each experiment comprised 10 pots per plant type per treatment. Treatments comprised of ten, weekly treatments, consisting of 100 ml per treatment per pot. The pots were soil drenched, with the required amount of compost tea extract. The treatments included the following application rates of the compost tea extracts:

Control = no added compost tea catalyst, no added compost extract.
Control 1 = no added compost tea catalyst with compost extract only.
T250:1 = 250 ml compost tea extract with compost tea catalyst to 1L water.
T500:1 = 500 ml compost tea extract with compost tea catalyst to 1L water.
T750:1 = 750 ml compost tea extract with compost tea catalyst to 1L water.
T1000:1 = 1000 ml compost tea extract with compost tea catalyst to 1L water.

5.3.2. Preparation of the compost tea extract

The compost tea catalyst and compost tea brewing was prepared according to the manufacturer's instructions. The recommended concentration by Growing Solutions System 10 (Anonymous 2011) is a 1:8 ratios, which translates into 500 g of compost: 40 L water. Municipal water was used and only mushroom compost was added to the filter and brewed aerobically for 24h for the first brew (Control 1). After each brew the brewer was cleaned and rinsed. The second aerobic brew consisted of a 100 mL compost tea catalyst added to the 500 g of mushroom compost per 40 L of water. After brewing, the abovementioned formulations were mixed separately using measuring cylinders and stored in lidded flasks until the specific formulations were ready to be applied to the plant samples

The readings were taken over the short growing period (September/October 2013- January 2014) of these two endangered plant species. The same compost tea extract with catalyst treatments (T1000:1, T750:1, T500:1, T250:1) and the Control 1 (compost tea extract with no catalyst) were applied to both species over 20 weeks. The Control received water only. TMC= no added compost tea catalyst just mushroom compost.

5.3.3 Data collection

Data was collected of fresh (wet) and dry weights of leaves, corm and rhizomes, roots and respective nutrient content were determined. The different components of the plant were recorded of both species at the end of the trial. The fresh weights were recorded. The excised leaves, bulb portions and roots were dried in a laboratory oven at 50° C for one week, and then weighed using a laboratory scale (Electronic Precision Balance, Model No: FR-H supplied by Scales Incorporated, Brackenfell, Cape Town). The dry soil samples were analysed using Inductively Coupled Plasma / Optical Emission Spectrometry (ICP-OES) techniques that included; Hot water extraction (B); Ammonium Acetate extraction (Ca, K, Mg, Na); Bray II (P); KCl method (pH); Walkey-Black method (C); Titrimetric method (H) and the EDTA extraction method (Cu, Zn, Mn). The dry leaf samples were analysed using Inductively Coupled Plasma / Optical Emission Spectrometry (ICP-OES) techniques that included Dry Ashing extraction (B, Ca, Cu, Fe, K, Mg, Mn, Na, P, and Zn) and Leco combustion (N) at Bemlab Laboratories, Somerset West, Cape Town (SANAS, 2015) and used to express the nutrient values of the plant material.

5.3.4 Statistical analysis

Mean replicate values (n=10) of the wet roots, bulbs (corms and rhizomes), and leaves were collected and analysed statistically using one-way analysis of variance (ANOVA). Mean replicate values (n=3) of the dry roots, bulbs (corms and rhizomes), and leaves were collected and analysed statistically using one-way analysis of variance (ANOVA). The Tukey HSD test was used to separate the means at a level of significance, $P < 0.05$, and these computations were done using the software of the PAST programme.

5.4 Results: *Hypoxis hemerocallidea*

5.4.1 The total wet analysis of *Hypoxis hemerocallidea*

The wet length of *Hypoxis hemerocallidea* leaf length, root length and corm diameter were not significantly different (df = 5, 54; and P < 0.05) among all of the treatments at 20 weeks post treatment (Table 5.1). The wet weight of *Hypoxis hemerocallidea* leaf weight, root weight and corm weight were not significantly different (df = 5, 54; and P < 0.05) among the treatments at 20 weeks post treatment (Table 5.1), However, total wet weight which consisted of the sum of leaves, roots and corms in T500:1 (396.81 g) was significantly higher (df = 5, 54; F = 2.896 and P < 0.01) than the control (241.3g) and T250:1 (245.93g) at 20 weeks post treatment (Table5.1).

Table 5.1 The total wet analysis (cm and g) of *Hypoxis hemerocallidea.*

Treatment	Mean ± SE Leaf length cm	Mean± SE Root length cm	Mean ± SE Corm diameter cm	Mean ± SE Leaf weight g	Mean ± SE Corm weight g	Mean ± SE Root weight g	Mean ± SE Total wet weight g
Control	25.9 ± 5.1a	30.75 ± 3.5a	6.91 ± 0.53a	15.9 ± 2.8a	167.3 ± 33.7a	58.09 ± 13.2a	241.3 ± 41.71a
Control 1	39.6 ± 6.1a	26.8 ± 3.1a	7.12 ± 0.4a	19.72 ± 3.7a	190.7 ± 28.6a	58.24 ± 14.8a	268.66 ± 32.15ab
T1000:1	45 ± 6.6a	29.5 ± 2.2a	6.9 ± 0.4a	34 ± 6.5a	212.1 ± 29.2a	72.14 ± 15.8a	318.2 ± 29.81ab
T750:1	40.85 ± 7.6a	27.2 ± 4.1a	7.23 ± 0.2a	31.64 ± 6.2a	241.5 ± 39.2a	79.13 ± 30.03a	352.22 ± 40.34ab
T500:1	46.6 ± 6.9a	29.1 ± 2.4a	7.39 ± 0.34a	40.01 ± 9.3a	271.8 ± 39.4a	84.98 ± 15.52a	396.81 ± 45.85b
T250:1	40.7 ± 6.2a	28.9 ± 3.3a	6.03 ± 0.30a	27.77 ± 6.9a	154.2 ± 19.2a	64 ± 12.33a	245.93 ± 27.72ab

Means followed by the same uppercase letter are not significantly different at P = 0.05 level of significance (df = 5, 54; P < 0.05) using Tukey test.

5.4.2 The total dry weight analysis (g) of *Hypoxis hemerocallidea*

Total dry weight of corms, roots and leaves in the treatments were not significantly different (df = 5, 54; F = 1.41 and P < 0.05) at 20 weeks post treatment, which yielded total weights ranging from 15.9– 25.2 g (Table 5.2).

Table 5.2 The total dry weight analysis (g) of *Hypoxis hemerocallidea*.

Treatments	Mean ± SE Corm dry weight/g	Mean ± SE Root dry weight/ g	Mean ± SE Leaves/g	Mean ± SE Total/g
Control	3.84 ± 0.73a	8.8 ± 2.23a	3.3 ± 0.53a	15.9 ± 2.6a
Control 1	7.21 ± 1.5a	7.5 ± 2.21a	4.2 ±0.93a	18.9 ± 3.1a
T1000:1	7.27 ± 0.1a	9 ± 2.22a	7.07 ± 1.4a	23.3 ± 2.6a
T750:1	8.65 ± 1.4a	9.3 ± 3.13a	5.5 ±1.04a	23.4 ± 3a
T500:1	8.39 ± 1.4a	10.3 ± 2.1a	6.5 ± 1.41a	25.2 ± 2.62a
T250:1	6.9 ± 1.2a	10 ± 2.2a	5.25 ± 1.3a	22.15 ± 3.43a

Means followed by the same uppercase letter per column are not significantly different at P < 0.05 level of significance (df = 5, 54; P < 0.05) using Tukey test.

5.4.3 The dry soil analysis (g.) soil sample of *Hypoxis hemerocallidea.*

The table below is utilised as baseline data only, as the same soil media was utilised during the experiment. The different nutrients did vary. (Table 5.3).

Table 5.3 The dry soil sample analysis (mg) of *Hypoxis hemerocallidea*.

Treatment	pH	N/ mg	P/ mg	K/mg	Ca (cmol(+)/kg	Mg (cmol(+)/kg	Na cmol(+)kg	Mn/mg	Fe/mg	Cu/mg	Zn/mg	B/mg
Control	5.0	2.8	137	292	2162	634.8	138	124.8	260.86	5.00	38.8	1.89
Control 1	5.4	3.5	195	441	2610	651.6	207	238.2	353.61	4.56	41.2	2.12
T1000:1	5.2	2.7	119	366	2288	604	131.1	246.6	265.28	4.03	51.4	1.45
T750:1	5.2	3.6	149	350	3080	662.4	133.4	263.5	411.56	5.61	54.6	2.25
T500:1	5.3	3.4	116	293	2282	662.4	151.8	161.6	288.33	4.57	37.8	1.56
T250:1	5.2	1.7	181	524	2768	972	317.4	197.3	381.50	5.88	57.0	1.78
TMC	6	2.83	58	616.2	818	52.8	5099.43	383.30	423.80	14.88	245.5	18.57

5.4.4 The total dry leaf nutrient analysis (mg.) of *Hypoxis hemerocallidea*

Generally, the calcium level was significantly lower in the control than in the other treatments. Calcium Control 1 was statistically higher than T1000:1, T750:1 and T500:1 (df = 5, 12; F =9.35 and P < 0.05) at 20 weeks post treatment; T250:1 was significantly higher than the Control, which yielded values ranging from 170-290 mg (Table 5.4). Calcium control was significantly lower than all the other treatments and T250: 1 significantly higher than T1000: 1; T750: 1 and T 500: 1 but similar to Control 1. No significant differences were revealed in the other nutrient concentrations between treatments as listed. Nonetheless, treatment T750:1 had the highest nitrogen, Control 1 had the highest phosphorus level; T500:1 had the highest potassium level; Control 1 had the highest calcium; T750:1 had the highest magnesium; T750:1 had the highest sodium; Control 1 had the highest manganese; T1000:1 had the highest iron content; Control 1 had the highest copper content; T500:1 had the highest zinc content; T250:1 had the highest boron content (Table 5.4).

Table 5.4 The total dry leaf nutrient analysis (mg.) of *Hypoxis hemerocallidea.*

Treatment	Mean ± SE N/mg	Mean ± SE P/mg	Mean ± SE K/mg	Mean ± SE Ca/mg	Mean ± SE Mg/mg	Mean ± SE Na/mg	Mean ± SE Mn/mg	Mean ± SE Fe/mg	Mean ± SE Cu/mg	Mean ± SE Zn/mg	Mean ± SE B/mg
Control	22 ± 0.74a	1.7± 0.017a	741 ± 0.03a	170 ± 0.03a	26.4 ± 0.02a	2085 ± 45.83a	176 ± 26.7a	106 ± 3.21a	8.3 ± 0.3a	32 ± 1.73a	34.3 ± 3.2a
Control 1	28 ± 0.92a	2.1± 0.048a	741 ± 0.5a	290 ± 0.11b	31.2± 0.03a	2198 ±232.53a	261.7 ±48.8a	300± 2.7a	12.7 ± 3.5a	45.7 ± 8.4a	41 ± 4.2a
T1000:1	24 ± 0.79a	1.4± 0.017a	780± 0.2a	208 ± 0.03c	28.8 ± 0.03a	2864 ± 550.1a	154.3 ±29.8a	496 ± 3a	12.3 ± 3.2a	41.3 ± 6.1a	32 ± 3.2a
T750:1	30 ± 1.01a	1.8± 0.0088a	780 ± 0.2a	222 ± 0.06c	38.4 ± 0.02a	3005 ± 499.8a	166.7 ±60.7a	133 ± 2.8a	10.3 ±1.8a	72 ± 3.4a	34 ± 1.9a
T500:1	23 ± 0.75a	1.56± 0.013a	943.8 ± 0.1a	202 ± 0.01c	36 ± 0.01a	2902 ± 121.1a	193.7 ±28.3a	191.3 ± 5.7a	9 ± 0.6a	108 ± 6.1a	36.7 ± 1.4a
T250:1	22 ± 0.73a	1.6± 0.0057a	819 ± 0.21a	236 ± 0.1bc	31.2 ± 0.02a	2760 ± 429.6a	188.3 ± 45a	271 ± 3.1a	7.3 ± 0.9a	52.3 ± 9.3a	42 ± 4.7a

Means followed by the same uppercase letter per column are not significantly different at $P < 0.05$ level of significance ($df = 5, 12$; $P < 0.05$) using Tukey test.

