PROPAGATING SOME COMMONLY-USED SOUTH AFRICAN MEDICINAL PLANTS WITH COMPOST AND VERMITEA

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PROPAGATING SOME COMMONLY-USED SOUTH AFRICAN MEDICINAL PLANTS WITH COMPOST AND VERMITEA

by

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DECLARATION

I, Steven Craig Faulconbridge, 20502042, hereby declare that the dissertation for *Magister Technologiae* is my own work and that it has not previously been submitted for assessment or completion of any postgraduate qualification to another University or for another qualification.

Steven Craig Faulconbridge

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ABSTRACT

The use of many of South Africa's medicinal plants has shown marked increase with over 27 million users in South Africa alone. Most plants are still being unsustainably wild-harvested, a major concern for biodiversity conservation. Commercial interest in certain more commonly-used species has increased, with potential to cultivate medicinal plants on a more sustainable basis. Focus has shifted from conventional use of synthetic fertilisers, pesticides and fungicides to more organic methods of plant propagation. Aqueous extract derived from earthworm composted food waste (vermitea) was used to study the germination and rooting success of selected species. Also survival and growth performance of selected plants grown in a medium amended with commercial NPK fertiliser was compared to those grown in the same medium amended with compost and to those grown in the same medium amended with compost with weekly applications of vermitea. No change in germination success was noted. Vermitea showed promising results on the rooting of cuttings. The application of NPK improved growth performance (biomass) significantly for all species tested. However, they had lower root:shoot ratios as well as lower survival rates compared to plants under the compost and compost/vermitea treatments. The improved survival of these plants highlights the potential of these organic treatments on the propagation of selected medicinal plants.

Key words: Compost, fertiliser, vermitea, medicinal plant cultivation, *Cyclopia genistoides, Pelargonium citronellum, Artemisia afra, Lessertia frutescens*.

CHAPTER 1 – GENERAL INTRODUCTION

1.1 Rationale and scope for the study

In recent years there has been an increase in scientific and commercial interest in the flora of South Africa (Van Wyk & Viljoen 2011) due to an ever-increasing demand for medicinal plants (Ndhlala *et al.* 2011). South Africa has seen a rapid increase in the research and development of these resources (Makunga *et al.* 2008; Gericke 2011). However, there are concerns for the sustainability of the raw materials supplied (Ndhlala *et al.* 2011). Subsequently, the need to protect and conserve the country's rich biological diversity has been noted (Gericke 2011).

> *'With unsurpassed botanical diversity, Southern Africa holds natural resources of global significance'* (Smith *et al.* 1996).

The National Environmental Management: Biodiversity Act or NEMBA (Act 10 of 2004) makes provision for the management and conservation of South Africa's biodiversity (NEMBA 2004). The primary focus of NEMBA is the protection of species and ecosystems under threat. NEMBA brings attention to the sustainable use and equal sharing of the benefits derived from 'bioprospecting', where it concerns these indigenous biological resources (Gericke 2011).

As defined by NEMBA (2004), 'bioprospecting' relates to research and development on indigenous biological resources or to the application of these resources for commercial or industrial use. Currently, many of the activities associated with the South African medicinal trade are in direct contravention of NEMBA (Ndhlala *et al.* 2011).

The National Research Foundation (NRF) initiated both the Indigenous Knowledge Systems research initiative and the Indigenous Plant Use forum, which put the focus on medicinal plant research in South Africa (Van Wyk & Viljoen 2011). Funding has been made available from state-funded organisations – such as the Department of Health, Medical Research Council, Agricultural Research Council (ARC) and NRF – to expand further on current research into the cultivation and development of important species (Ndhlala *et al.* 2011). Importantly, however, collaboration should be encouraged between researchers and universities working in this field (Gericke 2011).

This study forms part of a project funded by NRF to improve the propagation potential of four selected medicinal plant species. The four species studied, which are all endemic to South Africa, are *Lessertia (Sutherlandia) frutescens* (L.) R. Br. (Fabaceae); *Artemisia afra* (Asteraceae); *Cyclopia genistoides* (L) R. Br. var. *genistoides* (Fabaceae) and *Pelargonium citronellum* J. J. A. van der Walt (Geraniaceae)(Goldblatt & Manning 2000). These species have been chosen because they have a wide range of medicinal value and use in South Africa (Watt & Breyer-Brandwijk 1962; Van Wyk *et al.* 1997; Van Wyk & Gericke 2000), are relatively fast growing and harvesting focuses on the leaves and stems of the plant.

All of these species have already been partly or fully developed as commercial crops and products (Van Wyk 2011), with increased cultivation of *L. frutescens* (Van Wyk & Albrecht 2008) and *Cyclopia* species (honeybush) (Joubert *et al.* 2011) over the last 10-15 years. Furthermore, the extraction of essential oils from the leaves of certain *Pelargonium* species has led to the commercial exploitation of these species for the cosmetic, perfumery and pharmaceutical industries (Lalli *et al.* 2008). Gericke (2011) has highlighted two important points with regard to medicinal plant research. Firstly, selected species should either be very abundant in the wild, which will allow for sustainable harvesting, or they should show suitable characteristics for commercial propagation. For example, the majority of research carried out on *Sceletium tortuosum* N.E.Br. has used cultivated stock plants only (Gericke 2011).

The ARC and the South African National Botanical Institute (SANBI) have approached the propagation of the *Cyclopia* species from a nature conservation standpoint, focusing on the sustainability of the product (Joubert *et al.* 2011) as the majority of these are plants being wild-harvested (McKay & Blumberg 2007). *Cyclopia* species have attracted the attention of Cape Farmers (Van Wyk & Gericke 2000), where the use of organic production principles has been recommended due to their use as a health drink (Joubert *et al.* 2011). Organic amendments show great potential in increasing the nutritional quality as well as the antioxidant activity of plants intended for the medicinal and food industries (Theunissen *et al.* 2010).

These recommendations gave rise to the question: ‘Can a more practical, sustainable and environmentally-friendly propagation technology be implemented for medicinal plant propagation in South Africa?’ This project aimed to highlight the

advantages of using more organic and sustainable methods, which would utilise a local waste resource for the propagation of plants intended for the medicinal trade.

This study compared these four species through both seed and cutting propagation under three treatments: (i) growing medium with slow release fertiliser added (conventional [synthetic fertiliser] cultivation/propagation practice) (F); (ii) growing medium with compost added (C), and (iii) growing medium with compost and additional weekly vermitea applications (V).

1.2 Research question

What would be a practical, cost effective, environmentally-friendly and innovative propagation method of commonly used South African medicinal plants? The aim would be to further investigate the effectiveness of compost on plant performance (survival and growth) of selected South African medicinal plants, and to assess any additional effects of vermitea.

Objectives

Experiment 1:

(1) To determine whether the application of vermitea to the homogenous rooting medium (coconut coir) can improve seed germination, compared to no treatment;

(2) To determine whether the application of vermitea to the homogenous rooting medium (coconut coir) can improve cutting strike rate/rooting success compared to no treatment.

Hyp$_1$: There is a significant difference in germination/rooting between plants treated with vermitea and those receiving no treatment.

Experiment 2:

(3) To compare plant performance (survival and growth) of seedlings and cuttings grown with: (i) slow-release fertiliser (F); (ii) compost (C) and (iii) compost receiving vermitea treatments (V).

The fertiliser (F) propagation method is a common practice in the propagation of many plants in the nursery/horticultural industry, and can therefore be seen as the standard fertility practice for Experiment 2 of this study. Plant performance for

Experiment 2 will be quantified by overall plant survival, and by dry root biomass, dry shoot biomass and dry root: shoot ratios per species per treatment.

Hyp$_2$: There is a significant difference in plant performance (survival/growth) between treatments.

CHAPTER 2 – LITERATURE REVIEW

2.1 Exploitation of medicinal plants

Globally, the demand for medicinal plants and their remedies has been on the increase, with approximately 80% of developing countries dependent on these for health care (Mander 1998; Canter *et al.* 2005; McKay & Blumberg 2007). Herbal medicines are those derived from the leaves, roots, fruit or bark of a plant (Cocks & Møller 2002; Oloyede 2011) and are still used by many South Africans for primary healthcare and well-being (Cunningham 1988; Mander 1998; Cocks & Møller 2002; Oloyede 2011).

Traditional medicines hold high cultural value, with over 27 million users of traditional medicines in South Africa alone. This multi-million Rand industry has been well documented in the literature (Williams *et al.* 1997; Mander 1998; Williams *et al.* 2000; Dold & Cocks 2002; Hamilton 2003; Mander & Le Breton 2005; Mander & McKenzie 2005; Mander *et al.* 2006) and contributes to the livelihood of many families (Berry *et al.* 1994; Hamilton 2003; Botha *et al.* 2004a; Botha *et al.* 2004b; Mander & McKenzie 2005; Mander *et al.* 2006).

The increasing demand for medicinal plants in South Africa was reported in the 1980s (Cunningham 1989) and through to the late 1990s (Williams *et al.* 1997; Mander 1998), with the trend noticeably increasing across the country (Williams *et al.* 2000; Cocks & Møller 2002; Dold & Cocks 2002; Hamilton 2003; Botha *et al.* 2004a; Botha *et al.* 2004b; Canter *et al.* 2005; Williams *et al.* 2007; Makunga *et al.* 2008; Van Wyk 2011). As a result, many medicinal plants are becoming increasingly scarce in their natural habitats (Cunningham 1997; Botha *et al.* 2004a; Botha *et al.* 2004b). Most medicinal plants are still being harvested from the wild (Geldenhuys 2004a; Canter *et al.* 2005) in an unsustainable manner (Cunningham 1997; Mander *et al.* 2006), which results in major concerns for biodiversity (Dold & Cocks 2002).

Overexploitation caused by uncontrolled harvesting makes this one of the most complex aspects of resource management facing conservation authorities (Cunningham 1997; Botha *et al.* 2004b; Geldenhuys 2004b), health care professionals (Cunningham 1997) and resource users in the country (Cunningham 1997; Dold & Cocks 2002). Increased legislative controls and the development of

more sustainable harvesting methods have been largely unsuccessful in protecting medicinal plants in general (Dold & Cocks 2002; Botha *et al.* 2004a).

Cultivation seems to offer the only long-term alternative for the many species in the medicinal trade (Dold & Cocks 2002; Botha *et al.* 2004b; Canter *et al.* 2005); especially high-demand and conservation-priority species (Cunningham 1997). Being dependent on plants, humans are therefore dependent on the ability of plants to be propagated (Beyl & Trigiano 2008). However, there are only a few cultivation programmes currently in place in South Africa (Geldenhuys 2004a; Mander *et al.* 2006) and few African models exist of successful cultivation projects for medicinal plants (Dold & Cocks 2002).

2.2 Conventional - organic plant propagation

There is a large volume of literature available on the successful propagation of plants by either sexual or asexual methods (Van Wyk 1994; Beyl 2008a; Beyl & Trigiano 2008; Hoover 2008). These authors highlight the importance of providing the proper environment with regard to adequate water, oxygen, temperature and the correct range of pH and light intensity (Beyl & Trigiano 2008; Chong *et al.* 2008; Hoover 2008; Klingman 2008a; Tignor 2008). However, success lies in the selection of an appropriate growing medium which provides a good balance of the physiological and chemical properties required for plant metabolic processes (Chong 2008; Chong *et al.* 2008).

Over the last 25 years much propagation success has been recorded using various combinations of inorganic (soil-less) and organic growing media for both indoor and outdoor propagation (Chong 2008). In this regard, the ideal growing medium must provide support for anchorage while being friable enough to allow for root penetration, adequate aeration and drainage (Grey *et al.* 1994; Chong 2008; Holloway 2008). Importantly, the growing medium must also provide a non-toxic, pest- and disease-free environment for root initiation (Chong 2008; Ruter 2008). The presence of pests and/or disease pathogens can be very destructive (Stapleton 2008), affecting overall plant performance negatively (Donald *et al.* 1994; Windham 2008).

Proper sanitisation of the growing media (Donald *et al.* 1994; McQuilken 2008; Windham 2008), combined with the addition of fertilisers and pesticides aimed at

improving plant performance, has been a widely accepted practice in horticultural propagation (Donald *et al.* 1994; Klingman 2008b; McQuilken 2008; Stapleton 2008) as well as conventional agricultural practices (Fageria 2002). Grouped together, these conventional practices are, however, proving to be environmentally unsustainable due to the complete removal of beneficial soil microorganisms and natural pest enemies (Esterhuyse 1994; Klingman 2008b), ground water pollution (Zandonadi & Busato 2012) and human and wildlife health concerns (Siddiqui *et al.* 2008).

When planning a propagation project for medicinal plants intended for human use, propagators and plant growers need to take into account not only the financial implications (Donald *et al.* 1994; Ruter 2008), but also the health (Botha *et al*. 2004a) and environmental impacts (Esterhuyse 1994; Klingman 2008b) of their business on the end user and the environment respectively.