5.4.5 The total nutrient analysis (mg) of *Hypoxis hemerocallidea* dried roots

Treatments did not significantly (df = 5, 12; and P > 0.05) affect any of the nutrients measured in the corms. The following treatments had the highest nutrient content/values, namely or Control and T1000:1 had the highest nitrogen content; T750:1 had the highest phosphorus content; T750:1 had the highest potassium content; T250:1 had the highest calcium content; T750:1 had the highest magnesium content; control had the highest sodium content; Control 1 had the highest manganese content; T250:1 had the highest iron content; Control had the highest copper content; T500:1 had the highest zinc content and Control had the highest boron content (Table 5.5).

Table 5.5 The total nutrient analysis (mg.) of *Hypoxis hemerocallidea* dried roots.

Treat-ment	Mean ± SE N/ mg	Mean ± SE P/mg	Mean ± SE K/mg	Mean ± SE Ca/mg	Mean ± SE Mg/mg	Mean ± SE Na/mg	Mean ± SE Mn/mg	Mean ± SE Fe/mg	Mean ± SE Cu/mg	Mean ± SE Zn/mg	Mean ± SE B/mg
Control	5.1± 0.02a	1.5 ± 0.02a	390 ± 0.07a	170 ± 0.08a	38.4 ±0.01a	1557 ±212a	233 ± 2.3a	5328 ± 1731a	15 ± 1.2a	77 ± 1a	16 ± 1.5a
Control 1	4.6± 0.03a	1.3 ± 0.01a	312 ± 0.07a	168 ± 0.1a	38.4 ±0.03a	1542 ±100a	343 ± 61.8a	4669 ± 1332a	11 ± 1a	81 ± 4a	12 ± 1.5a
T1000:1	5.1± 0.03a	1.2± 0.01a	429 ± 0.03a	152 ± 0.06a	36 ± 0.04a	1501 ± 68a	306 ± 26.4a	2572 ±491a	10 ± 0.3a	97 ± 3a	14 ± 0.6a
T750:1	4.5± 0.03a	1.6± 0.02a	468 ± 0.2a	160 ± 0.05a	43.2 ±0.02a	1537± 53a	297 ± 72.4a	2231 ±759a	9 ± 0.7a	84 ± 4a	14 ± 0.3a
T500:1	4.3± 0.03a	1.1 ± 0.01a	273 ± 0.05a	160 ± 0.2a	38.4 ±0.01a	1345 ± 88a	295 ± 39a	4153 ± 1323a	11 ± 1.5a	98 ± 16a	13 ± 1.7a
T250:1	4.2± 0.03a	1.5± 0.02a	351 ± 0.13a	240 ± 0.5a	31± 0.01a	1465± 49a	222 ± 46.7a	7212 ± 3041a	13 ± 2a	93 ± 17a	13 ± 0.8a

Means followed by the same uppercase letter per column are not significantly different at P = 0.05 level of significance (df = 5, 12; P < 0.05) using Tukey test.

5.5 Results: *Siphonochilus aethiopicus*

5.5.1 The total wet analysis (cm. and g.) of *Siphonochilus aethiopicus*

Control and Control 1 had significantly longer leaf lengths of 29.65 and 25.15cm respectively that the rest of the treatments. Control 1 and control had significantly better heights of 44.3cm and 40.1cm respectively than the rest of the treatments. Control 1, Control, T750: 1, T1000: 1 had significantly longer root lengths of 25.2, 25, 23.5, and 22.45cm respectively than T500: 1 and T250:1. Control 1 and Control had significantly higher leaf and stem weights of 31.77 and 20.56g than treatments T1000:1, T750:1, T500: 1, T250: 1.

Table 5.6 The total wet analysis (cm and g) of *Siphonochilus aethiopicus*.

Treatment	leaf length cm	height in cm cm	root length cm	rhizome diameter cm	leaf & stem weight g	rhizome weight g	root weight g	total weight g
Control	29.65 ± 3.3a	40.1 ± 3.1ab	25 ± 1.80a	3.22 ± 0.24a	20.76 ± 2.9ab	16.57 ± 2.6a	33.5 ± 4.3a	70.9 ± 9.1a
Control 1	25.15 ± 2ab	44.3 ± 5.5a	25.2 ± 1.9a	3.22 ± 0.22a	31.77 ± 6.7a	20.38 ± 4.5a	38 ± 7.7a	90.1 ± 18.1a
T1000:1	21.65 ± 1.6b	30.1 ± 3.7b	22.45 ± 1.8ab	2.28 ± 0.21a	16.37 ± 4.41b	11.03 ± 2.5a	22 ± 6.1a	49.2 ± 12.8a
T750:1	20.65 ± 2b	29.2 ± 4b	23.5± 2.3ab	2.26 ± 0.22a	15.17 ± 5.02b	12.86 ± 3.8a	22.5 ± 7a	50.5 ± 15.4a
T500:1	20 ± 2.4b	27.9 ± 4.8b	20.95 ±1.5b	2.28 ± 0.27a	15.04 ± 5.11b	12.31 ± 5.6a	18.2± 6.2a	45.5 ± 16.8a
T250:1	20.5 ± 1.5b	26.0 ± 2.6b	20.4 ± 1.6b	2.26 ± 0.23a	9.54 ± 1.7b	9.93 ± 1.3a	15.4 ± 2.7a	34.9 ± 4.9a

Means followed by the same uppercase letter per column is not significantly different at $P < 0.05$ level of significance ($df = 5, 54$; $P < 0.05$) using Tukey test. Control 1 depicts mushroom compost tea extract only.

5.5.2 The total dry analysis (g) of *Siphonochilus aethiopicus*.

Total dry weights of leaves roots and rhizomes, in all treatments were not significantly different (df = 5, 54; F = 1.13 and P < 0.05) between all the treatments at 20 weeks post treatment (Table 5.7) and ranged: Rhizomes (2.5 to 1.4g), Root (2.9 to1.2g), leaves (4.2 to 1.3g).

Table 5.7 The total dry weight analysis (g) of *Siphonochilus aethiopicus*

Treatment	Mean ± SE Rhizomes	Mean ± SE Root	Mean ± SE leaves	Mean ± SE Total
Control	2.2 ± 0.4a	2.7 ± 0.4a	3.1 ± 0.4a	8 ± 1.1a
Control 1	2.5 ± 0.6a	2.9 ± 0.7a	4.2 ± 0.9a	9.55 ± 2.a
T1000:1	1.4 ± 0.3a	2 ± 0.72a	2.2 ± 0.7a	5.6 ± 1.7a
T750:1	2.5 ± 1a	2.4 ± 1a	2.3 ± 0.9a	7.1 ±2.7a
T500:1	1.4 ± 0.7a	1.4 ± 0.6a	2.1 ± 0.82a	5 ± 2.1a
T250:1	2.0 ± 0.6a	1.2 ± 0.2a	1.3 ± 0.22a	4.4 ± 0.6a

Means followed by the same uppercase letter are not significantly different at P < 0.05 level of significance (df = 5, 54; P < 0.05) using Tukey test.

5.5.3 The dry soil sample analysis (mg.) of *Siphonochilus aethiopicus*

The table below is utilised as baseline data only, as the same soil media was utilised during the experiment. The different nutrients levels varied between treatments. (Table 5.8).

Table 5.8 The dry soil sample analysis (mg.) of *Siphonochilus aethiopicus*

Treat-ment	pH	N/mg	P/ Mg	K/mg	Ca /mg	Mg/mg	Na/mg	Mn/mg	Fe/mg	Cu/mg	Zn/mg	B/mg
Control	4.6	3.3	74	259	2662	642	124.2	969.6	327.29	3.70	176.4	2.10
Control 1	4.5	4.1	122	674	3092	870	342.7	735.3	275.83	2.53	125.8	2.20
T1000:1	4.5	3.1	116	476	2334	642	262.2	718.8	247.17	2.41	120.1	1.77
T750:1	4.5	3.7	79	302	2958	633.6	124.2	864.2	298.51	2.96	128.6	2.06
T500:1	4.4	2.7	93	272	2760	661.2	126.5	478.3	191.67	1.75	74.4	1.58
T250:1	4.4	3.1	66	191	2254	530.4	98.9	856.0	281.50	2.35	141.4	1.70
TMC	6	2.83	5.8	616.2	818	52.8	5099.43	383.30	423.80	14.88	245.5	18.57

5.5.4 The total dry leaf nutrient analysis (mg.) of *Siphonochilus aethiopicus*.

The sodium (Na) levels 477 and 456 mg in treatments T500:1 and T250:1 respectively were significantly lower (df = 5, 12; F = 5.18 and P < 0.01) than the Control 1 treatment at 20 weeks post treatment, which yielded levels ranging from 594- 653 mg. The manganese (Mn) levels 1646 mg in the Control treatment was significantly lower (df = 5, 12; F = 5.71 and P < 0.01) than treatments T750:1, T500:1, T250:1 at 20 weeks post treatment, which yielded ranging from 2962- 5976 mg. The Zinc (Zn) levels in the control treatment, as shown below, at 29 mg was significantly lower (df = 5, 12; F = 3.54 and P < 0.01) than treatments T1000:1 and T500:1 at 20 weeks post treatment, which yielded ranging from 40.3- 50 mg. The nitrogen, phosphorus, potassium, calcium, magnesium, iron, copper, and boron dry leaf nutrient levels were non-significantly different between the treatments. (Table 5.9).

Table 5.9 The total dry leaf nutrient analysis (mg.) of *Siphonochilus aethiopicus*.

Treatment	Mean ± SE N/mg	Mean ± SE P/mg	Mean ± SE K/mg	Mean ± SE Ca/mg	Mean ± SE Mg/mg	Mean ± SE Na/mg	Mean ± SE Mn/mg	Mean ± SE Fe/mg	Mean ± SE Cu/mg	Mean ± SE Zn/mg	Mean ± SE B/mg
Control	13 ± 0.04a	3± 0.01a	1212.9± 0.09a	120 ± 0.02a	37.2 ± 0.03a	653 ± 39b	1646 ± 549c	243 ± 120a	3 ± 0.3a	29 ± 3.6c	10 ± 0.6a
Control 1	13 ± 0.03a	3.1± 0.01a	1329.9±0.11a	124 ± 0.02a	38.4 ± 0.01a	791 ± 24a	2962 ± 987b	121 ± 28a	3 ± 0.3a	42 ± 2.5ab	10 ± 0.3a
T1000:1	13 ± 0.06a	3.2± 0.003a	1755 ± 0.53a	106 ± 0.03a	37.2 ± 0.02a	594 ± 37.3b	4591±1530ab	101 ± 6.9a	3 ± 0.3a	50 ± 2.5a	11 ± 0.3a
T750:1	14 ± 0.04a	3.1± 0.015a	1560 ± 0.3a	120 ± 0.03a	37.2 ± 0.01a	602 ±115.4b	5546 ± 1849a	80 ± 6.2a	2 ± 0a	46 ± 4.3a	10 ± 0a
T500:1	13 ± 0.15a	3.1± 0.02a	1677 ± 0.6a	100 ± 0.05a	38.4 ± 0.03a	477 ± 5.6c	5976 ± 1992a	87 ± 9.8a	2.3 ± 0.3a	50± 6.3a	10 ±0.5a
T250:1	13 ± 0.04a	3± 0.003a	1638 ± 0.54a	104 ± 0.05a	33.6 ± 0.01a	456 ±23.5c	5284 ± 1761a	87 ± 2.1a	2 ± 0a	40.3±4.41ab	10 ± 0.3a

Means followed by the same uppercase letter are not significantly different at P = 0.05 level of significance (df = 5, 12; P > 0.05) using Tukey test. Control 1 depicts mushroom compost tea extract only.

5.6 Discussion

Compost tea extract applications to the soil over the short growing period (September/October 2013- January 2014) of these two endangered plant species had a positive effect on growth over time. The reasons for growth include the applications of compost tea, the greenhouse environment, and the short summer growth season of these two endangered species. The soil media was the same for both species during the experiment and its analysis revealed that soil nutrients varied across treatments, as both species appear to absorb nutrients through their storage organs and utilise these nutrients' differently during their growth season The nutrients extracted during brewing played a role in the growth of these slow growing corm and rhizome structures respectively, and can play a role in the antioxidant content and capacity. Nutrients were absorbed via the roots and its structures, ending in the leaves and occur in varying quantities throughout the plant.

5.6.1 *Hypoxis hemerocallidea*

Results from the total wet analysis of *Hypoxis hemerocallidea* from the current study show that treatment T500:1 was significantly higher than Control 1, because the corms were larger and heavier thereby producing the longest leaves, the largest corm diameter and the highest leaf, corm and root weight. It therefore appears that although T500:1 is the most suitable dosage, it does not imply that the other dosages, except for control, were not beneficial to plant growth.

Hypoxis corms varied in weight and size from the start of the experiment and this also resulted in the treatment T500:1 weighing more in total than the control. Statistically there was no difference between Control 1, T1000:1, T750:1 and treatment T250:1 and the Control. The total dry weights were non-significant. The dry leaf nutrient analysis revealed significant difference in the calcium (Ca) content of Hypoxis after treatment. Ca content was non-significant in the dried root analysis and although lower these Ca content levels were higher at the required rate for normal human growth and skeletal development, of about 150 mg per day. Roots play a vital role in the uptake of nutrients especially Iron (Fe), Zinc, (Zn), and Calcium, (Ca) and the availability of the nutrients to the leaves and corm is a function of the soil physiochemical and biological properties, where the release is from a solid phase of the soil into a soluble state (Frossard *et al*., 2000). The dried leaf nutrient analysis also revealed the following treatments had the highest nutrient content namely: Control 1: phosphorus (P), calcium (Ca), manganese (Mn) and copper (Cu); T1000:1 had the highest iron (Fe). T750:1 had the highest nitrogen (N), magnesium (Mg) and sodium (Na). T500:1 had the highest potassium (K) and zinc (Zn). Treatment T250:1 had the highest boron (B) level.

No significant differences were found between the treatments in the dried root analysis. The results do indicate nutrient levels differ across all treatments. The nitrogen (N) was highest in the Control and T1000:1. The Control also had the highest sodium (Na) and boron (B) levels. Control 1 had the highest manganese (Mn) and copper levels (Cu). Treatment T750:1 had the highest phosphorus (P), potassium (K), magnesium (Mg) levels. Treatment T500:1 had the highest zinc (Zn) levels and T250:1 had the highest calcium (Ca) and iron (Fe) levels.

5.6.2 *Siphonochilus aethiopicus*

The total wet analysis of *Siphonochilus aethiopicus* revealed significant differences in the total leaf length, plant height, root length and leaf and stem weights. The total leaf length in treatment T500:1 was significantly lower. The total plant height in T250:1 was significantly lower. Total root length (20.4 cm) in T250ml:1 was significantly lower. Total wet leaf and stem weights (9.54 cm) in T250:1 was significantly lower.

No significant differences were detected in the rhizome diameter, rhizome weight, root weight and total weight in the total wet analysis of SA. The results indicate that the compost tea had a significant effect on the wet height, leaf length, root length and leaf and stem wet weight, over time, yet wet rhizome diameter, weight, root weight and total weight were non-significant. There were no significant differences in the dry weight analysis. Control 1 had the highest total dry mass which consisted on the rhizomes, roots and leaves. Treatment T250:1 had the lowest total dry mass due to smaller rhizomes. The dry soil analysis revealed various results between treatments. The results do indicate nutrient levels differ across all treatments, with Control 1 with the highest nitrogen (N), phosphorous (P), potassium (K), calcium (Ca), magnesium (Mg) and sodium (Na) levels. Control had the highest manganese (Mn), iron (Fe), copper (Cu), zinc (Zn) and boron (B) levels. Soil pH varied between treatments and was slightly more acidic than the hypoxis dry soil analysis, in comparison to the mushroom compost, and possibly due the interaction of the nutrients, as compost tea was absorbed. Nutrient levels in the total dry leaf analysis differed. Control had the highest iron (Fe) and shared the highest copper (Cu) with T1000:1. Treatment T1000:1 also had the highest zinc (Zn), boron (B), potassium (K) and phosphorous (P) content. Control 1 had the highest calcium (Ca), magnesium (Mg) and sodium (Na) content. Treatment T750:1 had the highest nitrogen (N) levels. Treatment T500:1 had the highest manganese (Mn) content. The dry leaf analysis revealed significant differences between the treatments in sodium (Na) (in T500:1 and T250:1 - the lowest), manganese (Mn) where Control 1 and T1000:1 had the lowest levels. Control had the lowest zinc (Zn) levels, whilst the rest of the treatments were higher ranging from 40.3 mg to 50 mg.

In general, when comparing the nutrient levels (N; P; K; Ca) in Lachenalia and Ornithogalum (Claassens, 1990), also two indigenous bulbs to Hypoxis and Siphonochilus, the nitrogen (N), potassium (K) and Calcium (Ca) levels were lower in Lachenalia and Ornithogalum than Hypoxis and Siphonochilus. Phosphorus (P) levels were higher in Lachenalia and Ornithogalum *than* Hypoxis *and* Siphonochilus. Sparaxis corms were found to have highly flexible storage capacities of nutrients (Ruiters & Mckenzie, 1994). Mushroom compost nutrient levels (N, P, K, Ca, Mg, Na, Fe, Mn, Cu, Zn, B) and pH vary (Unzun, I., 2004; Jordon *et al*., 2006; Polat *et al*., 2009; Lou *et al*., 2015) and increase or decrease in plants. High sodium levels are generally found in most mushroom compost types and mixes.

5.7 CONCLUSION

Nutrient levels/content increased in total when comparing mushroom compost nutrient levels to dried plant part and soil of *H. hemerocallidea* and *S. aethiopicus*, and there are not many studies available which focus on the nutrient concentration within these two species. Boron, zinc, copper, iron, manganese, magnesium and potassium increased in both species in total, as compost tea extracts' were applied over 20 weeks. Excluding the total dried leaf of *H. hemerocallidea* and the dried leaf of *S. aethiopicus* the nitrogen levels were lower in the dried soil and roots of *H. hemerocallidea* and dried soil of *S. aethiopicus*, when compared to the nitrogen level in mushroom compost of 28.3 mg. Total phosphorus levels were lower in *H. hemerocallidea* corms & roots and leaves and in *Siphonochilus aethiopicus* dry leaves, but increased in both soils of *H. hemerocallidea* and *S. aethiopicus*. Total calcium levels increased in both soils of *H. hemerocallidea* and *S. aethiopicus*, and in the leaves of *H. hemerocallidea* but was lower in the leaves of *S. aethiopicus*. Total sodium levels increased in the corm/roots and leaf of *H. hemerocallidea* but decreased in the soil of *H. hemerocallidea*, and the soil and leaf content of *S. aethiopicus*. Mushroom compost had the highest sodium (Na), manganese (Mn), iron (Fe), copper (Cu), and zinc (Zn) and boron (B). The compost tea extracts had an effect on the nutrient levels/content even though nutrient levels did not differ in all treatments, as nutrients are dependent of compost tea makeup, source of compost, available nutrients in the compost before brewing, the brewing method, the plant species selected and its physiological attributes/characteristics, how and when the plant species absorbs the nutrients and where it will be stored within the plant itself. In spite of these nutrient variances mushroom compost tea is recommended as an organic fertilizer. Further research should be undertaken to determine the nutrient levels within the corms of *H. hemerocallidea* and rhizomes of *S. aethiopicus* over 2-3 growth seasons and its impact/ use in traditional medical remedies, and pharmacological medicines.

5.8 ACKNOWLEDGEMENTS

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Chapter Six

THE EFFECT OF COMPOST TEA EXTRACTS ON THE ANTIOXIDANT-CAPACITY AND -CONTENT OF TWO SOUTH AFRICAN GEOPHYTES, *HYPOXIS HEMEROCALLIDEA* AND *SIPHONOCHILUS AETHIOPICUS*.

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6.1 Abstract

Compost tea extracts are gaining popularity over conventional agriculture as the demand for organic production increases for crops to be healthier and less susceptible to disease. Further advances are the improvement of soil conditions, cost saving on pesticides and reduced fertilizer degraded ecosystems. The increased demand for African medicinal species such as *Hypoxis hemerocallidea* and *Siphonochilus aethiopicus* have led to their over exploitation because of overharvesting, degradation and habitat loss. The use of compost tea extracts in their cultivation can lead to the enhancement of their medicinal properties with the improvement of nutrient content of each species root structures. The total polyphenol, flavonol and flavanol content, and ORAC, FRAP, ABTS capacity were measured in the underground structures of *H. hemerocallidea* and *S. aethiopicus* grown in a tropical greenhouse environment. The polyphenol antioxidant content was not significant in both species. There was an increased flavonol content observed in the corms of *H. hemerocallidea*. In the rhizomes of *Siphonochilus* there were significant differences in flavonol content between CEO, 250:1 and 1000:1 compost tea treatments. The corms of *H. hemerocallidea* showed higher flavonol antioxidant activity. No flavanol content was detected in *H. hemerocallidea*. Significant differences were detected in the flavanol content in *S. aethiopicus*. ORAC (oxygen radical absorbance capacity) was not significant in both species. Significant differences were detected in the FRAP (ferric reducing antioxidant power) of the *H. hemerocallidea* compost tea treatments, but not in *S. aethiopicus*. ABTS (2,2′-azino-di-3-ethylbenzthialozine sulphonate) values were non-significant in both species. Based on the results in this study, it is recommended that a full strength mushroom compost tea be utilised to, generally, increase the antioxidant activity of *H. hemerocallidea* and *S. aethiopicus* underground storage organs.

Keywords: Polyphenols; Flavonols; ORAC, FRAP; ABTS; fertilizer; plant nutrition, compost extract only (CEO)

6.2 Introduction

6.2.1 General background

Over the past few decades, and more recently, the medicinal value and importance of various plants has led to a heightened interest in them, especially where extracts are discovered that contain a variety of secondary metabolites, which have antioxidant potential and protect against oxidative damage by free radicals (Larson, 1988; Akinmoladun *et al*., 2007). There are many differences between phytochemicals and antioxidants in their functions, location and usage. Phytochemicals are found in many plant foods such as vegetables, seed, beans, nuts, fruits etc. whilst antioxidants are found in both animal and plant foods. Antioxidants are vitamins and minerals, or phytochemicals that help prevent damage to cells from highly unstable and reactive molecules, commonly known as 'free radicals' (Lobo *et al*., 2010). Another source (Fang *et al*., 2002 and Liu, 2004) describes antioxidants as phytochemicals, vitamins and other nutrients that protect cells from damage caused by free radicals. In the Zingerberaceae family, these antioxidants can be accumulated in the rhizomes. This can result in the rhizomes having a higher antioxidant activity/ content and capacity. This can vary as indicated by a study done by Herrmann (1988) and Chan *et al.* (2008).

6.2.2 *Hypoxis hemerocallidea*

H. hemerocallidea (Family: Hypoxidaceae) is a cormous perennial with long, hairy, strap-like leaves and yellow star-shaped flowers borne on long stalks during spring (Van Wyk *et al*., 1997). The name Hypoxis is derived from the Greek words, hypo (below) and oxy (sharp), with reference to the ovary which is pointed at the base. The specific epithet is derived from the Greek hemera (a day) and kallos (beauty) presumably referring to the flowers that are short-lived and bears resemblance to the day lily, *Hemerocallis* sp. Formerly known as *H. rooperii*, the broad leaves are arranged one above the other, resulting in three distinct sections spreading outwards from the centre of the plant (Van Wyk *et al*., 1997). The genus Hypoxis consists of approximately 90 species, distributed on all continents, except Europe. Approximately 30 species are found in eastern southern Africa (Singh, 2007). The name 'African Potato' was given to the species after the Afrikaans, 'Afrika patat'. The plant is however a compressed underground corm which grows vertically and is commonly known as

'Inkomfe' among the African Zulu speaking people (Van Wyk *et al.,* 1997). Tubers are dark brown in colour, large and covered with bristly hairs. When freshly cut, the tubers are bright yellow in colour, with a bitter taste (SANBI, 2009).