2.3 Cultivation (propagation) of medicinal plants

There is a cultural belief, supported by science (Botha *et al.* 2004b; Oloyede 2011), that plants harvested from the wild for *muthi* (medicine) are more effective than cultivated ones, and are thus used in preference (Berry *et al.* 1994; Mander *et al.* 2006). As this trend continues to increase, it is not possible to use medicinal plants on a large scale without impacting on local wild populations (Dold & Cocks 2002; Canter *et al.* 2005). A number of South African studies have reported that the majority of the local urban traditional healers, traders and vendors have broken ties with some traditional practices and would readily accept cultivated plants (Cunningham 1997; Dold & Cocks 2002; Botha *et al.* 2004b; Geldenhuys 2004b).

Through cultivation an attempt can be made to use species in demand more sustainably (Cunningham 1997; Dold & Cocks 2002; Canter *et al.* 2005), as plants can be seen as a renewable resource that can be replenished over time (Esterhuyse 1994). Mass propagation of plants through cuttings has been used for easy-to-root species (Van Wyk 1994), and is the most common form of propagation for herbaceous plants (Ruter 2008). Cuttings have been particularly important in the conservation of rare and endangered plants (Beyl & Trigiano 2008). In Kwa-Zulu Natal, Botha *et al.* (2004b) detail success with the use of shoot tip cuttings of the endangered medicinal plant *Warburgia salutaris* (Bertol.f.) Chiov. (Canellaceae).

The conservation of wild populations can ensure the provision of seed and cutting material for propagation programmes (Cunningham 1997), providing good motivation for the development of small-scale farming and village gardens for species in high demand (Geldenhuys 2000; Cunningham *et al.* 2002; Dold & Cocks 2002; Geldenhuys 2004a; 2004b; Geldenhuys & Delvaux 2007).

Rural cultivation potentially offers considerable social and economic benefits (Botha *et al.* 2004b). However, there is a lack of rural tree planting culture in South Africa (Geldenhuys 2004b); rural nurseries usually lack the necessary funding, skills and sophistication of commercial nurseries (Donald *et al.* 1994). There is an opportunity to promote grass-roots nurseries (Van der Zel 1994) to help local users, once adequately trained, to cultivate local resources (Botha *et al.* 2004b; Geldenhuys 2004a; 2004b).

Plant propagation must be efficient and reliable, and the aim should be to improve plant performance or rectify problems (Compton 2008). Due to the numerous negative effects on the environment and human health (Avis 2007; Siddiqui *et al.* 2008; Zandonadi & Busato 2012), focus has shifted from conventional applications of synthetic (inorganic) fertilisers, pesticides and fungicides to more organic methods of maintaining soil fertility, plant production, and also pest and disease management (Lotter 2008; Litterick *et al.* 2010; Siddiqui *et al.* 2011; Zandonadi & Busato 2012).

Many years of ongoing research have highlighted a number of economic and ecological advantages of organic material additions to the soil (Jenkinson & Rayner 1977; Zhang *et al.* 1996; Zhang *et al.* 1998; Stolze *et al.* 2000; Lotter 2008; Zandonadi & Busato 2012). Consequently, there is a growing global trend to follow more environmentally-friendly practices of plant propagation in horticulture (Chong 2008; Klingman 2008b; Lazcano *et al.* 2009), agriculture (Lazcano & Domínguez 2011; Zandonadi & Busato 2012) and even forestry (Arriagada *et al.* 2009; Lazcano *et al.*2010a; 2010b; Kandari *et al.* 2011).

With increasing populations worldwide (Holdren & Ehrlich 1974; Meyer & Turner 1992), there has been an emerging need for ’innovation and green/eco-friendly technologies of organic waste management’ (Zandonadi & Busato 2012). Organic amendments sourced from local input (waste) materials have been regarded as viable (St Martin & Brathwaite 2012) and eco-friendly (Edwards 1988 in Zandonadi &

Busato 2012), but are also cost-effective organic fertiliser options (Litterick *et al.* 2010).

2.4 Benefits of organic material (compost and compost tea) additions to the soil/growing medium

> *'Soil quality has largely been defined by soil function and represents a composite of its physical, chemical and biological properties that provide a medium for plant growth...*' (Fageria 2002).

Historically, applications of composts and manures to the soil in either solid or liquid (extracts or teas) form were used as a soil fertility practice dating back to the times of the Romans and Egyptians (Koepf 1992 in Litterick *et al.* 2010). Additions of organic matter to the soil have long since been observed to influence the physical, chemical and biological properties of the soil positively over time (Jenkinson & Rayner 1977; Clark *et al.* 1999; Brady & Weil 2008a; Siddiqui *et al.* 2008). Organic matter has been well documented to provide for most of the soil cation exchange capacity (CEC) (Brady & Weil 2008a), which has been described as the most important chemical reaction in nature (Brady & Weil 2008b).

Soils treated with organic materials (compost or compost tea) are generally of a better quality than conventionally treated (fertilised) soils, having a higher microbial biomass, water-holding capacity, total nitrogen, organic matter (carbon), aggregate stability, permeability and pH (Brady & Weil 2008b; Lotter 2008; Litterick *et al.* 2010; Zandonadi & Busato 2012). Such practices have replaced conventional applications and inputs of synthetic fertilisers and pesticides in sustainable or organic farming and are now common strategies used in fertility, and pest and disease management (Fageria 2002; Lotter 2008; Siddiqui *et al.* 2008; Zandonadi & Busato 2012).

> *Compost tea is described as 'the product of showering re-circulated* [sic] *water through a porous bag of compost suspended over an open tank with the intent of maintaining aerobic conditions'* (Riggle 1996 in Scheuerell & Mahaffee 2002).

Compost teas are made from compost extracts that have been brewed aerobically, with additions of one or more sources of microbial food (Diver 2002). These can be called aerated compost teas (Scheuerell & Mahaffee 2002). Non-aerated compost

teas differ in that they are usually more passively produced (Scheuerell & Mahaffee 2002), and take longer to brew (7–14 days) without supplementary aeration (Diver 2002). The basic methods of aerated and non-aerated compost tea production have been well documented in the literature (Brinton & Droffner 1995; Ingham & Alms 1999; Diver 2002; Scheuerell & Mahaffee 2002).

It is well-known that organic materials contain beneficial biological properties (plant growth regulators and beneficial micro-organisms [predatory, antifungal and antibacterial]) which are useful for plant growth and development (Edwards *et al.* 2004; Brady & Weil 2008a; Siddiqui *et al.* 2008; Siddiqui *et al.* 2011; Zandonadi & Busato 2012). Plant growth regulators, which include compounds such as humic and fulvic acids, are produced naturally during composting (Brady & Weil 2008a). Plant growth regulators play an important role in root initiation, cell division, flowering and fruiting (Atiyeh *et al.* 2002a; Arancon *et al.* 2004; Paparozzi 2008). Furthermore, these biological properties have been attributed to disease and pest resistance (Zhang *et al.* 1998; Zandonadi & Busato 2012). Scheuerell & Mahaffee (2002) provide a comprehensive review on the benefits of compost teas with regard to disease and pest resistance. Furthermore, the following authors provide a comprehensive account of an increased resistance to many associated diseases and pests which occur in numerous edible food crops: Zhang *et al.* (1998); Scheuerell & Mahaffee (2004); Siddiqui *et al.* (2008); and Siddiqui *et al.* (2009).

Organic carbon, derived from organic materials, provides the energy and body-building constituents for the soil ecological community (Zhang *et al.* 1998; Clark *et al.* 1999; Barne & Striganova 2004; Brady & Weil 2008c) which regulate the release and retention of nutrients (Clark *et al.* 1999). Many of the positive effects attributed to compost and compost tea additions to the soil have been linked to the diverse community of beneficial microorganisms present (Litterick *et al.* 2010), albeit much of this is anecdotal (Scheuerell & Mahaffee 2002). Zhang *et al*. (1996 & 1998), however, observed that additions of compost and compost teas induced plant systemic acquired resistance, where plants produced defence compounds (polyphenols) that inhibited insects and disease attack.

Compost teas have been used extensively as a soil conditioner and a means of microorganism enrichment (Scheuerell & Mahaffee 2002; Zandonadi & Busato

2012). It can, however, take several years for the total soil organic matter and microbial community structure to increase after conversion to an organic system, although the aggregate microbial biomass and activity can change almost immediately (Clark *et al.* 1999).

The use of compost teas has proven to increase the health (Scheuerell & Mahaffee 2002), quality (Litterick *et al.* 2010; Siddiqui *et al.* 2011) and condition (Zandonadi & Busato 2012) of the soil through changes to the soil's chemical and physical properties (Scheuerell & Mahaffee 2004). We can measure the condition of the soil through standard laboratory analyses, but the costs involved in measuring microbial diversity are high, and consequently there is no standard method for reporting on compost tea microbiology (Scheuerell & Mahaffee 2002).

Microbial diversity is depressed by chemicals, especially fumigants. Container media, amended with compost, have been effective enough to replace methyl bromide with regard to disease suppression in propagation trials (Zhang *et al.* 1996). The use of locally-composted organic waste may therefore be more favourable than commercially-produced organic and inorganic growing media (Chong 2008), both as a soil conditioner and as a slow release fertiliser (Brady & Weil 2008a). With this extensive background, we can expect to observe some potential improvement in both the performance of plants and the quality of soil treated with organic amendments, over those not treated.

2.5 Composting organic materials

Traditional methods of compost-making follow a process of intense decomposition (thermophyllic), where heat build-up can reach extreme temperatures (45–65°C) (Brady & Weil 2008a; Dominguez & Edwards 2011a), resulting in the elimination of pathogenic microorganisms and subsequently the loss of nitrogen through volatilisation of ammonia (Dominguez & Edwards 2011a). Thermophyllic composting does not always make good quality compost (Edwards 2011), and the production of high quality compost requires technical skill and knowledge (Goyal *et al.* 2005). Thermophyllic compost production of large quantities of organic waste requires regular turning and management to increase aeration, often needing expensive machinery to do so (Edwards 2011). On the other hand, high quality compost can be made at lower temperatures (20–35°C) through slow decomposition or with the use

of earthworms (Brady & Weil 2008a). Earthworms promote microbial biomass and activity as well as retaining higher levels of nutrients in plant available form in the soil (Dominguez & Edwards 2011a).

> *'Worms prepare the ground in an excellent manner for the growth of fibrous-rooted plants and for seedlings of all kinds'* (Darwin 1881).

Earthworm-produced compost, or vermicompost, has been well documented in promoting plant growth and yield of a variety of field crops (Diver 2002; Edwards *et al.* 2006; Carlos *et al.* 2008; Oliva-Llaven *et al.* 2010) and ornamental plants, independent of nutrient supply (Atiyeh *et al.* 2000a; Atiyeh *et al.* 2001; Arancon *et al.* 2004; Edwards *et al.* 2006). The production of high quality vermicompost is relatively easier than thermophyllic compost, as the earthworms aerate and turn the organic waste, reducing the need for expensive machinery (Edwards 2011). Vermicomposting is a growing industry worldwide, and offers massive potential in local organic waste management and also as a growing medium (Atiyeh *et al.* 2001; Arancon *et al.* 2011).

2.6 Vermicomposting

Across the world, the practice of vermicomposting has become increasingly popular (Atiyeh *et al.* 2000a). Described by some as a new agricultural paradigm (Raytsak & Verkuijlen 2006; Oliva-Llaven *et al.* 2010), vermicomposting has been suggested as an answer to replacing commercial chemical fertilisers and pesticides in agriculture (Atiyeh *et al.* 2001; Arancon *et al.* 2007a). Vermicomposting can be described as a biotechnological process in which earthworms interact with microorganisms to oxidise and stabilise the energy-rich and complex organic waste materials into useable humus-like material (humification), known as vermicompost (Arancon *et al.* 2002; Carlos *et al.* 2008; Arancon *et al.* 2011).

The earthworms fragment the waste substrate and enhance the microbial activity and rates of decomposition (Nath *et al.* 2009; Arancon *et al.* 2011), while recycling the numerous waste types into highly desirable organic products (Ortega & Fernández 2007; Yadav *et al.* 2010). Many authors have subsequently demonstrated significant improvements in seed germination (emergence), rates of seedling growth and development (Atiyeh *et al.* 2000a; Atiyeh *et al.* 2000b; Zaller 2007a; Lazcano *et al.* 2010b) – as well as the stimulation of rooting, time of flowering and the

lengthening of internodes – by using vermicompost (Edwards *et al.* 2004). These effects should consequently result in an increase in the efficiency of the propagation system with improvements to the rooting and vigour of cuttings (Klingman 2008a), as well as germination rates.

As with compost, much evidence exists regarding improvements to the physical properties and the fertility of soil by the addition of vermicompost (Atiyeh *et al.* 2000b; Atiyeh *et al.* 2001; Arancon *et al.* 2003a; Edwards *et al.* 2004). Vermicompost has also been shown to suppress a wide variety of pests (Arancon *et al.* 2002; Arancon *et al.* 2003a; Edwards *et al.* 2004; Arancon *et al.* 2005a; Arancon *et al.* 2007a; Edwards *et al.* 2007) and disease pathogens with subsequent improvements to plant performance (Edwards *et al.* 2004; Edwards *et al.* 2006). In the review by Theunissen *et al.* (2010), consistently higher phenolic compounds were reported in plants treated with vermicomposts compared with standard fertilisers. These increases in plant phenolic compounds have been said to increase the antioxidant activity of medicinal plants, and thus show good potential to increase medicinal quality (Theunissen *et al.* 2010).