The species is valued by traditional healers for treating tuberculosis (TB), cancer, urinary tract infections, anxiety, palpitations, and rheumatoid arthritis, amongst others. Some studies reported that the aqueous extract has anticonvulsant properties, thereby lending credence to traditional herbal use to treat childhood convulsions and epilepsy (Ojewole, 2008). It is also used to restore immune systems of patients recovering from HIV and cancer. Hypoxis phytosterols, used to treat benign prostrate hypertrophy was successfully marketed, and later phytosterols (based on industrial sources) claiming to have immune system stimulating effects, became and still is a huge marketing success (Drewes *et al*., 2008). The phytochemical, hypoxoside, is an inactive compound that is converted to rooperol. The species has thus potent pharmacological properties to treat relevant inflammations as well as anti-infective, anti-diabetic, antioxidant, and antineoplastic conditions in patients (Owira & Ojewole, 2009). In a review article by Street and Prinsloo (2012), a few medicinal compounds found in the plant, which include hypoxoside, sitosterol and an aglycone derivative, rooperol were reported. Chemical analysis by Oluwule *et al.* (2007) revealed different classes of secondary metabolites namely glycosides, polyphenols, saponnins, steroids and tannins. Three cytokinins, namely zeatin, zeatin riboside and zeatin glucoside were reported by Hutchings (1996).

Using the corms raw could be toxic and any extract must be used with caution (SANBI, 2009). The market and trade usage by traditional healers of *Hypoxis* sp. that has been investigated, found that it is one of the most frequently traded plant species in KwaZulu-Natal (Dold & Cocks, 2002). *H. hemerocallidea* corms continue to decline in South Africa, mainly due to overharvesting of this miracle muthi and wonder potato for its prized medicinal value. As found in studies of *Gethyllis* species, urban development also contributes to its decline (Daniels *et al*., 2013).

6.2.3 *Siphonochilus aethiopicus*

S. aethiopicus (Schweinf.) B.L. Burtt, (Family: Zingerberaceae) is a rhizome with a restricted distribution to Mpumalanga and Northern Province while becoming extinct in Kwa-Zulu Natal province of South Africa (Van Wyk *et al.,* 1997). Siphonochilus is derived from the Greek 'siphono' meaning tube and 'chilus' meaning lip, referring to the shape of the flower and 'aethiopicus' meaning from southern Africa (Gordon-Gray *et al.,* 1989). Known as wild ginger,

the deciduous, small, aromatic cone-shaped rhizomes and leaves when crushed, smell similar to that of real ginger, *Zingiber officinale* (Van Wyk *et al*., 1997). This species was used traditionally by the Zulu people for a variety of medicinal ailments such as asthma, hysteria, colds, coughs, flu, dyspepsia, and travel sickness, amongst others (Cumes *et al*., 2009). Besides its unique and distinctive morphology, the Swati people use wild ginger to treat malaria and menstruation by chewing the fresh rhizomes and roots (Van Wyk *et al*., 1997). The aromatic roots are reportedly used by the Zulu people to ward off lightening (Van Wyk *et al*., 1997). Wild ginger has similar culinary uses as true ginger and the plant family has a number of valuable spice plants such as cardamom and turmeric. In an anti-inflammatory and antibacterial study, it was discovered that fresh rhizomes of wild ginger exhibited better efficacy than leaves, despite the fact that aqueous rhizome extracts displayed moderate to high levels of cytotoxicity (Light *et al*., 2002). Further medicinal studies on wild ginger confirmed the treatment for pain and inflammation (Fennell *et al*., 2004). In KwaZulu Natal, the market and trade usage amongst traditional healers of wild ginger was and is regarded as one of the most popular traded plant species (Dold & Cocks, 2002; Institute of Natural Resources, 2003). During earlier studies wild ginger presented antiplasmodial compounds in dried rhizomes which may play a role in the traditional use of wild ginger to treat malaria (Lategan *et al*., 2008). Further research confirmed both antibacterial and antifungal activities in the rhizomes and leaves of wild ginger (Coopoosamy *et al*., 2010). With these advances new medicinal products have been developed, where wild ginger is commercially grown, harvested using freeze dried roots and rhizomes, then marketed and sold in the form of tablets to treat asthma, candida, coughs, colds, headaches and malaria (Van Wyk, 2011). Antioxidant studies indicated that the species to have one of the highest hydroxyl radical scavenging ability in water extracts, and 'substantial potentiation of lipid peroxidation in the membranes of normal peripheral blood mononuclear cells' (Steenkamp *et al*., 2005). Overharvesting of this sought after medicinal plant, has led to the plant almost being lost to total extinction as the cone shaped rhizomes and fleshy roots are dug up and sold on muthi markets throughout South Africa (Raimondo *et al.,* 2009). While micro propagation has saved the species from extinction where wild populations were thought to be almost totally depleted, the species remain on the listed data for Red Listed species of South African plants (SANBI, 2002). Essential oils have been extracted from Siphonochilus roots and rhizomes as conducted by Viljoen *et al.* (2002).

Previous studies have been conducted on the antioxidant activity of both species but the effect of the application of various concentrations of compost tea extracts on antioxidant activity has never been evaluated. The use of compost tea extracts could possibly enhance the medicinal properties, specifically its antioxidant activity, of both the above mentioned

species as well as promoting the future commercial cultivation to supply the growing demand for the species.

6.2.4. Compost tea extracts

Compost tea is a water extract of compost that is brewed so that the beneficial organisms, i.e. the beneficial bacteria, fungi, protozoa and nematodes, are extracted from the compost and given the right environment to increase in number and activity by providing soluble food sources and the nutrients present in the water (Scheuerell & Mahaffee, 2002; Ingham 2005). The quality of the compost will determine how large a diversity of these beneficial organisms will be present (Ingham, 2003). Compost extract is obtained when adding water to compost and allow excess water to drain from the compost. Typically, this means the compost is over saturated (Scheuerell and Mahaffee, 2002; Ingham 2005). A typical extract contains only soluble nutrients and few organisms. More organisms may be pulled from the compost surfaces by cycling the water through the compost a number of times; thereby allowing more organisms to protect leaf and root surfaces (Ingham, 2003).

The use of a commercial compost tea extract as part of an organic, environmentally sound approach, in crop growing has slowly gained momentum (Bess, 2000; Touart, 2000, Ingham, 2005). There are various approaches to compost tea extraction and utilization, in the horticultural, turf care, agriculture, landscaping, and viticulture sectors. It is good practice to start with good, high quality compost (El Hanafi Sebti, 2005). This research project was specifically focused on the effect of the various compost tea extract concentrations on the antioxidant content-and -capacity of two southern African bulbous species.

6.3. Materials and methods

6.3.1. Plant material

The plant samples (n=60) for both *Hypoxis hemerocallidea* corms and *Siphonochilus aethiopicus* rhizomes were purchased from Afro Indigenous Nursery, KwaZulu-Natal. The experiments were conducted at the nursery of the Department of Horticultural Sciences, Cape Peninsula University of Technology (CPUT), Bellville - 33°55’56” S, 18°38’25” E. The plant samples were grown in the tropical greenhouse under controlled environmental conditions with an average relative humidity of 70 % and an average temperature of 26°C daily. The plant soil media consisted of equal parts of fine pine bark, perlite and vermiculite in 15 cm plastic pots. The experiment was laid out in a randomized complete block design in a factorial arrangement. Each experiment comprised 10 pots per plant type per treatment.

Treatments comprised of ten, weekly treatments, consisting of 100 ml per treatment per pot. The pots were soil drenched, with the required amount of compost tea extract. The treatments included the following application rates of the compost tea extracts:

Control = no added compost tea catalyst, no added compost extract.
Control 1 = no added compost tea catalyst with compost extract only = CEO.
T250:1 = 250 ml compost tea extract with compost tea catalyst to 1L water.
T500:1 = 500 ml compost tea extract with compost tea catalyst to 1L water.
T750:1 = 750 ml compost tea extract with compost tea catalyst to 1L water.
T1000:1 = 1000 ml compost tea extract with compost tea catalyst to 1L water.

6.3.2. Preparation of the compost tea extract

The compost tea catalyst and compost tea brewing was prepared according to the manufacturer's instructions. The recommended concentration by Growing Solutions System 10 is a 1:8 ratio, which translates into 500 g of compost: 40 L water. Municipal water was used and only mushroom compost was added to the filter and brewed aerobically for 24h for the first brew (Control 1). After each brew the brewer was cleaned and rinsed. The second aerobic brew consisted of a 100 mL compost tea catalyst added to the 500 g of mushroom compost per 40 L of water. After brewing, the abovementioned formulations were mixed separately using measuring cylinders and stored in lidded flasks until the specific formulations were ready to be applied to the plant samples.

6.3.3. Plant sample preparation

Fresh material was harvested and the plant samples were excised into leaves, bulbs and roots for both species. The plant parts of both species were dried in a laboratory oven (Drying oven 220. 40 L (Labotec, Cape Town) at 50 °C for 48h. The drying period for the bulb components of both species were extended because of its sizes. The various plant parts were ground into a powder using a laboratory mill (Kinematica AG POLYMIX PX-MFC 90 D lab mill, supplied by United Scientific) and then weighed using a laboratory scale (Electronic precision balance, Model: FR-H: 6000g x 0.01, Scales Incorporated, Cape Town). During the drying process the weights of both species decreased and samples were combined according to their treatment groups in order to bulk up their weights for the required antioxidant analysis, resulting in four samples (N=4) per treatment group.

6.3.4. Antioxidant analysis sample preparation

The antioxidant capacity-and -content analyses was conducted at the Oxidative Stress Research Centre, Faculty of Health and Wellness Sciences, CPUT, Bellville campus. Crude

extracts of the underground plant parts of both species were prepared by stirring the various dried parts (0.05g of each) in 80% (v/v) ethanol (50ml) (EtOH) (Saarchem, South Africa), thereafter it was centrifuged at 4000 rpm for 5 min. The supernatants were used for all analyses. The same sample preparation technique was followed for all assays and all analyses were done in triplicate. The sample handling and preparation is similar to studies done on *Gethyllis multifolia* and *G. villosa* by Daniels *et al*. (2011).

6.3.5. Determination of the antioxidant content

6.3.5.1 Total polyphenol, flavonol and flavanone content

Various crude extracts were determined by the Folin Ciocalteu method (Singleton *et al*., 1974; Swain and Hills, 1959). The method of Swain and Hills (1959) were adapted for use in a plate reader. Using a 96 well microplate, 25 µL of sample was mixed with 125 µL Folin–Ciocalteu reagent (Merck, South Africa), diluted 1:10 with distilled water. 100 µL (7.5%) aqueous Sodium Carbonate (Na_2CO_3) (Sigma-Aldrich, South Africa) was added to the well, after 5min. The plates were incubated for 2h, before the absorbance was read at 765 nm at room temperature using a Multiskan plate reader (Thermo Electron Corporation, USA). The standard curve was prepared using 0, 20, 50, 100, 250 and 500 mg/L gallic acid in 10% EtOH and the results were expressed as mg gallic acid equivalents per g dry weight (mg GAE/g DW).

The use of quercetin at 0, 5, 10, 20, 40, and 80 mg/L in 95% ethanol (Sigma-Aldrich, South Africa) was used as a standard to determine the flavonol content. In the sample wells, 12.5 µL of the crude sample extracts was mixed with 12.5 µL 0.1% HCl (Merck, South Africa) in 95% ethanol, 225 µL 2% HCl and incubated for 30 min at room temperature. The absorbance was read at 360 nm, at a temperature of 25°C (Mazza *et al*., 1999). The results were expressed as mg quercetin equivalent per g dry weight (mg QE/g DW).

An adapted method of Kosalek *et al*. (2004) was used to determine the flavanone content. Briefly, 100 µL of sample was mixed with 200 µL 1% 2.4-dinitrophenylhydrazine (DNPH) (2% H_2SO_4 in methanol (MeOH). After incubation at 50°C for 50 min., 700 µL of 10% Potassium hydroxide (KOH) in 70% MeOH was added. The samples were centrifuged and 30 µL of the resulting supernatant mixed with 270 µL MeOH in a 96-well plate and the absorbance read at 495 nm. A linear standard curve using 0, 0.2, 0.5, 1.0, 1.5, and 2.0 mg/mL naringenin (Sigma-Aldrich, South Africa) in methanol was included. The results were expressed as mg naringenin equivalent per g dry weight (mg NE/g DW).

6.3.6. Determination of the antioxidant capacity

6.3.6.1 Ferric reducing antioxidant power (FRAP)

The FRAP assay was performed using the method of Benzie and Strain (1999). In a 96-well microplate, 10 µL of the crude sample extract was mixed with 300 µL FRAP reagent [0.3 M acetate buffer, pH 3.6 (Saarchem, South Africa), 10 mM 2,4,6- tripyridyl-s-triazine (TPTZ) in 0.1 M HCl (Sigma-Aldrich, South Africa), 20 mM Iron (III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$) (Sigma-Aldrich, South Africa), 6.6 mL distilled water and incubated for at 37°C for 30 min in the plate reader. Absorbance was measured at 593 nm. L-Ascorbic acid (Sigma- Aldrich, South Africa) was used as a standard with concentrations varying between 0 and 1000 µM. The results were expressed as µM ascorbic acid equivalent per g dry weight (µM AAE/g DW).

6.3.6.2 Oxygen radical absorbance capacity (ORAC)

The H-$ORAC_{FL}$ values were determined according to the methods as described by Prior *et al*. (2003). A stock standard solution of Trolox (500 µM) was diluted in phosphate buffer (75 mM, pH 7.4) to provide calibration standards ranging from 5 to 25 µM. The Fluoroskan ascent plate reader (Thermo Fisher Scientific, Waltham, USA) was set at 37ºC. Fluorescence filters with an excitation wavelength of 485 nm and emission wavelength of 538 nm were used. A fluorescein stock solution was prepared in a phosphate buffer and further diluted to provide a final concentration of 14 µM per well. The peroxyl generator, 2, 2'-azobis (2-amidino-propane) dihydrochloride (AAPH) (25 mg/mL in phosphate buffer), was added with a multichannel pipette to give a final AAPH concentration of 4.8 mM per well. The fluorescence per well, containing 12 µL diluted hydrophilic extract, was read every 5 min. for 2h. The final ORACFL values were calculated using the regression equation $y = ax^2 + bx + c$ between the Trolox concentration (µM) and the area under the curve. The results were expressed as µM Trolox equivalents per g dry weight (µM TE/g DW).

6.3.6.3 2,2′-azino-di-3-ethylbenzthialozine sulphonate (ABTS)

The ABTS assay was performed following the method of Re *et al*. (1999). The stock solutions included a 7 mM ABTS and 140 mM Potassium-peroxodisulphate ($K_2S_2O_8$) (Merck, South Africa) solution. By adding 88 µL $K_2S_2O_8$ to 5 mL ABTS solution, a working solution was prepared to perform the analysis according to the plant sample quantities provided. The two solutions were mixed well and allowed to react for 24h at room temperature in the dark. Trolox (6-Hydrox-2, 5, 7, 8-tetramethyl- chroman-2-carboxylic acid) was used as the standard

with concentrations ranging between 0 and 500 µM. Crude sample extracts (25 µL) were allowed to react with 300 µL ABTS in the dark at room temperature for 30 min. before the absorbance was read at 734 nm at 25°C in a plate reader. The results were expressed as µM Trolox equivalent per g dry weight (µM TE/g DW).

6.3.7 Statistical analysis

The statistical significance between antioxidant activity values of the various crude plant extracts was determined by an analysis of variance (ANOVA) where $P \leq 0.05$ was considered to be statistically significant. The computer program employed for the statistical analysis was Medcalc version 9.4.2.0 (Medcalc, Belgium). Microsoft Office Excel 2014, version 16.0.6324.1000 (Microsoft Corporation, USA) was employed to determine the correlation between antioxidant contents and activity.

6.4 Results

This section will be discussed according to the following keys:

Control = no added compost tea catalyst, no added compost extract.
Control 1 = no added compost tea catalyst with compost extract only (CEO).
T250:1 = 250 ml compost tea extract with compost tea catalyst to 1L water.
T500:1 = 500 ml compost tea extract with compost tea catalyst to 1L water.
T750:1 = 750 ml compost tea extract with compost tea catalyst to 1L water.
T1000:1 = 1000 ml compost tea extract with compost tea catalyst to 1L water.

The reason for using *H. hemerocallidea* (HH) corms and *S. aethiopicus* (SA) rhizomes was that both these underground plants structures are used by traditional healers for the preparation of homeopathic remedies. At the commencement of the experiment, the corms and rhizomes had different weights, resulting in inconsistent growth both in the leaves and stems and in the bulb structures below the ground.

6.4.1 Antioxidant content

Total polyphenol, flavonol and flavanone content in *H. hemerocallidea* and *S. aethiopicus*

Polyphenols

The total polyphenol content in *H. hemerocallidea* (HH) plant underground structures revealed no significant differences (P<0.05) across all treatments, including both controls. Increased polyphenol content was found in the 750:1 and 1000:1 compost tea treatments with values of 67 and 65mg GAE, respectively. The lowest total polyphenol content was observed in the 500:1 compost tea treatment (Figure 6.1).

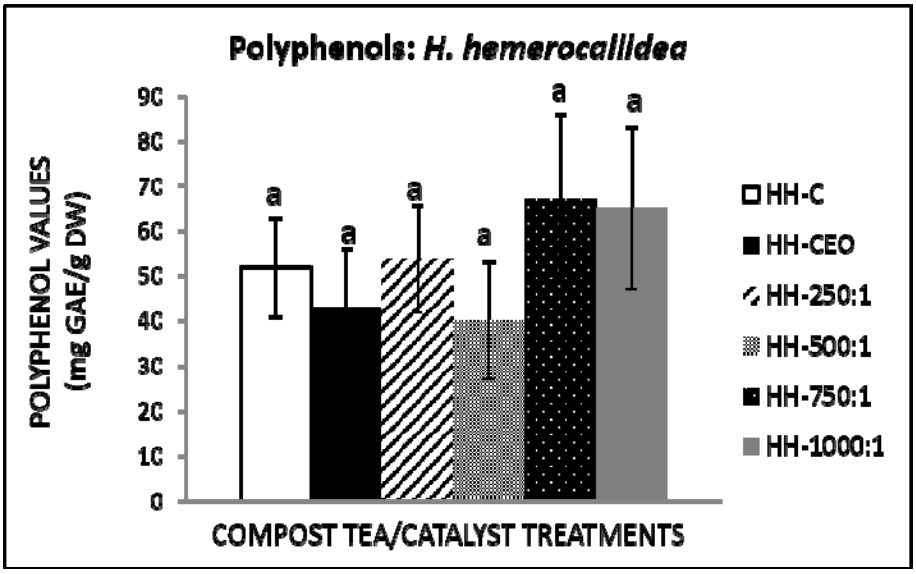

Figure 6.1 The total polyphenol (mg GAE/g dry weight) content of *H. hemerocallidea* corms. Values represent the means ± SD for the different treatments (n=4). Different treatments are compared to each other per plant species. The means of treatments with different letters on bars are significantly (P<0.05) different.

For *S. aethiopicus* similar total polyphenol results were recorded with no significant differences across all treatments and both controls. The highest total polyphenol content was recorded in the T250:1, Control 1(CEO) and T750:1 compost tea treatments with readings of 7.1, 6.8 and 6.5 mg GAE, respectively. The polyphenol content was similar but were non-significant (Figure 6.2).

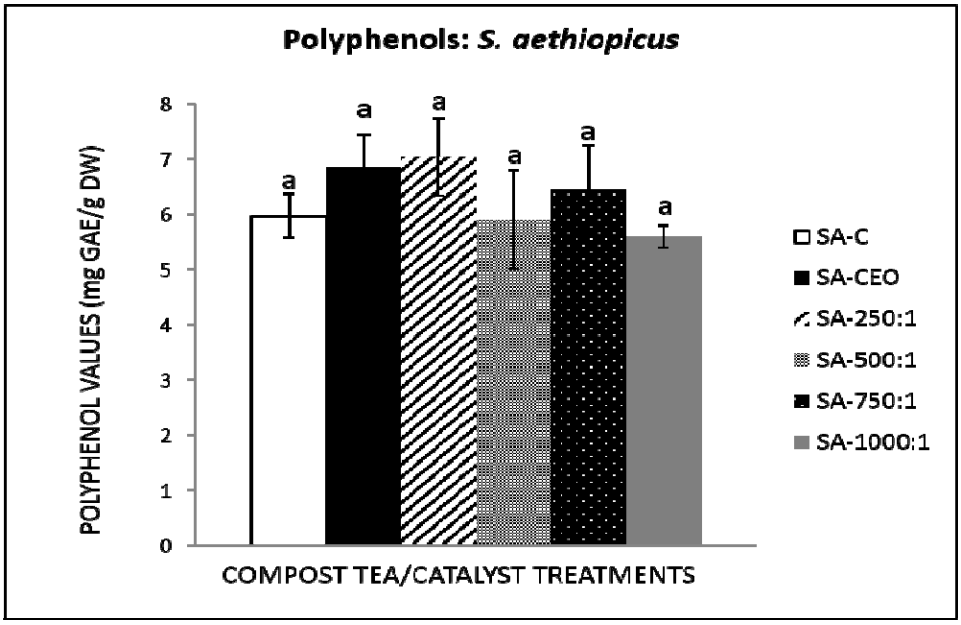

Figure 6.2 The total polyphenol (mg GAE/g dry weight) content of *S. aethiopicus* rhizomes. Values represent the means ± SD for the different treatments (n=4). Different treatments are compared to each other per plant species. The means of treatments with different letters on bars are significantly (P<0.05) different.

Flavonols

In *H. hemerocallidea* the total flavonol content of the underground corms revealed significant differences (P<0.05) between the Hypoxis Control (C) and the Control 1 (CEO) treatment with values of 6.19mg and 2.66mg QE, respectively. The T500:1 treatment was found to be significantly (P<0.05) lower than the T1000:1 and T750:1 treatments, with values of 2.8 mg, 7.94 mg and 7.82 mg QE, respectively. The lowest total flavonol content was found in the compost treatment with no catalyst (CEO). The CEO was significantly (P<0.05) lower, compared to the control (C), T1000:1, T750:1 and T250:1, with values of 2.66 mg, 6.19 mg, 7.94 mg, 7.82 mg and 5.08 mg QE, respectively (Figure 6.3).

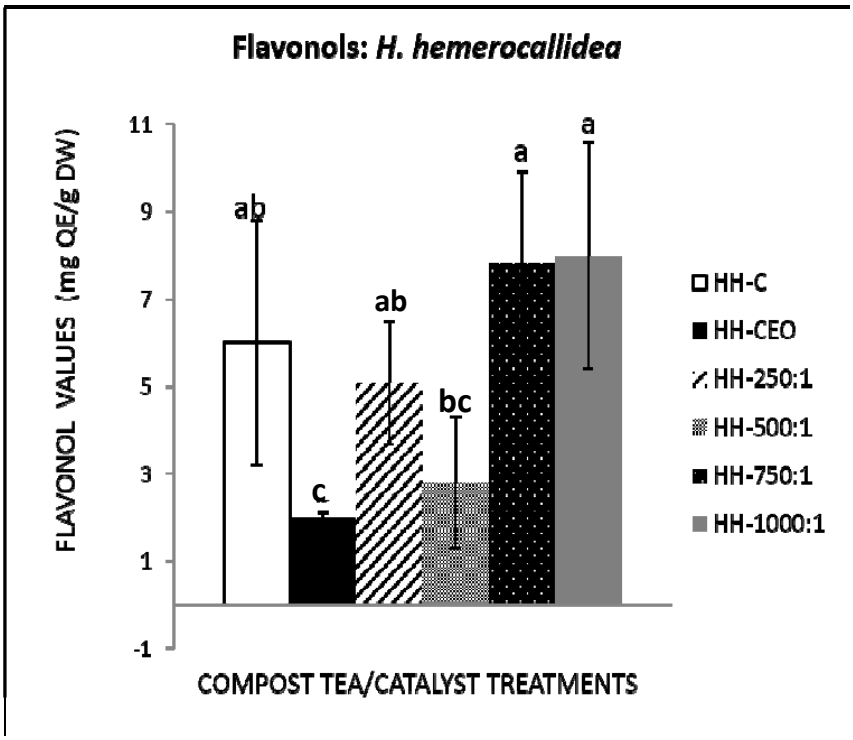

Figure 6.3 The total flavonol (mg QE/g dry weight) content of *H. hemerocallidea* corms. Values represent the means ± SD for the different treatments (n=4). Different treatments are compared to each other per plant species. The means of treatments with different letters on bars are significantly (P<0.05) different.