Vermicomposting has been recognised as an innovative technology through valuable aqueous by-products, also known as vermicompost extracts or teas (Gutierrez-Miceli *et al.* 2011). Vermiteas contain lower concentrations of soluble plant nutrients (nitrate and phosphate), beneficial microorganisms and plant growth regulators, when compared with solid vermicompost (Edwards *et al.* 2006; Nath *et al.* 2009). However, vermiteas have also shown similar results to solid vermicompost in a number of field and pot trials (Atiyeh *et al.* 2001; Edwards *et al.* 2006; Carlos *et al.* 2008).

CHAPTER 3 – MATERIALS AND METHODS

3.1 Study area

The Nelson Mandela Metropolitan University (NMMU) George campus is situated near the town of George in the southern Cape of South Africa (33°57'50.39"S, 22°32'8.01"E). The area receives orographic rain throughout the year, with definite peaks in autumn and early summer. The rainfall for the area ranges from 700–1200 mm per annum and temperatures may average from 14.5°C in the winter months to 21°C in the summer months (South African Weather Service 2013).

Rainfall and temperature data suggest a moist, warm temperate climate while localised low pressure systems may produce hot and dry north-westerly winds from May to August (Geldenhuys 1982). The site has an altitude of 220 m above sea level (Google™ Earth). During the study period (December to June) a total of 226 mm of rainfall occurred, with average daily maximum and minimum temperatures of 23.7°C and 13.3°C, respectively (South African Weather Service 2013).

3.2 Experimental design

3.2.1 Study site

A shade house growing area, measuring 5 m x 8 m x 2 m, was used for this study. The shade house was covered entirely with 40% shade-density cloth, which aimed to decrease the solar radiation while also providing a form of wind protection (Ruter 2008). Importantly, the wind was filtered and not blocked, therefore allowing adequate ventilation of the growing area (Ruter 2008). The entire area was enclosed to prevent entry by wild animals.

The site has access to both rainwater and municipal water. An irrigation system was installed and connected to the municipal system, and watering was controlled automatically for the duration of the study.

3.2.2 Growing conditions

This study comprised two experiments for selected seedlings and cuttings. Experiment 1 evaluated seedling and cutting survival (germination and rooting) in a homogenous rooting medium so as to limit variability (Rayner 1967), while Experiment 2 evaluated seedlings and cuttings in a pot trial experiment.

An extra layer of shade cloth (35% shade-density) was temporarily used to cover the northern side of the growing area. Once they had taken, cuttings were kept shaded for up to two weeks under mist in this area during Experiment 1 until adequate rooting had occurred (Von Krosigk 1994). During this period, increased shade of 75% for cuttings was recommended; firstly, to increase humidity, and secondly, to reduce light intensity (Ruter 2008). The extra shade cloth was removed for Experiment 2.

With no artificial heating or lighting, similar conditions with regard to light, wind and water were maintained as far as possible for the duration of both growing experiments (Burger 2008; Klingman 2008a). Replications were arranged in a randomised design so as to cover the range of light, temperature and wind conditions present in the shade house.

For Experiment 1, all cuttings were kept moist by daily misting for 10 minutes (late morning, midday and afternoon) (Burger 2008; Klingman 2008a) using a Hunter X-Core Indoor Irrigation Controller. Seedlings were kept moist under polyethylene plastic and watered when necessary. Using the controller, a standardised watering schedule was maintained during Experiment 2 (morning and afternoon), and over-watering was avoided by using measured drip irrigation (at a rate of 3.8 litres per hour).

For quality testing purposes, water samples were taken from both sources in December of 2012 prior to commencement of the experiments, and again in June 2013, six months after commencement (Donald *et al.* 1994; Klingman 2008b; Ruter 2008). These were sent to the Western Cape Department of Agriculture laboratory at Elsenberg to test for irrigation suitability.

3.2.3 Plant material

Shade house experiments were conducted for four indigenous medicinal plant species: *Lessertia frutescens*, *Artemisia afra, Cyclopia genistoides,* and *Pelargonium citronellum.* The following section elaborates where necessary on any specific propagation methods required for each species. Standard methods were followed with regard to the growing media, watering, shading and layout in the growing area for both experiments.

Lessertia (Sutherlandia) frutescens

Lessertia frutescens (Fabaceae), or cancer bush, is an endemic and fast-growing South African shrub (Van Wyk *et al.* 1997). The leaves and stems are harvested (Van Wyk & Albrecht 2008) and the species has been regarded as a multipurpose medicinal plant, known to treat a wide variety of ailments (Van Wyk *et al.* 1997).

Flowering in summer, *L. frutescens* is said to grow easily from seed sown in autumn or spring (Xaba & Notten 2003). The seeds were placed in boiling water (100°C) and left for ten hours to soak to aid in fracturing the seed coat (Brown & Duncan 2006). Only mature and fully-formed seeds were used. Seeds were sown into a well-drained rooting medium (Brown & Duncan 2006) in late spring and incubated under 40% shade. There is a common belief that this plant does not germinate well under nursery conditions, but more successfully when sown sporadically under wild or natural conditions (Mr J. Turner, permaculture/nursery practitioner, *pers. comm.*).

Lessertia frutescens is also said to grow well from cuttings (Mr D. de Wet, curator, George Botanical Gardens, *pers. comm*. & Mrs L. Leggit, nursery practitioner, Blue Mountain Nursery, *pers. comm.*), and semi-hard wood cuttings were taken in summer and planted into a well-drained rooting medium.

Cyclopia genistoides

Cyclopia genistoides (Fabaceae), or honeybush, is endemic to the Cape Fynbos biome (Joubert *et al.* 2008). *C. genistoides* is a robust resprouting shrub, occurring in lowland Fynbos from Malmesbury to Albertinia in the Western Cape (Goldblatt & Manning 2000). Honeybush tea has been derived from several *Cyclopia* spp. (including *C. intermedia*, *C. sessilifolia*, and *C. subternata*), of which *C. genistoides* was the original species used for making tea (Van Wyk & Gericke 2000).

Cyclopia genistoides grows in low-nutrient sandy to loam soil characterised by low levels of phosphorus and low pH (Joubert *et al.* 2007). This species has a relatively high moisture requirement in the winter months (Brown & Duncan 2006). After the methods of Brown & Duncan (2006), the seeds were placed in boiling water (100°C) and left for ten hours to soak to aid in fracturing the seed coat. Only mature and fully-formed seeds were used. Seeds were sown into a well-drained growing medium in late spring and incubated under 40% shade. Cuttings were taken and sown into a well-drained medium in summer (Mbangcolo *et al.* 2013a).

Pelargonium citronellum

Pelargonium citronellum (Geraniaceae), or lemon-scented pelargonium, is a rare South African plant and is endemic to the Western Cape between Herbertsdale and Ladismith (Raimondo & Helme 2007). This evergreen bushy shrub is heavily lemon-scented and grows up to two metres in height (Goldblatt & Manning 2000; Mjuleni 2007). This habitat specialist occurs in scattered populations, on alluvial soils, but is not currently threatened (Raimondo & Helme 2007).

Pelargonium citronellum grows well from cuttings all year round (Mjuleni 2007). Cuttings were taken in summer and placed into well drained rooting medium. Seed was sown in summer in a well-drained rooting medium (Raimondo & Helme 2007) and left to incubate under 40% shade with adequate watering (Mjuleni 2007).

Artemisia afra

Artemisia afra (Asteraceae), or African wormwood, is an endemic South African herbaceous perennial shrub (Van der Walt 2004). *A. afra* has been described as a universal medicinal plant and has been commonly used for many years to treat a wide range of ailments (Van Wyk & Gericke 2000).

This species is generally located along streams and on damp slopes (Goldblatt & Manning 2000), growing well in full sun and well-drained soils (Van der Walt 2004). Seeds were sown in a well-drained growing medium in late spring and left to incubate under 40% shade. Due to the small nature of the seeds, a folded piece of paper was used, with the seeds collected along the fold; the paper was tapped gently so as to distribute the seeds as evenly as possible. Semi-hardwood cuttings were taken in late summer and planted in a well-drained rooting medium (Bremness 1988).

3.2.4 Experiment 1: Seedling and cutting propagation

Growing methods are described in the following sections. Detailed analyses of the vermitea batches are presented in Chapter 4 under Results. Analyses of the irrigation water can be found in Appendix 1, and detailed analyses of the compost and growing media are presented in Chapter 5 under Results.

Growing media: Coconut coir dust

Coir is the name given to the outer fibrous husk or mesocarp of the coconut (*Cocos nucifera* L.) (Offord *et al.* 1998). Coir dust, the waste product derived from the rope and mat industry (Abad *et al.* 2002), has been described as an environmentally-friendly substitute for peat as a growing medium for containerised plant propagation (Offord *et al.* 1998). Supplied in brick form, coir is readily available and used in the horticultural industry (Konduru *et al.* 1999).

Coir dries out relatively slowly in comparison to other media, but maintains a high air content even when wet (Offord *et al.* 1998). Coir has excellent water-holding capacity, requiring less watering, and also has a suitable pH for plant growth (5.6). Coir shows great value as a growing medium (Israel *et al.* 2011) and is used extensively in Australia for the propagation of cuttings, with no adverse effects noted (Offord *et al.* 1998).

Before use, the coir bricks were rehydrated with rainwater and broken up, each brick producing approximately 50 litres of growing medium. Once saturated, the coir was squeezed to remove any excess water and then used as the primary growing medium for seeds and cuttings in Experiment 1.

Amendments

Compost tea (vermitea) production

To make a compost tea, compost (inoculum) is soaked in water (incubated) in a fermentation vessel (in this case a 20-litre plastic bucket with a lid) and later filtered to remove the sludge component (filtration) (Brinton & Droffner 1995; Ingham & Alms 1999; Diver 2002; Scheuerell & Mahaffee 2002). Table 3.1 lists the standard components used for the production of compost tea for this study.

Table 3.1: The standard components used to produce compost tea at a concentration of 20% with supplementary nutrition (the scale of production was matched to the application needs per week).

Components	Volume (L)
Aerated rainwater (chlorine free)	100
Compost (kitchen and paper waste vermicompost)	20
Molasses (unsulphured)	0.9

Methods followed: On a Tuesday afternoon, each batch of compost tea was made in a 20-litre bucket containing rainwater provided with constant aeration (Brinton & Droffner 1995; Diver 2002; Scheuerell & Mahaffee 2002; Litterick *et al.* 2010). The compost source was wrapped in cheesecloth and suspended below water level in the fermentation vessel (Diver 2002). The compost was initially submersed in the aerated water for 30 seconds, before allowing it to drain for 15 seconds; this process was repeated three times at the start of each brewed batch of compost tea (Scheuerell & Mahaffee 2004; 2006).

Supplementary molasses (unsulphured) was added (0.9 L 100 L^{-1}) (Table 3.1) as a source of microbial food (Scheuerell & Mahaffee 2004), and the solution was left to ferment (brew) (Scheuerell & Mahaffee 2002; Salter & Edwards 2011) with the lid on for a period of 34 hours (Scheuerell & Mahaffee 2004; 2006) at a constant temperature of 22°C (± 2°C) (Scheuerell & Mahaffee 2002). On Thursday morning, after the brewing period, the solution was filtered through cheesecloth to remove the solid (sludge) component (Scheuerell & Mahaffee 2002; Litterick *et al.* 2010; PDEP 2012) and then applied directly for each plant application (Arancon *et al.* 2003a; Edwards *et al.* 2006)

Rationale: The chemical and physical characteristics of compost teas can vary quite a bit between batches (St Martin & Brathwaite 2012), due to a number of input and output variables. These variables, which will be discussed later in more detail, include the compost source material, the extraction method used, additions of supplementary nutrients, as well as water quality and brewing time (Scheuerell & Mahaffee 2002).

All of the above-mentioned variables and the ratios used were maintained as constant as possible for each batch produced. A sample was taken from each batch

and sent to the Western Cape Department of Agriculture for chemical analysis. All laboratory analyses for pH used KCl. The following section covers the important input and output variables of this process in more detail, and provides some insight into the actual process followed for this study.

Compost source material: vermicompost

Vermicompost was used as the compost-inoculum source for compost tea production in this study. The vermicompost was sourced from the NMMU George campus's onsite vermiculture programme, which was set up by the author at the end of 2010. Kitchen (food) and paper waste made up the bulk of feedstock used in this programme. Kitchen waste was collected from the university canteen and comprised approximately 65–70% fruit (no pineapple) and vegetable peels, with the remainder being made up of tea bags, bread, rice, pasta, egg shells, coffee grounds and paper filters.

Cooked foods, such as meat and oily, fatty foods were removed and not included as a feedstock. Paper was collected from the university offices (approximately 30% newspaper and 70% office discards). A paper shredder was used to shred the paper to a width of 2 cm. The kitchen waste was buffered with shredded paper at a ratio of 5:1 (kitchen waste: paper) (Warman & AngLopez 2010) before being fed to the epigeic earthworm species *Eisenia fetida* (Savigny) in containerised worm farm bins.