In *S. aethiopicus* the total flavonol content of the underground structures revealed significant differences (P<0.05) between the CEO and T250:1 compost tea treatments with the following values 4.06 mg and 2.62 mg, respectively. No significant differences were found in the control (C), T250:1, T500:1, T750:1 and T1000:1 compost tea treatments. The highest flavonol reading was found in the CEO treatment with a value of 4.06 mg (Figure 6.4).

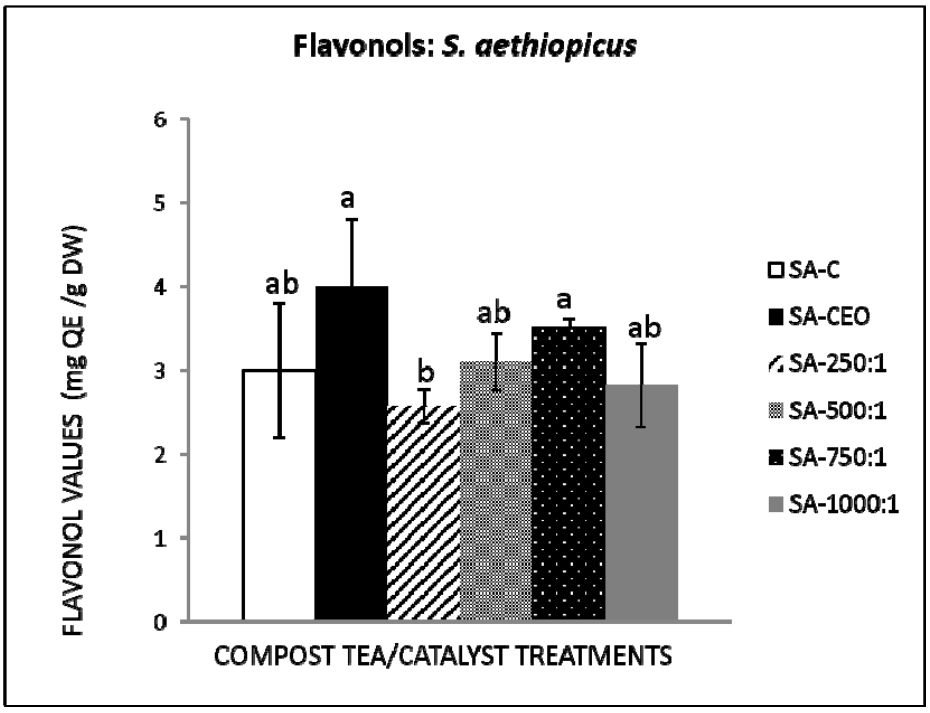

Figure 6.4 The total flavonol (mg QE/g dry weight) content of *S. aethiopicus* rhizomes. Values represent the means ± SD for the different treatments (n=4). Different treatments are compared to each other per plant species. The means of treatments with different letters on bars are significantly (P<0.05) different.

Flavanols

No Flavanols were detected in the *H. hemerocallidea* corms.

In *S. aethiopicus* the total flavanol content of the underground structures revealed a significant difference (P<0.05) between the T250:1 when compared to the T1000:1, T500:1 and CEO treatments with the following values: 1.06 mg, 1.42 mg and 1.9 mg, respectively. No significant differences were found in the flavanol content of the control (C), T250:1 and T750:1 compost tea treatments. The highest flavanol value (1.9 mg) was found in the T500:1 and the lowest value (1.06 mg) in the T250:1 compost tea treatment (Figure 6.5).

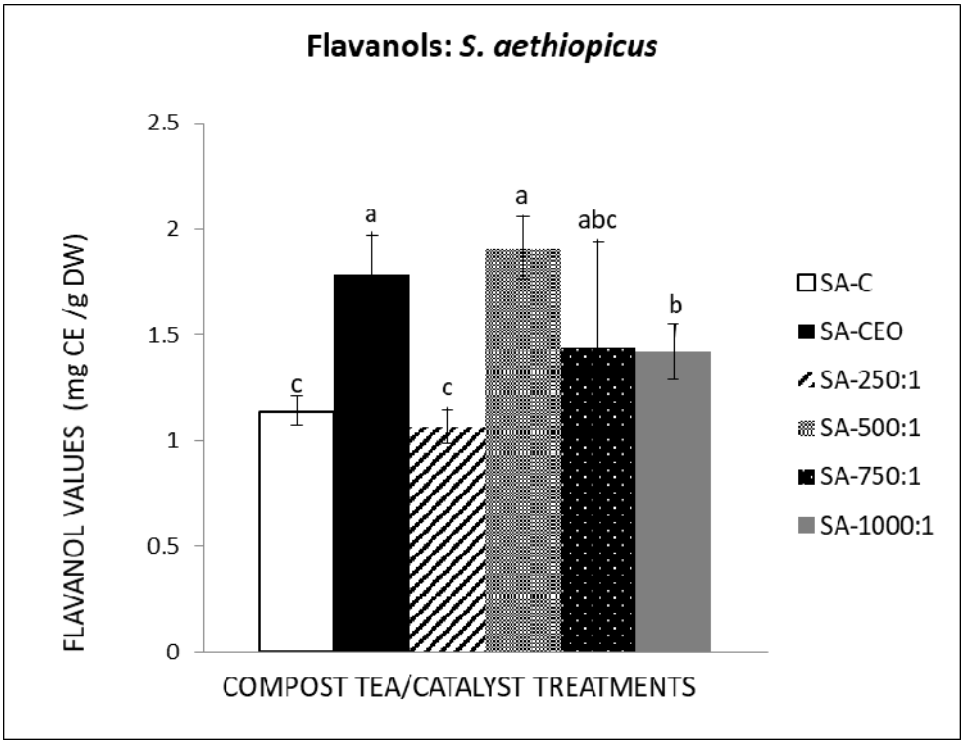

Figure 6.5 The total flavanol (mg QE/g dry weight) content of *S. aethiopicus* rhizomes. Values represent the means ± SD for the different treatments (n=4). Different treatments are compared to each other per plant species. The means of treatments with different letters on bars are significantly (P<0.05) different.

6.4.2 Antioxidant capacity

6.4.2.1 Ferric reducing antioxidant power (FRAP)

The FRAP value of the T1000:1 treatment in *H. hemerocallidea* was found to be significantly (P<0.05) higher when compared to the T500:1 with values of 349.30 and 199.84 µM AA/g respectively. The T1000:1 and T750:1 treatments showed the highest FRAP values followed by the T250:1 compost tea treatment. In general, there were no significant differences in the FRAP values for the Control (C), Control 1 (CEO), T250:1, T750:1 and T1000:1 compost tea treatments. The lowest FRAP reading (199.84 µM) was observed in the T500:1 compost tea treatment (Figure 6.6).

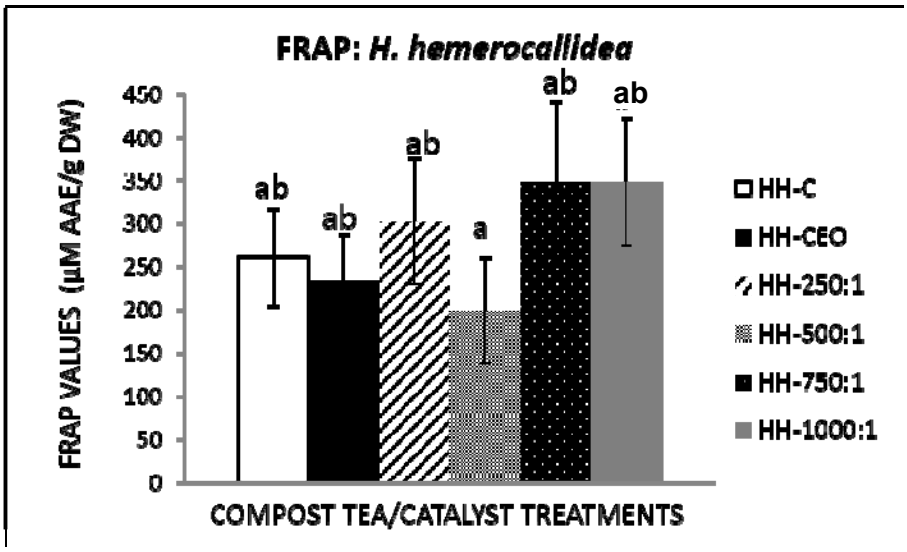

Figure 6.6 The total ferric reducing antioxidant power (FRAP) (µM AAE/g dry weight) of *H. hemerocallidea* corms. Values represent the means ± SD for the different treatments (n=4). Different treatments are compared to each other per plant species. The means of treatments with different letters on bars are significantly (P<0.05) different.

There were no significant differences in the FRAP values in the all the compost tea treatments for *S. aethiopicus*. FRAP values for the Control 1 (CEO), T250:1, T500:1 and T750:1 compost treatments were found to be similar and ranged from 28.22 to 29.18 µM. On the other hand, the FRAP values for the control (C) and T1000:1 treatments were similar but lower than the previously mentioned treatments and ranged from 24.85 to 25.55 µM AA/g, respectively (Figure 6.7).

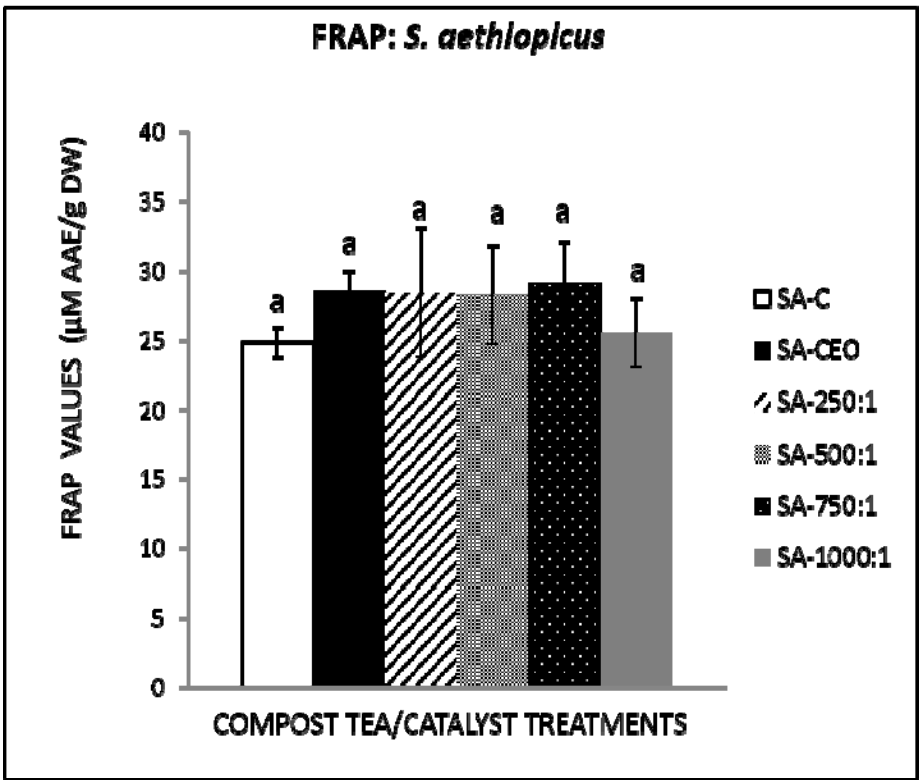

Figure 6.7 The total ferric reducing antioxidant power (FRAP) (µM AAE/g dry weight) of *S. aethiopicus* rhizomes. Values represent the means ± SD for the different treatments (n=4). Different treatments are compared to each other per plant species. The means of treatments with different letters on bars are significantly (P<0.05) different.