Eight plastic bins (measuring 1.5 m x 0.5 m x 0.5 m) have been housed in a shed, and the important environmental requirements pertaining to earthworm production (pH control, aeration and rain protection) as described by various authors have been met (Reinecke *et al.* 1992; Munroe 2007; Oliva-Llaven *et al.* 2010; Dominguez & Edwards 2011b). Covered with plastic, the worm farms have been protected from evaporation and drying out, and the pH has been maintained (at approximately 7 KCl) by periodic additions of dolomitic lime every two to three weeks. The soil moisture has been maintained in the range of 75–90% (Dominguez & Edwards 2011b) with periodic watering, taking care not to overwater or allow drying-out to occur. Drainage has been adequately provided for each farm (Gutiérrez-Miceli *et al.* 2008) and earthworms are at present thriving.

Eisenia fetida have been very successful in processing a wide range of organic materials (Atiyeh *et al.* 2001; Barne & Striganova 2004; Edwards *et al.* 2004; Carlos *et al.* 2008; Nath *et al.* 2009). However, vermicompost quality differs greatly depending on the parent source material (Zaller 2007a; Salter & Edwards 2011). A reliable source of vermicompost with consistent characteristics will therefore help to minimise the variability of teas produced (Salter & Edwards 2011).

Aerated compost tea (vermitea) production

Aerated compost tea is actively brewed with aeration and has a shorter production time than non-aerated compost tea (Scheuerell & Mahaffee 2002). Compost tea produced with aeration has shown a more positive impact on plant growth than tea produced without aeration. Compost teas, which include vermiteas, which are produced with aeration are more stable and effective than those produced without aeration (Diver 2002; Edwards *et al.* 2006; Arancon *et al.* 2011; Edwards *et al.* 2011a). Furthermore, aeration promotes the proliferation and survival of microorganisms (Scheuerell & Mahaffee 2002; Salter & Edwards 2011).

Aeration has been provided by a Daro Twin aquarium air pump, attached with tubing (Diver 2002; Ryan 2003). Two tubes, attached to aeration stones, force air though a double stream into the bucket (Ingham & Alms 1999; Diver 2002). Importantly, the tubes reach to (and stay at) the bottom of the bucket, facilitating maximum agitation of the suspended compost component (PDEP 2012). Aeration was provided during the entire brewing process.

Research has shown that aerated vermiteas are more effective on plant growth when used sooner after production, rather than later (Arancon *et al.* 2003a). Edwards *et al.* (2006) recommend the use of freshly made vermiteas – 24 hours after production – for each plant application.

Water quality and temperature

The physical and chemical properties of the water used for vermitea production will affect the growth of microorganisms (for example, negative effects of chlorine, pesticides, pathogens and suspended solids) (Edwards *et al.* 2006; Edwards *et al.* 2011a; Salter & Edwards 2011). The rainwater used had a pH of 5.0, tested low for salt, sodium and chloride content and was deemed suitable for irrigation purposes (Appendix 1).

Temperature has been shown to affect the dissolved oxygen concentration, as well as the growth rates and types of organisms found in compost tea (Scheuerell & Mahaffee 2002; Salter & Edwards 2011). To benefit the proliferation of bacteria, without undue environmental stress to microorganisms after plant application, Scheuerell & Mahaffee (2002) recommend that temperatures be maintained at ambient temperatures (20–22°C) during the brewing process. Temperature was regulated in the fermentation vessel with the use of a 100 watt Dophin submersible thermostat heater with fixed temperature settings, suitable for over 100 litres of water.

Optimum vermicompost tea concentration

Studies indicate that low concentrations of vermiteas (< 30% vermicompost) tend to promote plant growth, while higher rates can lead to depressed plant growth (> 40%) (Arancon *et al.* 2003a; Edwards *et al.* 2004; Edwards *et al.* 2006). This may be explained by the fact that high concentrations of certain plant growth regulators have been shown to inhibit plant growth (Cheng *et al.* 2008). Arancon *et al.* (2003b) have, however, suggested that depressed plant growth may be due to the higher levels of salts associated with higher concentrations of vermicompost. This can be supported by the fact that seedlings and cuttings are very sensitive to high levels of salt during the early stages (Donald *et al.* 1994; Chong 2008; Klingman 2008a).

Extensive research into optimum vermitea concentrations has been carried out at the Soil Ecology Laboratory at Ohio State University in America. Edwards and colleagues (Edwards *et al.* 2004 & 2006) found significant results with regard to plant performance with anything from 5–30% dilutions of vermicompost in water. Numerous other studies have observed best results at concentrations of 20% (Atiyeh *et al.* 2001; Zaller 2007a; Oliva-Llaven *et al.* 2010; Salter & Edwards 2011). For these reasons vermitea was produced under standard methods at a concentration of 20% for both experiments.

Seedling propagation

On 15 December 2012, approximately 900 seeds – per species – were planted into eight sterilised (soaked in bleached water solution) (Chong 2008) non-compartmentalised nursery flats of equal size (16.5 cm x 23 cm x 6 cm), each containing an homogenous (100% coconut coir) rooting medium. This was repeated for all four species, numbering a total of 32 nursery flats. Seed for *P. citronellum* and *A. afra* were obtained from Kirstenbosch Botanical Gardens, while *C. genistoides* and *L. Frutescens* seeds were obtained from New Plant Nursery and George Botanical Gardens respectively. All seed sown were deemed relatively fresh.

All seeds apart from *A. afra* were planted into individual pre-made holes of an approximately equal depth (± 2 cm) before being covered with a thin layer of coir, and pressed down with the hand. With very small seeds it was necessary to divide the seeds of *A. afra* into eight approximately equal parts. These were then scattered evenly over the surface of the growing medium using a folded piece of paper – tapping seeds out individually – before being covered with a thin layer of coir. One half of each species (four nursery flats, n = 450), totalling 16 nursery flats for all species, were treated by soaking the nursery flat with a 20% vermitea solution (Table 3.1), while the other half were treated (soaked) with an equal volume of rainwater water only.

Nursery flats were arranged in a random complete block design, with each nursery flat as a block. These were kept on a sterilised metal framed bench of 1.2 m in height to aid in aeration and drainage (Klingman 2008a; Ruter 2008). Each flat was covered with polyethylene sheeting and kept equally moist and under similar conditions until germination (Donald *et al.* 1994; Compton 2008). After germination – and once seedlings showed their second set of leaves – they were transplanted directly into 12 cm pots to avoid stress (Donald *et al.* 1994; Chong 2008).

Cutting propagation

Uniform stem tip cuttings of approximately 5–10 cm in length (species dependent) were taken for each species (n = 396) on the morning of propagation and kept moist in a black plastic bag in a cooler box (Ruter 2008). Only vigorous and healthy looking stock plants free of disease or pest symptoms were used (Van Wyk 1994; Ruter 2008; Windham 2008). Cutting selection focused on juvenile plant parts and suckers

(Beyl 2008b; Ruter 2008) derived from between six and twelve stock plants per species. *C. genistoides* & *P. citronellum* were taken on 13 December 2012 from New Plant Nursery (Victoria Bay Heights 33°59'41.49"S, 22°32'15.68"E). *A. afra* were taken on 5 February 2013 from Blue Mountain Nursery (Wilderness Heights 33°58'25.24"S, 22°33'15.68"E). *L. frutescens* were wild harvested on 18 February 2013 with the ranger from Fransmanshoek Conservancy (Vleesbaai 34°18'1.82"S, 21°55'52.89"E) as part of a co-operative rehabilitation project.

All flowers were removed, as well as all the leaves from the proximal 2–5 cm from each cutting (after the methods of Holloway 2008). A sterilised pair of scissors was used and the base of each cutting was cut at a 45º angle to assist in rooting (Holloway 2008). All cuttings were submerged in water prior to being planted, in an attempt to increase the humidity around each plant (Mrs L. Leggit, nursery practitioner, Blue Mountain Nursery, *pers. comm.*).

A total of 396 cuttings – for each species – were planted into 66 sterilised (soaked in a bleached water solution) (Chong 2008) and prefilled six-pack plant trays with a 100% coconut coir rooting medium. This was repeated for all four species, with a total number of 264 six-pack trays. Each tray was moistened to field capacity prior to planting (Donald *et al.* 1994). Each cutting was planted in the centre of each compartment, and care was taken not to push them to the bottom, so as to allow for adequate space for root development. The medium around the base of each cutting was firmed down with the fingers and any leaves that were partially buried were removed (Holloway 2008).

Twenty-four trays per species were selected at random and grouped into threes (n = 18 cuttings per group), each group representing a block. Trays were arranged on a sterilised metal-framed bench and kept moist through misting. Twelve of these trays per species (n = 72 cuttings) were treated by soaking the nursery flat with a 20% vermitea solution (Table 3.1), while the other twelve were treated with an equal volume of rainwater water only.

This experiment comprised four replications of three trays of six cuttings with vermitea treatment, and four with no treatment, per species. The remaining 42 cutting trays were watered equally and maintained under similar conditions to provide enough stock for Experiment 2. Cuttings were left for two weeks, after which

a test cutting was selected per treatment and checked every three days until the first roots appeared (verified by tugging on the cutting to dislodge it from the medium). Once cuttings were sufficiently rooted to enable lifting with the root system intact, they were transplanted directly into 12 cm pots to avoid stress (Chong 2008).

3.2.5 Experiment 2: Seedling and cutting pot trial

Growing media:

Coarse builders' sand and hammer-milled pine bark (sourced from Barco Ltd., George) was used as the primary growing media for this experiment at the ratios listed in Table 3.2. No fungicides, herbicides or pesticides were used during this experiment.

Table 3.2: The proportions of the different constituents with their additives used during Experiment 2 growth phase (measurements are volume to volume ratio).

Growing medium	Treatment		
	Fertiliser	Compost	Vermitea
Bark: Sand: Compost ratio	50:50:0	40:40:20	40:40:20
Additions	Multicoat® 5g L^{-1}	Compost	Compost

Chemical analyses were conducted on all the growth media used prior to experimentation. Laboratory analysis did not measure for total N concentrations, and thus all estimations of total N, and calculated C:N ratios are approximations only and are not reliable. For the purpose of comparability across treatments, the approximate NPK concentrations of the growing media were converted from mg kg^{-1} to mg L^{-1} to represent the concentrations delivered per 650 ml pot of medium (value x 0.65). All NPK values are representative of the measure of a 650 ml pot. All observed values for resistance were converted to electrical conductivity (EC) for comparative purposes.

Amendments

Controlled-release fertiliser

The controlled-release NPK fertiliser, Multicoat®, was selected for this study due to its common use in nurseries in the Western Cape and Gauteng (Mr G. Burger, Agronomist, Haifa South Africa, *pers. comm.*). Multicoat® provides a completely balanced nutrient solution in one single application, allowing for maximum and even plant growth. Haifa South Africa (Pty) Ltd., the producers of Multicoat®, recommend

a blend of Multicoat® 8 with NPK ratio 15-7-15+Mg+Mn and Multicoat® 2 with NPK ratio 42-0-0 for container grown small shrubs (www.haifa-group.com).

Table 3.3: The approximate ratios and concentrations of NPK delivered per pot (650 ml medium), under treatment F. Each pot received an equal portion of the Multicoat® 2 & 8 blend at the recommended dose of 5 g L^{-1}. Values have been calculated from percentages provided by the manufacturer and rounded off to the nearest whole number.

Nutrients	Multicoat®		Weight	Volume	
	2 (29%)	8 (71%)	g in 5 g	mg L^{-1}	mg 650 ml^{-1}
N	42	15	1.14	1132	742
P	0	7	0.25	248	161
K	0	15	0.53	533	346

The manufacturer's recommended application rate for this blend is 5 g L^{-1} of growing medium for containerised propagation (www.haifa-group.com). To establish a standardised concentration ratio of NPK for this treatment which would be comparable with the other treatments, values were converted into mg L^{-1} and then calculated per pot of 650 ml medium (Table 3.3). This blend was homogenously premixed with coarse sand and sieved bark (Table 3.2) at the recommended rate and used for the fertiliser (F) treatment only.

Compost

Compost was obtained from the George Botanical Gardens and was produced through a thermophyllic process from garden waste in an outdoor system. The chemical and physical properties of the compost used were analysed. Compost was sieved and premixed with the growing media for treatments C and V prior to potting at a rate of 20% (Table 3.2).

Vermicompost tea

***Methods followed*:** Vermitea used for Experiment 2 was produced by the standard methods discussed in Section 3.2.4. All applications of vermitea for Experiment 2 were carried out weekly (every Thursday morning). Vermitea, produced at a concentration of 20%, was applied by means of soil drenching at a dosage of 100 ml per plant. The following section illustrates the reasons for this approach:

Optimum application dosage of vermitea for pot trials

For pot experiments on Brussels sprouts (*Brassica oleracea* L. *gemmifera* DC), Radin & Warman (2010) used an application dosage of 100 ml of vermitea at a concentration of 20% per plant. Brussels sprouts are known for their season-long nutrient demand, and the authors regarded this as a high dosage. In a further study on pak choi (*Brassica rapa* cv. *Bonsai*, Chinensis group), Pant *et al.* (2009) used a dose of 150 ml per plant; however, the vermiteas they produced were at a concentration of only 10%, which was half of that used in the previous study.

Optimum timing of vermitea applications

The suggested application timings of compost tea differ in the literature, from once weekly (Atiyeh *et al.* 2001; Pant *et al.* 2009; Siddiqui *et al.* 2009; Edwards *et al.* 2011a) to twice weekly (Edwards *et al.* 2006). However, weekly applications of vermitea specifically, have had dramatic effects on certain crop pests (Edwards *et al.* 2011a). Forestry fertigation trials of soluble fertilisers have shown best results using weekly applications. The author found that this provided enough time (four to five days) for salts to be leached to acceptable levels for the plant (Donald *et al.* 1994).

The time of day when compost tea should be applied is very important. The timing of application should occur when the environmental conditions such as ultra violet light and heat are relatively low (Scheuerell & Mahaffee 2002). These conditions should reduce the stress to the microbial populations found in the compost tea.

Methods of vermitea application

Compost teas may be applied as a foliar spray or as a soil drench (Pant *et al.* 2009), enabling easier application than the solid form (Edwards *et al.* 2004; Edwards *et al.* 2006). With high levels of micro and macro nutrients (Gutiérrez-Miceli *et al.* 2008; Siddiqui *et al.* 2011), as well as beneficial microorganisms (Diver 2002), compost teas applied to the base of the plant as a soil drench have been shown to increase the organic matter content as well as the water-holding capacity of the soil (Scheuerell & Mahaffee 2002; Scheuerell & Mahaffee 2004).

Conventional pesticide sprayer equipment has been recommended for the application of compost teas (Scheuerell & Mahaffee 2002). For the purpose of this study all plant applications were carried out with the use of a Rovic & Leers WS-16-litre knapsack sprayer. To facilitate an adequate means of soil drenching, the

sprayer piece was removed from the nozzle head of the knapsack. The wire gauze was kept in place, which not only prevented the unwanted spraying of adjacent plants, but also allowed for a more controlled release of vermitea. To ensure that an equal measure of treatment (100 ml) was delivered to each plant per application, the knapsack required a calibration.

Calibration of vermitea application equipment

The knapsack tank was filled with water, and four marked containers were used to determine the output from the knapsack over a period of one minute (UCIPM 2011). The calibration procedure was initially repeated three times and an average output of 1.2 litres was recorded per minute. It therefore took 5 seconds to deliver the required dose of 100 ml with the handle fully pressed. This calibration process was repeated over the course of the application period and provided similar results.

Seedling and cutting pot trial

Pots were sterilised and prefilled with an approximately equal amount of the growing media at the ratios listed in Table 3.2. Care was taken to leave one centimetre from the surface of the growing medium to the lip of the pot for treatment purposes. Fertilisation of treatment F was achieved by homogenously mixing the Multicoat® blend in with the sand and bark growing medium at the prescribed rate (www.haifa-group.com).

For treatments C and V, compost was first sieved using a compost sieve before mixing in with sand and bark at the appropriate ratio (Table 3.2). Treatment F therefore differs from treatment C and V in that a conventional slow release fertiliser mixture was added, whereas the latter two received an organic amendment in the form of compost, with subsequent vermitea applications to treatment V only.

Only vigorous and healthy seedlings and cuttings, not previously treated with vermitea during Experiment 1 of this study, were selected from each species and transplanted into pots. Once planted out, the pots were arranged into a randomised block design after Rayner (1967), with each pot representing an individual unit (n = 10 seedlings & n = 15 cuttings). Pots were watered equally to field capacity and kept under similar conditions (Burger 2008; Klingman 2008a) for the duration of the study.

The day after transplanting, vermitea applications were given to the seedlings and cuttings within the blocks allocated V only, for each species. Seedlings and cuttings within the blocks allocated F and C received an equal volume of water only. No additional artificial watering was carried out on treatment days, albeit some rainfall did occur.

Vermitea was applied to one-third (V) of each species, while one-third (F) had controlled-release fertiliser and one-third (C) compost only (n = 90 seedlings & n = 135 cuttings) (Table 3.4). This experiment comprised three replications for each species (seedlings each consisting of 10 pots of F, 10 of V and 10 of C and cuttings each consisting of 15 pots of F, 15 of V and 15 of C). Within each replication block, the individual pots were randomised to allow for site differences.

Table 3.4: The species matrix for Experiment 2, indicating the plants to be planted out into the conventional medium (F); the compost-applied medium (C) or compost-applied medium treated with vermitea (V).

Species	Seedlings			Cuttings		
Treatment	Fertiliser	Compost	Vermitea	Fertiliser	Compost	Vermitea
Pelargonium citronellum	30	30	30	45	45	45
Cyclopia genistoides	30	30	30	45	45	45
Artemisia afra	30	30	30	45	45	45

3.3 Data collection

3.3.1 Experiment 1

For seedlings, the four nursery flats treated with vermitea were compared with the four untreated nursery flats at the time of transplanting, and the germination rate of the two treatments was compared. For cuttings, the 12 six-pack trays treated with vermitea were compared with the 12 randomly selected untreated trays (n = 72) at the time of transplanting. The cutting rooting/strike rate of the two treatments was compared.

3.3.2 Experiment 2

Overall survival percentages for seedlings and cuttings were recorded per species per treatment 100 days after planting. All plants were harvested from the growing medium, all soil washed from the roots using rain water, and the plants were then air-dried (Ortega & Fernández 2007). The roots were separated from the above-ground parts before weighing to establish root and shoot wet weights (Bachman &

Metzger 2008). The separated parts were then oven-dried at 75℃ for 48 hours (Noble & Schumann 1993) and reweighed to determine the average dry weight per treatment. With these data, root to shoot ratios were established per species, per treatment. Soil samples were taken from the growing media of each treatment per species at the end of the study; these were sent for laboratory analysis.

3.4 Data analysis

3.4.1 Experiment 1

A chi-squared (2 x 2) goodness of fit test was used to compare the initial treatment of vermitea and control treatment for seedling germination as well as cutting strike rate (germinated: Yes or No; cutting strike: Yes or No) at a significance level of 0.05 for each selected species. Comparisons were made of the overall average seedling and cutting survival with or without vermitea treatment.

3.4.2 Experiment 2

A chi-squared (2 x 3) goodness of fit test was used to compare the survival of each species between treatments F, C and V at a significance level of 0.05. A one-way analysis of variance (ANOVA) was used to compare the mean dry biomass (roots and shoots), the dry root to shoot ratios as well as the final samples of the growing media between treatments F, C and V at a significance level of 0.05 for each species using the Statistica software (StatSoft 2010, www.statsoft.com). The Tukey HSD test was used to determine significance of differences between treatment means.

CHAPTER 4 – EXPERIMENT 1: PROPAGATION TRIAL

4.1 Results

4.1.1 Chemical properties of vermicompost and vermitea batches

Table 4.1: The chemical properties of the kitchen waste vermicompost batches used for vermitea production (mean ± standard error; n = 2; EC = electrical conductivity).

				mg kg^{-1}		
pH (KCl)	EC mS m^{-1}	C:N	Carbon (%)	NH_4	P	K
6.8 ± 0.5	100 ± 22	14:1	24 ± 0.05	17600 ± 3900	4950 ± 1450	11400 ± 200

The chemical characteristics of the kitchen waste vermicompost are presented in Table 4.1, and a full analysis can be found in Appendix 2. Laboratory analysis provided a mean pH of 6.6 and an electrical conductivity (EC) of 100 mS m^{-1}. However, total N was not measured. Lacking values for NO_3, it was not possible to accurately establish the C:N ratio for the vermicompost. However, with the available NH_4 and carbon content a rough C:N ratio of 14:1 was established.

The chemical characteristics of the aerated vermitea batches are presented in Table 4.2, and a full analysis can be found in Appendix 3. The vermiteas had a mean pH of 6.3 across batches, with an average EC of 350 mS m^{-1}. Laboratory analysis provided consistent values for $NO_3 < 3.0$ mg L^{-1} and for NH_4 at approximately 0.6 mg L^{-1}.

Table 4.2: The chemical properties of the aerated vermitea batches used in this study (mean ± standard error; n = 18).

		mg L^{-1}			
pH (KCl)	EC (mS m^{-1})	NO_3	NH_4	P	K
6.3 ± 0.2	350 ± 48	± 2.5	0.6 ± 0.1	31.4 ± 3	654.2 ± 20

4.1.2 Germination and rooting success

The germination success of the four species is presented in Table 4.3. There was a negative response towards the vermitea treatment for *C. genistoides,* with the control providing a more successful germination score ($p < 0.05$) of 44% vs. 27%. No other significant differences were observed between any of the other species with or without treatment. For seeds of *L. frutescens* as many as four plantings took place, all of which failed to provide enough viable seedlings (< 60 out of 450) for further experimentation. Germination of *A. afra* was a complete failure.

The rooting success of the four cutting species is presented in Table 4.4. There were differences observed in the rooting success of all cuttings, but these were not statistically significant ($p > 0.05$). The vermitea did, however, provide an approximately 20% higher rooting success for all species, apart from *L. frutescens*. Although *L. frutescens* had poor rooting success in both treatments, the control treatment was twice as successful as the vermitea treatment.

Table 4.3: Comparisons of the germination success of four medicinal plant seedlings with or without vermitea treatment (n = 450). χ^2 goodness of fit test was done with 1 degree of freedom at significance level of 0.05. No result was obtained for *A. afra* seeds due to poor germination.

Species	Treatment		Statistic
	Control	Vermitea	χ^2
C. genistoides	191	123	14.30 ($p < 0.05$)
P. citronellum	125	156	3.12 ($p > 0.05$)
A. afra	0	0	-
L. frutescens	57	57	0.00 ($p > 0.05$)
	373	336	

Table 4.4: Comparison of the rooting success of four medicinal plant species with or without vermitea treatment (n = 72). χ^2 goodness of fit test was done with 1 degree of freedom at significance level of 0.05.

Species	Treatment		Statistic
	Control	Vermitea	χ^2
C. genistoides	54	68	1.39 ($p > 0.05$)
P. citronellum	31	45	2.22 ($p > 0.05$)
A. afra	44	59	1.90 ($p > 0.05$)
L. frutescens	18	9	2.37 ($p > 0.05$)
	147	181	

4.2 Discussion

4.2.1 Chemical properties of vermicompost and vermitea batches

Variability was observed in the produced vermiteas, although the vermicompost used was produced from comparatively similar parent stock derived from the university canteen (Appendix 2). This variability was to be expected (St. Martin & Brathwaite 2012), and for this reason standard production methods were maintained throughout the study. Differences in processes and feedstock have shown marked difference in produced vermicomposts (Edwards *et al.* 2011b), and the canteen waste would have differed from time to time. C:N ratios are a useful indicator of the stability of organic materials (Goyal *et al.* 2005); however, without total N it was not possible to accurately establish the C:N ratio, or even the NO_3 potential of the vermiteas. With the use of total C and NH_4 the C:N ratio, concentrations of available P and the level of EC, are acceptable according to Edwards *et al.* (2011b).

Through laboratory analyses, the observed pH averages of the vermiteas were lower than would be expected from aerated vermitea derived from food waste (7.5–7.8). The values observed are closer to the range of non-aerated vermiteas (6.6–6.8) (Edwards *et al.* 2011c) and are more acidic than the vermicompost source. The observed average EC of the vermiteas was, however, very high compared with the EC of the vermicompost. Aeration is necessary to increase the levels of EC compared with non-aerated vermiteas (Edwards *et al.* 2011c) and, as far as possible, constant aeration was provided for all batches. Laboratory analyses did not measure for total dissolved oxygen, and it was therefore difficult to compare samples and assess whether sufficient oxygen was provided during brewing.

In a comparable study, the results of the vermitea analyses are similar with regard to NH_4 content; however, the levels of NO_3 are far lower (Pant *et al.* 2009). The study conducted by Pant *et al.* (2009) and work done at the Soil Ecology Laboratory at Ohio State University (Edwards *et al.* 2011c), however, utilised vermiteas brewed for 12 and 24 hours respectively, whereas this study followed methodology of 34 hours. Increased levels of NO_3 have subsequently been observed in aerated vs. non-aerated vermiteas (Edwards *et al.* 2011c). These discrepancies in pH and NO_3, as well as the high levels of EC, might be as a result of the longer brewing times used in this study. Furthermore, molasses was added as a microbial enhancer to the

vermiteas without any nitrogen boosting supplement. Increases in microbial populations have resulted in losses of NO_3 in solution, through denitrification (Allaby 1998).

Organic materials have been shown to contain biological properties such as soluble plant growth regulators (humic and fulvic acids) and plant growth hormones (gibberellins, kinetins and auxins) which have been hypothesised as being responsible for increased germination and growth (Atiyeh *et al.* 2000a; Atiyeh *et al.* 2002a; Arancon *et al.* 2004; Edwards *et al.* 2006). This study did not assess the actual presence or concentrations of plant growth regulators/hormones in the vermitea, but a number of authors have looked at their roles in plant growth.