6.4.2.2 Oxygen radical absorbance capacity (ORAC)

The ORAC values for the compost tea treatments on *H. hemerocallidea* were found not to be significantly different with values ranging from 1330.72 to 2007.88 µM TE/g. The highest value amongst all the compost tea treatments were recorded in the T750:1 and the lowest in the T500:1 treatment (Fig. 6.8).

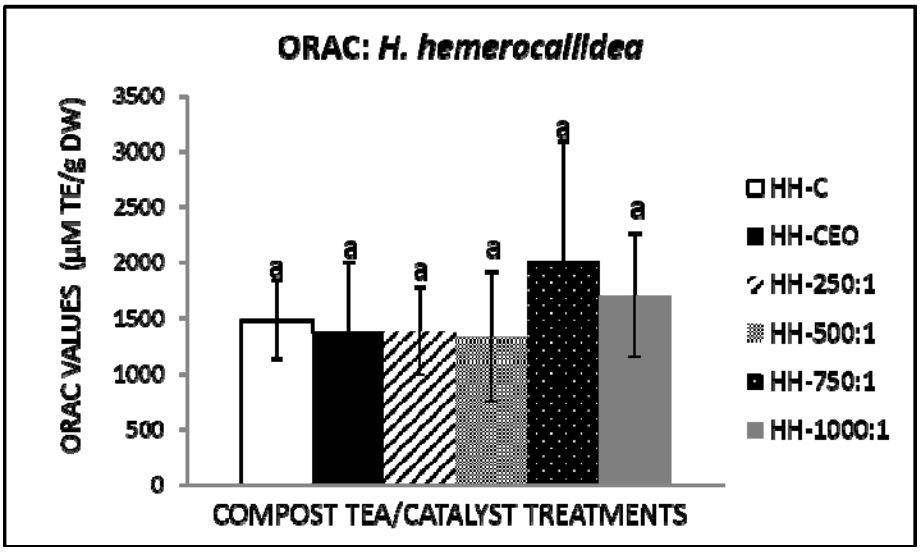

Figure 6.8 The total oxygen radical absorbance capacity (ORAC) (µM TE/g dry weight) of *H. hemerocallidea* corms. Values represent the means ± SD for the different treatments (n=4). Different treatments are compared to each other per plant species. The means of treatments with different letters on bars are significantly (P<0.05) different.

Similarly, for *S. aethiopicus,* there were no significant differences in the ORAC values across all compost tea treatments. The values ranged from 140.25 to 201 µM TE/g. The highest ORAC value (201 µmol) was recorded in the CEO and the lowest (140.25 µM) in the control (C) (Figure 6.9).

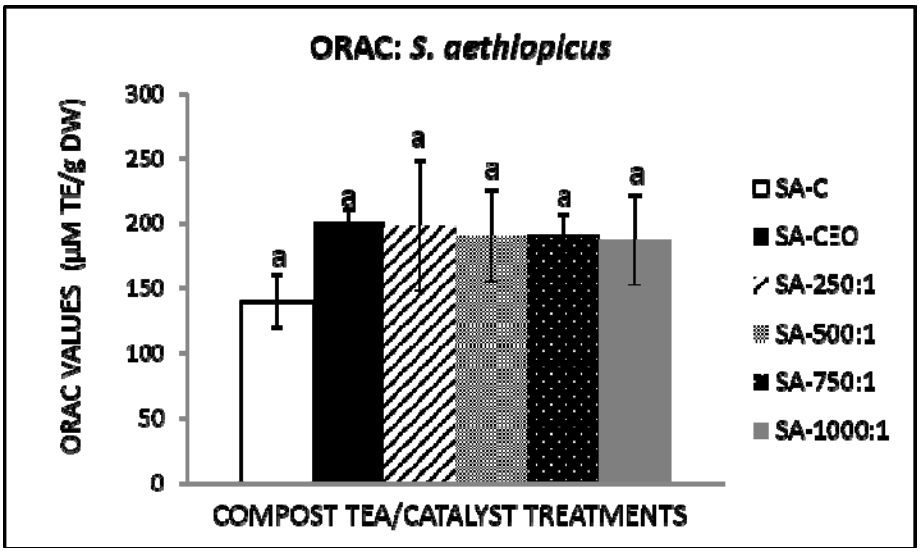

Figure 6.9 The total oxygen radical absorbance capacity (ORAC) (µM TE/g dry weight) of *S. aethiopicus* rhizomes. Values represent the means ± SD for the different treatments (n=4). Different treatments are compared to each other per plant species. The means of treatments with different letters on bars are significantly (P<0.05) different.

6.4.2.3 2,2′-azino-di-3-ethylbenzthialozine sulphonate (ABTS)

The ABTS values of *H. hemerocallidea* were found to be significantly (P<0.001) higher in the T1000:1 treatment compared to the T500:1 treatment with values of 274.91 and 177.45 µM, respectively. Furthermore, there were no significant differences amongst all the other treatments. The lowest value (177.45 µM) was recorded in the T500:1 treatment and the highest value (274.91 µM) in the1000:1 compost tea treatment (Figure 6.10).

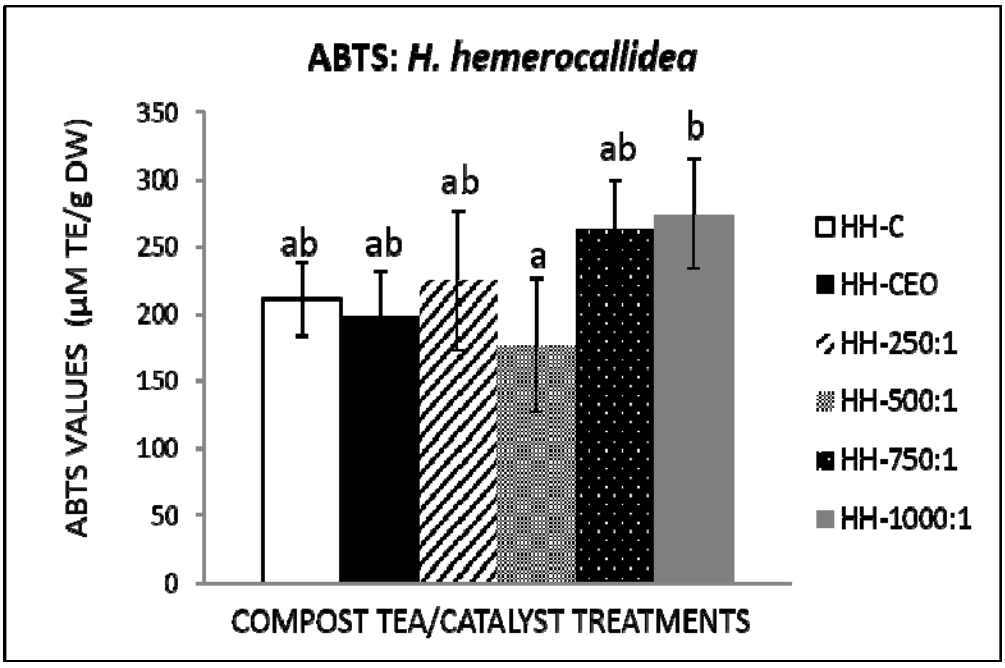

Figure 6.10 The total ABTS radical cation scavenging ability (µM TE/g dry weight) of *H. hemerocallidea* corms. Values represent the means ± SD for the different treatments (n=4). Different treatments are compared to each other per plant species. The means of treatments with different letters on bars are significantly (P<0.05) different.

The ABTS values of *S. aethiopicus* rhizomes were found to be not significantly different amongst all the compost tea treatments. The Control 1 (CEO) and T250:1 showed similar values of 33.71 and 33.80 µM, respectively. The lowest ABTS value (28.99 µM) was recorded in the T750:1 treatment and the highest (33.80 µM) in the CEO compost tea treatment (Figure 6.11).

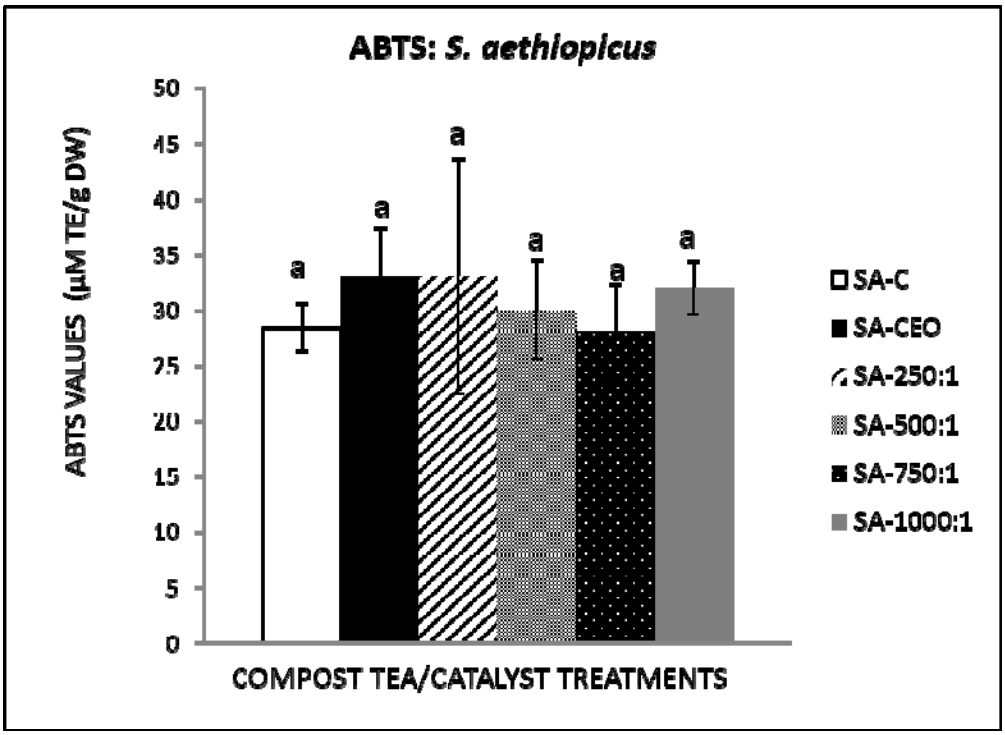

Figure 6.11 The total ABTS radical cation scavenging ability (µM TE/g dry weight) of *S. aethiopicus* rhizomes. Values represent the means ± SD for the different treatments (n=4). Different treatments are compared to each other per plant species. The means of treatments with different letters on bars are significantly (P<0.05) different.

6.5 DISCUSSION

A study on the effect of compost tea extracts on the antioxidant activity of these bulbous plants has never been conducted before. Thus, a direct comparison to similar studies cannot be made due to unavailable literature, and therefore what is seen as good antioxidant activity is difficult to describe. The different antioxidant test methods further complicate studies in general. This study will make comparisons to antioxidant studies on other plant species where similar plant structures were used as the basis of their research. In Lilium bulbs, the total phenolic (Jin *et al*. 2012), flavonoid and flavonol content was higher when compared to the Hypoxis and Siphonochilus. Sultana & Anwar (2008), conducted a flavonol (kaempeferol, quercetin and myricetin) study on vegetables, fruit and other medicinal plants, revealing that spinach and cauliflower, as leafy vegetables had a higher flavonol content, whilst the medicinal plants such as *Aloe vera* and moringa leaves, contained a higher flavonol content in this category. The flavonol content was higher when compared to the Hypoxis and Siphonochilus.