These studies found increased seedling germination with the use of organic amendments and specifically vermiteas for a number of ornamental (Atiyeh *et al.* 2002b), vegetable (Atiyeh *et al.* 2000a; Atiyeh *et al.* 2000b; Atiyeh *et al.* 2001; Arancon *et al.* 2003a; Arancon *et al.* 2006a), horticultural (Arancon *et al.* 2007b; Zandonadi & Busato 2012) and forestry plants (Lazcano *et al.* 2010b; Kandari *et al.* 2011). One would therefore assume that the use of vermitea in the rooting medium should improve the germination/rooting success of selected species.

4.2.2 Germination and rooting success

Light and nutrients are generally not required for seedling germination or for the rooting of cuttings (Chong *et al.* 2008), as adequate nutrients are usually derived from the seed coat or from storage organs located in the plant (Windham 2008; Nichols *et al.* 2012). This study showed statistically significant effects with regard to increased seedling emergence for *C. genistoides* only. Seeds of this species responded negatively to the vermitea treatment, providing the highest germination scores under the control treatment. Very poor overall germination was observed for all species under both treatments.

Studies have shown that at the early stage of development, some plants may respond negatively to vermiteas (Ievinsh 2011). This may possibly be explained by the fact that herbaceous seedlings are known to be sensitive to salts in the medium (Donald *et al.* 1994; Chong 2008). EC is a measure of the soluble salt concentration, and for sensitive plants and seedlings, the EC of saturated extracts of growth media should not exceed 100–200 mS m^{-1} (Edwards *et al.* 2011b). The observed EC of the

vermicompost falls in line with these recommendations; however, the observed EC of the vermiteas is far higher.

High EC has resulted in germination inhibition (Gutierrez-Miceli *et al.* 2011) and the high EC observed for the vermiteas seems to be the cause of the significant results observed for *C. genistoides.* However, higher germination was recorded for *P. citronellum* under the vermitea treatment. Only a few seeds of *A. afra* germinated and none survived past the initial cotyledon stage, although the control received no treatment and the results were the same. Furthermore, seeds of *L. frutescens* failed over numerous plantings to produce enough viable plants for further experimentation. We can assume that no treatment effects were responsible for failure of these two species. Seeds of *L. frutescens* have been said to perform poorly under nursery conditions, with best results observed with minimum intervention/care (Mrs L. Leggit, nursery practitioner, Blue Mountain Nursery, *pers comm*. & Mr J. Turner, nursery and permaculture consultant, *pers comm.*)

The cuttings of all species, apart from *L. frutescens*, although not significant had a 20% increase in rooting success under the vermitea treatment, with *C. genistoides* providing the highest scores. There has been rapid development of cutting technology (Van Wyk 1994) over the years with plant growth hormones such as auxins and gibberellins used commercially to assist in rooting (Beyl & Trigiano 2008; Cheng *et al.* 2008). These hormones have been shown to speed up rooting time, percentage survival and quality of the cutting (Ruter 2008). Cuttings can, however, be rooted without auxins, but they do benefit from their use (Paparozzi 2008).

In a comparable study by Mbangcolo *et al.* (2013a), no significant differences were observed in the rooting success for *C. genistoides,* with or without the use of commercial rooting agents. Interestingly, the vermitea used in this study produced a rooting success of 94%, while the study by Mbangcolo *et al.* (2013a) observed the highest rooting percentage of 86% with the use of a commercial rooting hormone IBA-indole-3butyric acid. Furthermore, being legumes, *Cyclopia* species have the potential to fix nitrogen through symbiosis with soil rhizobial bacteria (Spriggs & Dakora 2009). No inoculation was done in this study; however, excellent rooting success was observed under the vermitea treatment.

The dipping of cuttings and seeds in compost teas has already been used as a commercial practice (Scheuerell & Mahaffee 2002). Kandari *et al.* (2011) found that concentrations of vermitea produced at 20% provided the best results for seedling emergence. However, their study found this to be species-specific. Different crops should respond differently to vermitea treatments and therefore such practices should be used cautiously for crop propagation (levinsh 2011). In general, the cutting species responded positively to vermitea treatment and increased samples sizes may have resulted in more statistically significant results. Vermitea appears to show promise as a viable means of improving rooting success in the medium, but we cannot accept the hypothesis that vermitea can increase germination/rooting potential.

4.3 Conclusion

The use of a locally-sourced organic waste material for improving plant germination and rooting success has been observed in this study. Organic aqueous extracts or vermiteas derived from kitchen waste have been shown to provide a stable source of plant nutrients. Vermitea treatment to the rooting medium has been observed to improve the rooting potential of selected cuttings species; however, increased sample sizes may have produced more statistically significant results. EC can be seen as an indirect measure of fertility status of the medium (Chong 2008) and high EC values observed in the vermiteas may have attributed to the poor germination scores observed for selected seeds. Seed can be seen as a very inexpensive part of a propagation system (Donald & Jacobs 1994). Monitoring and maintenance of EC within the range acceptable to sensitive plants and seedlings may produce more positive results.

CHAPTER 5 – EXPERIMENT 2: POT TRIAL

5.1 Results

5.1.1 Analysis of the growing media

Table 5.1: The chemical characteristics of the two growing media (with compost added at 20% to medium C but prior to the addition of fertiliser to medium F) prior to use in the medicinal plant trial. For comparability throughout the study, values have been converted from mg kg^{-1} to mg L^{-1} and multiplied by 0.65 to represent the approximate concentrations delivered per 650 ml pot of medium. Carbon has been presented in percentage form and the value is expressed per 650 ml pot.

Variable	Medium	
	Fertiliser	Compost (vermitea)
pH (KCl)	6.6	7.4
EC mS m^{-1}	2	2
NH_4 mg 650 ml^{-1}	416	535
P mg650 ml^{-1}	46	56
K mg650 ml^{-1}	89	108
Carbon (%)	1	1.3
C:N	24:1	24:1

The analyses of the initial growing media per treatment and pure compost used prior to experimentation are presented in Tables 5.1 and 5.2 respectively, and a full analysis may be found in Appendix 2. The available NH_4, P and exchangeable K concentrations observed for medium C were approximately only 22%, 17.5% and 17.5% higher, respectively, than those observed for medium F. The differences between medium C and F do not reflect the addition of the 20% compost in spite of its different composition. Very low EC was measured for both media and the pure compost.

Table 5.2: The chemical characteristics of the garden waste compost used as a component in the growing media of treatments C and V (n = 1).

				mg kg^{-1}		
pH (KCl)	EC mS m^{-1}	C:N	C (%)	NH_4	P	K
5.4	3	13:1	5.5	4117	274	610

5.1.2 Analysis of the amendments

Section 3.3.5 details the available NPK provided by the slow release fertiliser blend. The nutrient concentration of the vermitea batches are discussed in detail in Chapter 4 (Table 4.3). Constant values of < 3.0 mg L^{-1} NO_3 were observed across all vermitea batches with an average value of 0.59 mg L^{-1} observed for NH_4. Table 5.3 illustrates the approximate available concentrations of NPK found in the vermitea per 100 ml dose and for the 15 week test period. The average pH and EC of the vermiteas were detailed and discussed in Chapter 4, Sections 4.1.1 and 4.2.1 (Table 4.3).

Table 5.3: Analysis of the available $N0_3$, NH_4, P & K concentrations observed from 18 vermitea batches (mean ± standard error). Total concentrations have been calculated to estimate the approximate concentration of NPK delivered per treatment application (100 ml) and for the entire test period of 15 applications for pots under treatment V. All batches of vermitea provided an analysis of < 3.0 mg L^{-1} for NO_3, a value of 2.5 mg L^{-1} was subsequently used to estimate overall NO_3 concentration delivered per batch of vermitea.

Nutrients	Per batch mg L^{-1} (n = 18)	mg 100 ml^{-1}	Total mg L^{-1} (n = 15)
NO_3	2.5	± 0.25	± 3.75
NH_4	0.59 ± 0.07	0.059	0.89
P	31.39 ± 2.87	3.14	47.09
K	654.17 ± 19.45	64.52	967.76

5.1.3 Overall NPK concentrations delivered per treatment

The available NH_4, P and K values of the initial media (Table 5.1) and the NPK values of the two amendments (fertiliser and vermitea) were added to establish the approximate overall NPK concentrations delivered per pot per treatment. These approximate NPK values were calculated per pot for treatments F, C and V as < 1200 mg N, < 210 mg P and < 450 mg K; < 540 mg N, < 60 mg P and < 110 mg K and < 550 mg N, < 110 mg P and < 1100 mg K, respectively (Figure 5.1).

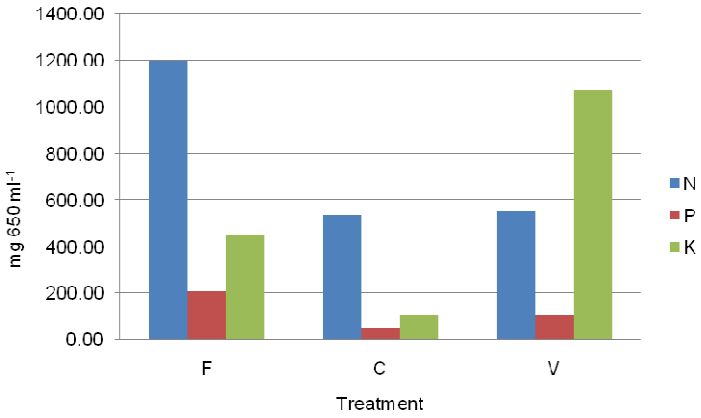

Figure 5.1: The approximate NPK concentrations delivered per 650 ml pot per treatment.

5.1.4 Plant survival

Seedlings

The overall survival of the two species of seedlings tested across treatments is presented in Table 5.4. No statistically significant differences were observed across treatments for either of the species tested ($p > 0.05$). Only small differences were observed across treatments with survival rates of 60–66% for *C. genistoides* and 76–80% for *P. citronellum* observed across treatments F, C and V respectively.

Table 5.4: Comparisons of the overall observed survival of seedlings of the two medicinal plant species undergoing three treatments (n = 30). χ^2 goodness of fit test was done with 2 degrees of freedom at significance level of 0.05.

Species	Treatment			Statistic
	Fertiliser	Compost	Vermitea	χ^2
C. genistoides	18	20	18	0.14 ($p > 0.05$)
P. citronellum	24	24	23	0.03 ($p > 0.05$)
	42	44	41	

Cuttings

The overall survival rates of the cuttings of the three species tested across the three treatments are presented in Table 5.5. *C. genistoides* had a high survival rate under all treatments, with no significant difference observed between them. Although not statistically significant, a higher survival rate of 71% was recorded for *P. citronellum* under treatment V compared with treatments F and C. A statistically significant difference was observed ($\chi^2 = 6.20$) between treatments V and F ($p < 0.05$) and between C and F ($p < 0.05$) for *A. afra*, while treatments C and V ($p > 0.05$) did not differ significantly. A very low overall survival rate of 13%, 35% and 40% was observed for *A. afra* across treatments F, C and V respectively.

Table 5.5: Comparisons of the overall observed survival of cuttings of the three medicinal plant species under three treatments (n = 45). χ^2 goodness of fit test was done with 2 degrees of freedom at significance level of 0.05.

Species	Treatment			Statistic
	Fertiliser	Compost	Vermitea	χ^2
C. genistoides	41	45	43	0.19 ($p > 0.05$)
P. citronellum	22	24	32	2.15 ($p > 0.05$)
A. afra	6	16	18	6.20 ($p < 0.05$)
	69	85	93	

5.1.5 Plant growth measurements

The results of the plant growth measurement for the medicinal plant seedlings and cuttings are illustrated in Figures 5.2, 5.3 and 5.4. Full statistical analysis are presented in Appendices 4 and 5. The mean dry root mass, dry shoot mass, both measured in grams, and the root to shoot ratio are compared across all three treatments. Figure 5.5 illustrates the significant differences observed in plant growth between treatments. The photos were taken prior to the plants being weighed and oven dried.

Seedlings

For seedlings of *C. genistoides*, dry root mass, dry shoot mass and root: shoot ratio values were significantly greater in treatment F then in treatments and C and V. No statistical significance was observed between treatments C and V for seedlings of this species. For seedlings of *P. citronellum*, dry root mass and dry shoot mass values were significantly greater in treatment F then in treatments C and V. root: shoot ratio values were significantly greater in treatment F than in treatment C for this species.

Cuttings

For cuttings of *C. genistoides,* dry root mass and dry shoot mass values were significantly greater in treatments F than in treatments C and V. root: shoot ratio values were significantly greater in treatment V than in treatment and F and C. For cuttings of *P. citronellum,* dry root mass, dry shoot mass and root: shoot ratio values were significantly greater in treatment F than in treatments C and V. For cuttings of *A. afra*, dry root mass and dry shoot mass values were significantly greater in treatment F than is treatments C and V.