The total phenolic content and antioxidant activity were found to be higher overall in the leaves (due to the exposure to sunlight) than the rhizomes of 14 ginger species. The rhizomes of *Zingiber officinale* had readings of 157 mg GAE/100 mg and 84 mg AA/100 mg. (Chan *et al.,* 2008). These levels were higher when compared to the Hypoxis and Siphonochilus.

Twelve onion cultivars', which were evaluated for fresh bulb yields and flavonoid content showed the highest total flavonoids (quercetin) in the red 'Tropea rossa' variety with 557.8 mg QE kg-1. Previous research has indicated that the colour of onion bulbs is related to quercetin presence (Marotti & Piccaglia, 2002). This level was higher when compared to the Hypoxis and Siphonochilus. Makris & Rossiter (2001) conducted research of the chopping verses the boiling of onion bulbs and revealed that chopping yielded better/higher flavonol content than boiling the onion bulbs. The flavonol contents were higher when compared to the Hypoxis and Siphonochilus.

Nuutila *et al*. (2003) compared the antioxidant activities of onion and garlic extracts on rat hepatocytes, revealing that red onions and red spring onions have a higher quercetin content, and the skin extract in particular, have a higher radical scavenging activity than garlic. The flavonol contents were higher when compared to the Hypoxis and Siphonochilus.

A study conducted on *Allium cepa*, revealed higher total flavonoid content and FRAP (ferric reducing antioxidant power) capacity values (Sulaiman *et al*., 2011) when compared to the Hypoxis and Siphonochilus values. Fresh fennel bulbs have higher antioxidant activity (Rawson *et al.*, 2011), when compared to the Hypoxis and Siphonochilus values. *Gethyllis multifolia* and *G. villosa* flowers and fruit revealed higher ORAC, FRAP and polyphenol levels than that of *S. aethiopicus*. However, *H. hemerocallidea* has higher levels of ORAC, FRAP, ABTS, polyphenols, and flavonols when compared to both *Gethyllis* species (Daniels *et al.,* 2011).

In *H. hemerocallidea* corms the flavonol content of the underground corms revealed that the T1000:1 and T750:1 were the most effective compost tea treatments. In *S. aethiopicus* rhizomes, the flavonol content of the underground rhizomes revealed that the compost tea extract with no catalyst (CEO) and the T750:1 compost tea treatment were the most effective.

No flavanols were found in the Hypoxis corms. In *S. aethiopicus* the flavanol content of the underground rhizomes was increased the highest in the T500:1 treatment and the CEO compost tea extract (Figure 6.5).

In *H. hemerocallidea* underground corms, the highest FRAP value was observed in the T1000:1 followed by the T750:1 treatment (Figure 6.6). The compost tea treatments had no definite significant effect on the FRAP value for *S. aethiopicus* (Figure 6.7). In both species the compost tea treatments had no significant effect on the ORAC of the underground storage organs (Figures 6.8 and 6.9). The highest significant ABTS value of *H. hemerocallidea* was observed in the T1000:1 treatment, but revealed no significant differences amongst all treatments for *S. aethiopicus* (Figures 6.10 and 6.11 respectively).

6.6. CONCLUSION

It is clear that both *H. hemerocallidea* and *S. aethiopicus* continue to decline due to overharvesting from their natural habitat and that the demand for their medicinal value exceeds the supply especially amongst African people (Cunningham, 1993, Williams *et al*., 2008). While both species are slow-growing, it becomes important to increase production with improvement of cultivation requirements. Although significant differences were discovered between treatments of both species, both the content and activity levels are lower when compare to other vegetables and bulb species. Similarly, growing both medicinal species organically and adding compost tea extract enhanced the growth over time and therefore could enhance both species qualities for medicinal use and thereby eliminate the need to use chemical fertilizers. The objective to investigate the antioxidant capacity and content of *H. hemerocallidea* and *S. aethiopicus* and was to record a standard compost tea extract to enhance /improve the antioxidant capacity and content of both these endangered species.

Based on the results above it is clear that Hypoxis corms have a higher antioxidant content and capacity when compared to Siphonochilus rhizomes, even though it was not intended to compare the two species due to the variety of differences between each other. Both species have complex physiological structures, the one being a corm, the other being a rhizome, and as such grow and yield differently. The complex physiological plant structures of both species contributed to the various antioxidant values, as well as the type of compost tea, and its source, could also have impacted on the results. With the current trends toward growing plants organically, many studies have focussed on the cultivation of a variety of ornamental and edible crops. Documentation remains limited on the application of compost tea on the cultivation of medicinal species. Further research projects specifically focusing on compost tea types using South African soils is recommended.

This study therefore gave insight into the growth habits and compost tea treatments of two complex plant species. It was therefore worthwhile to establish the effect and rate of application of mushroom compost tea extracts for future cultivation of *H. hemerocallidea* and *S. aethiopicus* to gain a better understanding of the effect of compost teas on the cultivation and survival of these important species. Although the species received the same dosage on the same day and time, they responded differently and subsequently yielded different results. This was expected as the two species have different root storage organs, namely Hypoxis corms vs. Siphonochilus rhizomes. Based on the results above it is recommended that a full strength mushroom compost tea be utilised to, in general, increase the antioxidant activity of *H. hemerocallidea* and *S. aethiopicus.*

6.7 Acknowledgements

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Chapter Seven

Conclusion and recommendations

This study aimed to investigate the potential effects of compost tea extracts on growth, nutritional value, antioxidant content and capacity and soil quality on the cultivation of *H. hemerocallidea* and *S. aethiopicus* under soil drench applications.

Chapter one describes and highlights the research problem, the hypotheses tested, the objectives, which guided the study and highlights the overview of the chapters.

In chapter two, the potential effects of organic compost tea extracts on the growth and medicinal potential of *H. hemerocallidea* and *S. aethiopicus* were investigated. From the background it was clear that both species have important medicinal potential for a traditional medicinal market in South Africa. Both these species are listed as endangered species in the Red list of endangered species for South Africa. Their populations continue to decline due to overharvesting from their natural habitat as the demand for their medicinal use exceeds the supply. Considering the above, this review recommended that there is a need for the advancement of improving cultivation techniques to increase commercial production. Little evidence was found that these species are cultivated commercially. Similarly, growing medicinal species organically could enhance their product quality for medicinal commercialisation and reduce the amount of chemical fertilizers being used. As found with other species, organic cultivation of these species could lead possibly to increase their yield, nutritional content and antioxidant values as these responses are controlled by essential growth promoting substances in the growth media. This potential cultivation is also in line with current trends toward growing plants organically as many studies have focussed on the cultivation of a variety of ornamental and edible crops. It was also found that studies on the application and use of compost tea extracts on medicinal species are limited. This chapter therefore emphasised that further research studies will not only gain a better understanding on the effect of compost tea but also to enhance the future cultivation potential for *H. hemerocallidea* and *S. aethiopicus* and contribute to their survival in their natural habitat.

Chapter three evaluated the effects of mushroom compost tea extracts on the growth of *H. hemerocallidea* and *S. aethiopicus* under different soil drench applications to improve their cultivation as traditional medicinal species. This study reported not only that both species are in decline due to overharvesting, but also found to be slow growing which could contribute to their scarcity. For these reasons improving their cultivation techniques become a priority. Compost teas were carefully prepared from mushroom compost, applied while growth was

monitored and variables measured. The study however found no significant difference in the growth variables of the species during the experimental period. Due to the possible slow growth rate of both species as reported early, plants did not respond significantly to the treatments. There was however a significant interaction between time (weeks) vs. treatment over the twenty-week period. The different dosages of CTE influenced the growth significantly over time. Therefore, mushroom compost tea could be recommended as an organic alternative to chemically based fertilizers for *H. hemerocallidea* and *S. aethiopicus.* It is however important that future studies be encouraged and undertaken with an extended growing period over two growing seasons or longer. As some positive growth signs were visible, only further research can establish a true rate of application and a better understanding of the effect of mushroom compost tea extracts on the cultivation of *H. hemerocallidea* and *S. aethiopicus.*

In chapter four the focus was on the effects of mushroom compost tea extract on chlorophyll content in *H. hemerocallidea* and *S. aethiopicus*. The importance of chlorophyll was stressed to determine the effectiveness of the CTE treatments on these two endangered plant species. Even though plants were exposed to various levels of CTE concentrations, both species exhibited no significant difference in chlorophyll production at the apical leaves. The leaves were slow to emerge but did show a change in greening visual observed and measured according to the Linkert scale. It was possible that the bulk of the CTE was absorbed into the Hypoxis corms and Siphonochilus rhizomes. The Siphonochilus chlorophyll readings across all the treatment groups were found to be non-significant between treatments. Siphonochilus was reported being slow growing and did increase in the chlorophyll levels towards the end of the experiment period, across all treatments. There was a significant interaction between time (weeks) vs. treatment over the twenty weeks, and the dosages of CTE did significantly influenced the growth in the rhizomes and subsequently the new shoot formation, over time. The study supported the application and use of CTE extract on medicinal species such as *H. hemerocallidea* and *S. aethiopicus* and therefore recommend further studies to be evaluated over longer growth periods and possibly more frequent applications of CTE.

Chapter five evaluated the effects of mushroom compost tea extracts on weight, soil media nutrient and mineral elements on the growth of *H. hemerocallidea* and *S. aethiopicus.* Nutrient content increased in this study in soil and dried plant parts compared to mushroom compost nutrient levels. Apparently no previous studies on nutrient levels were found on these two species. Several nutrient elements such as boron, zinc, copper, iron, manganese, magnesium and potassium increased in both species. The CTE had an effect on the nutrient levels within different concentrations and within the two species as both species grew over time. In spite of these nutrient variances mushroom compost tea is recommended as an organic fertilizer. Further research is recommended to determine the nutrient levels within the

corms of *H. hemerocallidea* and rhizomes of *S. aethiopicus*, how and when the plant species absorbs nutrients and where it will be stored within the plant parts. Using organic CTE can benefit the cultivation of medicinal species in future use of traditional medical remedies and pharmacological medicines.

Chapter six tested the effect of compost tea extracts on the antioxidant capacity and content of *H. hemerocallidea* and *S. aethiopicus*. This study reported that it has been well documented that both species continue to decline due to overharvesting from their natural habitat and that the demand for their medicinal value exceeds the supply especially amongst African people. Although significant differences were reported between treatments of both species, both the content and activity levels were lower when compared to other vegetables and bulb species. Similarly, growing both medicinal species organically and adding CTE enhanced the growth over time and therefore could enhance the quality for medicinal use of both species thereby eliminating the need for chemical fertilizers. Although the objective of this study was to investigate the antioxidant capacity and content of *H. hemerocallidea* and *S. aethiopicus* in response CTE applications, both the species have complex physiological structures, one being a corm, and the other being a rhizome, and as such grow, and yield differently. These plant structures could have contributed to the various antioxidant values measured. Based on these results *Hypoxis* corms had a higher antioxidant content and capacity when compared with *Siphonochilus* rhizomes, even though it was not intended to compare the two species due to the varietal differences between the two. With the current trends toward growing plants organically and while some studies have focused on a variety of ornamental and edible crops, documentation remains limited on the application of compost tea on the cultivation of medicinal species. Further research specifically focusing on compost tea types and possibly on various soil types is recommended.

In conclusion, this study gave new insight, and set a baseline into two important and complex medicinal species, *H. hemerocallidea* and *S. aethiopicus* and their growth responses to various concentrations of CTE. Due to the limited plant availability; endangerment of the species, both species having complex physiology, and having to work with non-uniform material presented quite a challenge. It is recommended that future studies be conducted over two full growth seasons. Although the species received the same dosages, they responded differently and subsequently yielded different results. The fact that both species are slow growing, have complex physiology, have a short growth period and that both have complex root storage organs, Hypoxis being a corm and Siphonochilus a rhizome respectively, emphasized that it was necessary to gain a better understanding of their growth responses to compost tea extracts. It was therefore worthwhile to establish the effect and rate of application of mushroom compost tea extracts on the cultivation of *H. hemerocallidea*

and *S. aethiopicus.* Based on these results, a full strength mushroom CTE is recommended for *H. hemerocallidea* and *S. aethiopicus* to support their cultivation and survival as indigenous and medicinal species in South Africa.

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Chapter Eight

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