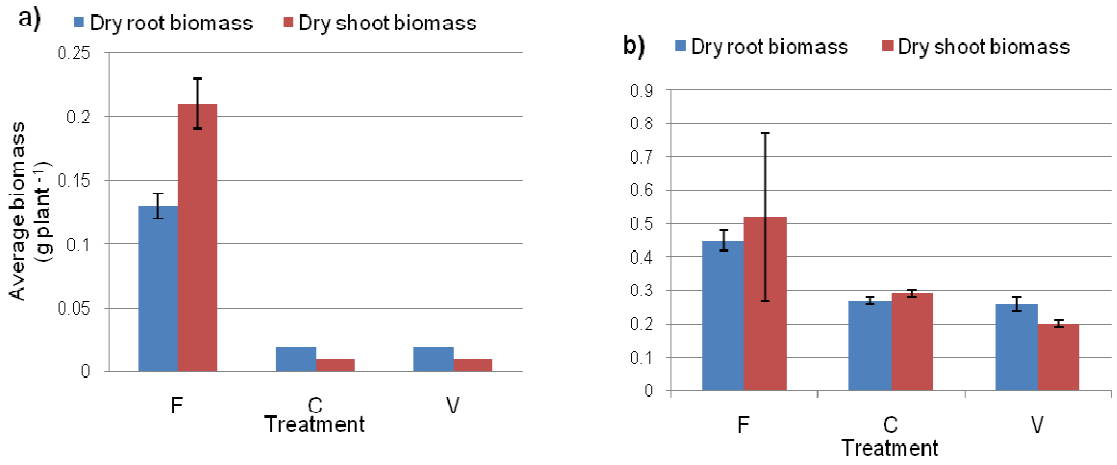

Figure 5.2: The dry root and shoot biomass averages observed for *C. genistoides* seedlings (a) and cuttings (b) across the three treatments (mean ± standard error). The standard error equated to zero for seedlings under treatment C and V and the bars are thus not visible.

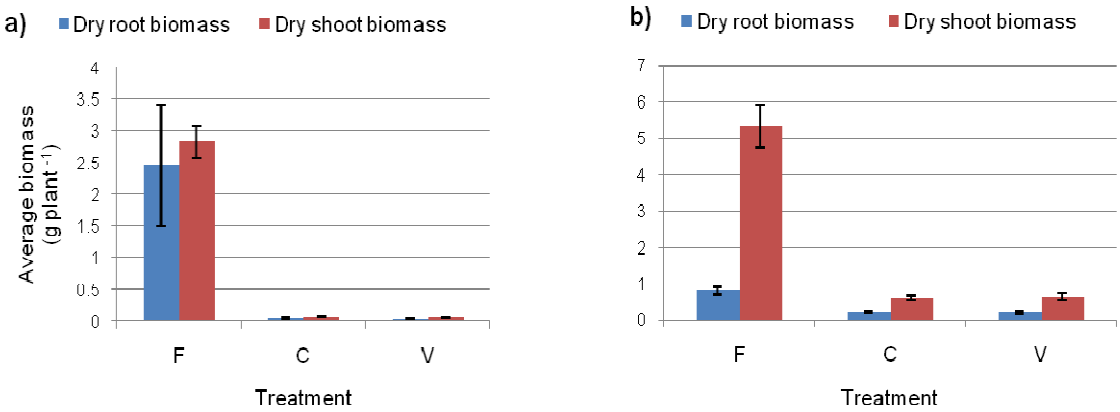

Figure 5.3: The dry root and shoot biomass averages observed for *P. citronellum* seedlings (a) and cuttings (b) across the three treatments (mean ± standard error).

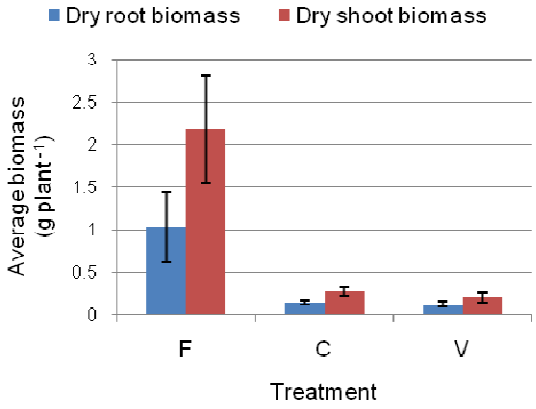

Figure 5.4: The dry root and shoot biomass averages observed for *A. afra* cuttings across the three treatments (mean ± standard error).

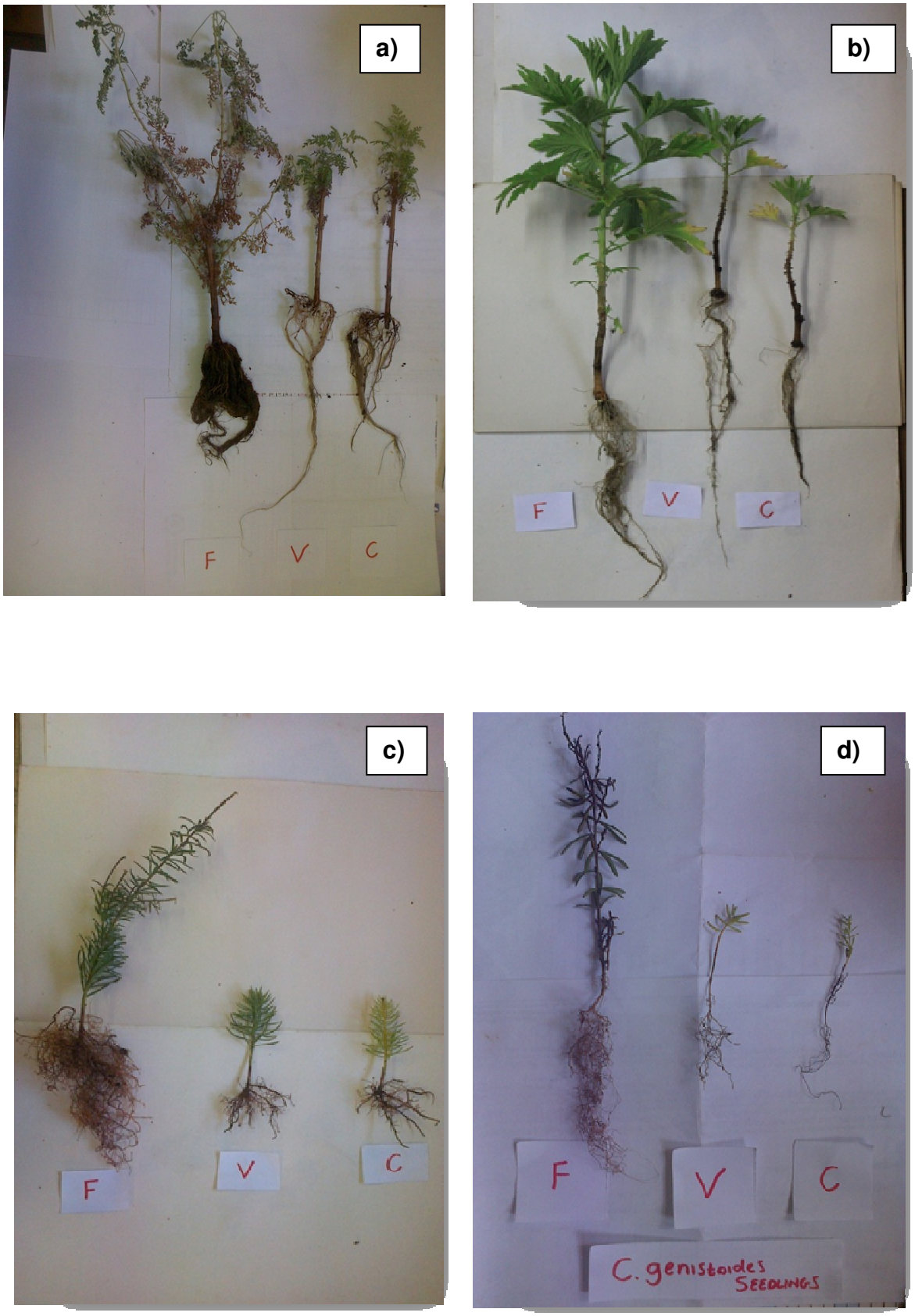

Figure 5.5: Photos taken after 15 weeks of growth for plant samples harvested from each treatment (F= fertiliser, V = vermitea; C = compost) prior to weighing and drying: (a) *A. afra* (cuttings), (b) *P. citronellum*(cuttings) (c) *C. genistoides* (cuttings) (d) *C. genistoides* (seedlings) (Photos by S. Faulconbridge 2013).

5.1.6 Analysis of final growing media

The results of the major nutrients observed in the three growing media at the end of the experiment are presented in Table 5.8. The fertiliser treatment had significantly greater NH_4 values than treatments C and V ($p < 0.001$). Observed values for P differed significantly between treatments V and F ($p = 0.002$) only. No significant differences were observed for K between treatments. A low significant difference was observed for carbon between treatments C and F ($p = 0.03$) only. A significant difference was observed for pH between treatments F and V ($p = 0.005$) and treatments F and C ($p = 0.01$).

Table 5.6: The available NH_4 exchangeable P and soluble K, carbon content and pH observed for soil samples taken from the growing media of all three treatments for each species (seedlings and cuttings) at the termination of the growing experiment (mean ± standard error, n = 5). Values with the same letter in the same row do not differ significantly ($p < 0.05$).

Variable	Treatment			Statistic
	Fertiliser	Compost	Vermitea	
NH_4 mg L^{-1}	311 ± 22.41^{b}	731 ± 57.86^{a}	750 ± 18.30^{a}	$F = 24.605$; df = 2; 12; $p < 0.001$
P mg L^{-1}	62.60 ± 3.67^{b}	76.80 ± 3.84^{ab}	87.60 ± 4.55^{a}	$F = 9.653$, df = 2, 12; $p = 0.003$
K mg L^{-1}	37.20 ± 5.49^{a}	43.60 ± 17.12^{a}	73.60 ± 14.39^{a}	$F = 2.137$; df = 2, 12; $p = 0.161$
Carbon %	1.86 ± 0.10^{a}	2.7 ± 0.32^{b}	2.36 ± 0.11^{ab}	$F = 4.244$, df = 2, 12; $p = 0.04$
pH (KCl)	7.76 ± 0.04^{a}	7.6 ± 0.03^{ab}	7.58 ± 0.02^{b}	$F = 9.700$; df = 2, 12; $p = 0.003$

5.2 Discussion

5.2.1 Growing media and amendments

All soil and compost/vermicompost analyses provided concentrations for available NH_4, available P and exchangeable K content NO_3 was not measured in any of the growing media or compost/vermicompost samples, and any assumptions of the actual concentrations of NPK delivered per treatment cannot be substantiated. The analysis of the compost, however, indicated that medium C should have seen an increase in available NH_4, soluble P and exchangeable K by as much as 52%, 33% and 38% respectively compared with medium F. The soil results for medium C did not, however, reflect this. This is surprising as all samples were bulked and dried according to standard procedure prior to laboratory analysis. The compost was of a relatively low pH with an acceptable C:N ratio.

These ratios have been established from the soil analyses and are approximations according to the available NH_4 concentration observed. C:N ratios have been described as important determinants of compost quality (Goyal *et al.* 2005), whereby a C:N ratio of 8:1 has been said to be most desirable for mature compost (Brady & Weil 2008a). Composts derived from garden wastes are generally low in nitrogen (Hashemimajd *et al.* 2004), and the compost used in this study was approximately 76% lower than the vermicompost analysis with regard to NH_4 content (Table 4.1).

In general, however, compost has been described as a suitable slow-release fertiliser and soil conditioner for container-grown plants in horticulture (Brady & Weil 2008a; Lazcano *et al.* 2009) and a 20% compost-amended growing medium has been deemed appropriate (Luxhøi *et al.* 2008). However, the methods of production are of importance in maintaining quality compost (Goyal *et al.* 2005), and additions of 10% (Zhang *et al.* 1996) and 20% garden waste compost have shown good results in other studies (Lazcano *et al.* 2009; St Martin & Brathwaite 2012). High approximate C:N ratios were observed for both growing media, possibly due to the high proportion of bark, and consequently low available NH_4 observed in the results. Surprisingly, the soil analyses indicated that both media C and F had comparable levels of Na and EC; however, the pH of medium C was markedly different from F and from that of the compost source. This pH variability is somewhat surprising.

The pH levels of growing media have been said to affect plant performance due to their influence on nutrient availability (Tisdale 1985a; Donald *et al.* 1994), especially with regard to available P and micro-nutrients (Bushman *et al.* 2009). Research has indicated that a pH (KCl) of 5.5–7.0 is optimal for woody plant growth (Mills & Cowling 2006) and 6.0–7.0 for potting media (Edwards *et al.* 2011b). This might be explained by the fact that P is most available at this range (Bushman *et al.* 2009) and that nitrification is enhanced at a pH of 5.5–6.5, as is microbial decomposition of organic matter (Tisdale 1985b), making nutrients (NH_4 and NO_3) available to plants (Mattson 2012).

Plant survival and growth can be seen as quantifiable measurements (Donald *et al.* 1994), both of which affect overall plant performance. Optimal plant performance can be achieved through the addition of sufficient nutrients, either organic or inorganic to the growing medium (Theunissen *et al.* 2010). The fertiliser (Multicoat®) treatment

can be seen as the standard fertiliser practice, and when applied at the manufacturer's recommended dosage, provided more than adequate nutrition for plant growth in this study.

Compost and vermitea can be seen as the organic treatments, and vermitea derived from vermicompost has been said to provide very little or no N (Handreck 1986). Consequently, vermitea has been said to contain lower levels of plant nutrients than vermicompost (Gutiérrez-Miceli *et al.* 2008; Gutierrez-Miceli *et al.* 2011), but they appear to stimulate macro and micro nutrient uptake (Arancon *et al.* 2005a). The chemistry of vermiteas is very complex (Pant *et al.* 2009; Edwards *et al.* 2011c) and there is very little published evidence of the actual NPK concentrations of produced vermiteas (Edwards *et al.* 2011c).

The produced vermiteas contained the full range of macro- and micro-nutrients described as essential for plant growth (Tisdale 1985a; Mattson 2012) (Appendix 3). Total N was not measured through laboratory analyses, and with no measurements for total N, total concentrations cannot be accurately calculated; merely approximations for comparative purposes can be made.

Nitrogen has been considered the major element limiting plant growth (Williams 2003; Dawson 2012). Significantly higher biomass readings were observed for all species at seedling and cutting level under the fertiliser treatment. These differences indicate that the fertiliser treatment received substantially higher available N compared with the other two treatments. Pots grown under the vermitea treatment received only a fraction more available N than the compost treatment, and biomass averages between these two treatments were very similar.

The average EC observed for the vermiteas was, however, higher than the recommended range of 200–300 mS m^{-1} for use on established plants (Edwards *et al.* 2011b). The initial pH of the media might have made certain nutrients unavailable to the plant, and a high EC could have affected water and thus nutrient uptake.

5.2.2 Plant survival

No statistically significant differences were observed for seedlings or cuttings. However, the vermitea treatment provided the highest survival values for cuttings of *P. citronellum* and *A. afra*, whereas, a higher survival was observed for *C. genistoides* cuttings under the compost treatment. Cuttings of *A. afra* showed very poor overall survival; however, the compost and vermitea treatments provided almost three times more viable plants than the fertiliser treatment. Even though similar conditions were maintained, major die back was observed for this species in the media during the study. This might have been as a result of transplant shock (Donald *et al.* 1994; Close *et al.* 2005) caused by potting. Koehorst *et al.* (2010) found that under hydroponic conditions, *A. afra* did not do well under acid or alkaline conditions with highest biomass observed at a pH of 6.5. Through heavier fertilisation, greater biomass might be observed. However, such an imbalance of nutrients has contributed to increased disease incidence (Tisdale 1985a).

Although this study did not assess pest or disease suppression, there is a large body of evidence that compost teas can decrease disease and pest incidence (Diver 2002; Scheuerell & Mahaffee 2002; Edwards *et al.* 2004; Scheuerell & Mahaffee 2006; Siddiqui *et al.* 2009). Furthermore, this study did not assess the biological component of the vermiteas. Improvements in plant survival have been attributed to bioactive (Siddiqui *et al.* 2011) and hormone-like compounds naturally found in organic materials (Atiyeh *et al.* 2002a; Arancon *et al.* 2003b; Arancon *et al.* 2006b; Siddiqui *et al.* 2009; Zandonadi & Busato 2012). Although the organic treatments did comparatively better than the fertiliser treatment, larger sample sizes might have produced more significant results with regard to survival.

The overall survival of plants in the organic treatments suggests that compost and vermitea can be a suitable means of plant propagation. Increased plant survival will lead to an increase in crop success (Lazcano *et al.* 2009) and will lead to a more efficient and reliable propagation programme (Compton 2008). Plant survival must, however, be considered in the context of plant growth to assess overall plant performance.

5.2.3 Plant growth measurements

Although the compost and vermitea treatments had better survival, seedlings and cuttings of all species showed much greater responses with regard to the fertiliser treatment for above and below ground biomass for all plants tested. The opposite was, however, observed for root to shoot ratios, with the exception of seedlings of *P. citronellum.* The examination of root to shoot ratios can be used to understand the relationship between root and shoot biomass and therefore might be used to indicate plant root biomass distribution (Mokany *et al.* 2006).

Root to shoot ratios indicate the position and quantity of plant tissue, with either a supportive or growth function (Allaby 1998). Plants with a higher proportion of roots will essentially be able to compete more effectively for soil nutrients (N and P), whereas plants with a higher proportion of shoots will be able to accumulate more light energy (Allaby 1998). This approach can be used as a rough indicator of physiological processes in plants and 'may thus reflect the cumulative response of plants to biotic, abiotic and management influences' (Mokany *et al.* 2006). The growth of new roots is essential for the plant to access sufficient P and K (Mattson 2012). Consequently, greater root biomass increases the exploitation potential of the soil by the plant and thus influences nutrient and water uptake (Lazcano *et al.* 2009) as was observed for all fertiliser treatment plants.

An increase in root to shoot ratio has been generally accepted as an indication of a healthier plant, provided that the increase is derived from a greater root weight and not a decrease in shoot weight (Wood & Roper 2000). Plants grown in high nutrient environments generally grow faster, and have lower root to shoot ratios than plants grown in low nutrient environments (Tilman 1988). This was observed in this study and can be explained by the fact that plants generally allocate more biomass to their leaves under high nutrient conditions (Tilman 1988), while the opposite seems true with root distribution under nutrient poor conditions (Chapin *et al.* 1987).

N fertilisation of non-leguminous plants has generally resulted in increased dry biomass (Dean & Clark 1980). This could explain the much greater biomass averages observed for both *A. afra* and *P. citronellum* and consequently the lower root to shoot ratios for both species under the fertiliser treatment. Increasing levels of

N have been directly related to increased plant growth of *A. afra* (Prinsloo *et al.* 2011) and *Pelargonium* species (Weiss 1997; Ram *et al.* 2003).

The compost and vermitea treatments provided comparable results for all parameters measured across all species with the exception of the root to shoot ratio for *C. genistoides* cuttings. Here the vermitea treatment provided a higher ratio compared with both other treatments. Very low nitrogen fixation has been observed in unfertilised legumes (Dean & Clark 1980) and the fertiliser treatment consequently provided greater biomass averages for cuttings compared with the seedlings of *C. genistoides* across treatments. Seedling nutrition is very important in the early stages; however, high levels of N can be detrimental (Donald *et al.* 1994).

In a recent study, Mbangcolo *et al.* (2013b) found that for cuttings of *C. genistoides,* both the dry root and shoot biomass averages were significantly increased with increasing additions of Nitrosol® organic plant NPK fertiliser (4:1:3) (1.6 & 3.33 mg L^{-1}) compared with the control. The average dry biomass trends

Figure 5.6: Dry root and shoot biomass averages of *C. genistoides* cuttings under different fertiliser treatments taken from two comparative studies. Results from this dissertation study (three columns on right) are compared with results observed in a recent study by Mbangcolo *et al* (2013) (three columns on left), who used two rates of Nitrosol® organic plant fertiliser (3.33 mg L^{-1}& 1.6 mg L^{-1}) over a period of 10 weeks.

observed in the study by Mbangcolo *et al.* (2013b) are comparable with those found in this study (Figure 5.6). The Nitrosol® 1.6 mg L^{-1} treatment produced marginally higher biomass averages than the compost and vermitea treatments of this study. The control in the Mbangcolo study provided a trend – with regard to root to shoot ratio – similar to the vermitea treatment in this study, which supports the statement by Chapin *et al*. (1987) of root allocation in low nutrient environments.

Spriggs & Dakora (2009) observed significant increases for all growth parameters measured when *C. genistoides* was inoculated with soil rhizobial bacteria. These authors stated that this species received approximately less than half of its N nutrition through symbiosis. Higher biomass readings might have been observed for this species as no rhizobial bacteria inoculations were carried out in this study.

There are few detailed reports where compost teas have been used on their own as sources of fertility (Scheuerell & Mahaffee 2002) and the effects on plant growth have been said to be quite anecdotal (Litterick *et al.* 2010). In a review by Theunissen *et al.* (2010), the contribution to plant growth by additions of vermicompost have been well documented. Many greenhouse trials have produced good results for a range of plants species with the use of vermicompost (Arancon *et al.* 2011) and vermiteas (Edwards *et al.* 2011c). Vermicomposts and vermiteas appear to have potential to improve plant growth and dry biomass yield when added to the soil (Atiyeh *et al.* 2000a; Zaller 2007b). However, in all of these studies, increases were observed with the combined use of synthetic or inorganic fertilisers, and therefore few pure organic examples exist (Arancon & Edwards 2011). Notably, this use increased the potential of organic fertiliser by increasing the efficiency of inorganic fertiliser (Kalantari *et al.* 2011; Siddiqui *et al.* 2011).

Vermiteas contain lower levels of plant nutrients, as has been observed in this study, and conventional wisdom suggests that they should be supplemented with nutrients for fast results (Pant *et al.* 2009; Gutierrez-Miceli *et al.* 2011). Losses of N through denitrification might have resulted in the lower NO_3 observed compared with other studies. Known organic additives such as kelp extract and humic acids increase the content of total N (Pant *et al.* 2009). The incorporation of good quality compost might have improved the biomass results under the two organic treatments. Other appropriate forms of organic N might be suitable for the organic market. Such

additives show promise for increasing plant biomass, while maintaining soil condition in the vermitea treatments.

5.2.4 Comparisons of final media

Results indicate that the overall pH of the growing media increased across treatments in comparison to the initial media. The observations of final pH are above 7.5 for all treatments and species. This is surprising, as the pH of the vermiteas averaged around 6.3, and could not have increased pH so significantly. The irrigation water was declared acceptable with regard to sodium; however, it did have a neutral pH (Appendix 1).

P becomes unavailable above a pH of 7.3 due to fixation (Lechmere-Oertal *et al.* 2005) and this has been observed in the final growing media, which shows comparable levels of P compared with the initial media for all treatments. Adequate and available P is required for root development, and plants deficient in P will exhibit slow growth, both in the roots and the shoots (Sposito *et al.* 2012). This was evident in both the compost and vermitea treatments, but not the fertiliser treatment. Significantly higher P was observed for samples under the vermitea treatment compared with the fertiliser treatment. As stated before, new root growth is essential for P uptake (Mattson 2012), and with greater root biomass, plants under the fertiliser treatments were able to access more P. Adequate P was provided for plants under the fertiliser treatment through the slow release fertiliser, regardless of the basic pH.

The fertiliser treatment also received higher levels of available N through the slow-release fertiliser. Significantly higher levels of NH_4 were observed in the media of the two organic treatments compared with that of the fertiliser treatment. These levels are comparable with the initial soil samples, however, NH_4 remains tied up in the soil as a slow release (Brady & Weil 2008c). Without measurements for total N, the data presented here are unreliable and it is not possible to form accurate conclusions with regard to total N between treatments.

The replenishment of nutrients is essential in maintaining plant growth (Windham 2008; Theunissen *et al.* 2010); however, the biomass averages observed here suggest that neither the compost nor vermitea treatments provided enough nutrients, specifically available N and P, for optimal plant growth. The fertiliser treatment

sustained plant growth throughout the study by providing adequate N and P in soluble form through the slow release fertiliser. Inoculation of soil rhizobial bacteria for *C. genistoides* might have improved the ability of these plants to fix atmospheric nitrogen, which might have resulted in increased biomass.

Compost and vermiteas have been well documented as soil conditioners (Zandonadi & Busato 2012), providing cost effective and environmentally sound alternatives to conventional fertilisers (Siddiqui *et al.* 2009). Soil health or condition are essential aspects of sustainable plant production (Litterick *et al.* 2010); however, both organic treatments did not provide enough nutrients for improved plant growth. Maintenance of an adequate pH and EC, in the range stated for optimal plant growth, could ensure that essential nutrients are made more available for the plant.

5.3 Conclusion

In this study, the overall survival of plants under the compost and vermitea treatments was promising. Biomass was significantly increased with the application of fertiliser for all species tested. The higher biomass averages corresponded with lower root to shoot ratios as well as lower overall survival. With improved plant survival, an increase in plant biomass potential should result from an increase in the number of viable stock plants produced.

Few studies have looked at using vermitea as a source of fertility on its own, and the combined use of fertilisers and vermitea has been recommended for biomass production. The use of high quality compost as a source of slow-release fertiliser might have increased the biomass averages under the organic treatments. Nitrogen-boosting amendments could further increase the potential of the organic treatments with regard to biomass production; however, these need to fit the organic label and be considered environmentally-friendly. Supplementing plants with organic fertilisers has economic as well as environmental advantages, but increased biomass does not necessarily reflect quality with regard to medicinal value. Even though the chemical composition of the plants was not looked at, there is evidence from other studies which shows significant increases in phenolic compounds of organically grown plants compared with fertilised plants, which might be of interest for the medicinal plant industry.

This study attempted to gauge the possibility of using an organic waste product as a natural plant fertiliser for medicinal plants. Plant survival was improved; however, biomass in the fertiliser treatment was stimulated by the supply of NPK. We cannot accept the hypothesis that plant performance was significantly different between treatments. Laboratory analyses indicated that the production of vermitea provided low overall N compared with other studies, and the methodology used might require attention. From a production point of view, effort is required to optimise these organic treatments so as to provide increased plant growth while producing the best quality plant (per species), while at the same time maintaining soil condition.

CHAPTER 6 – GENERAL DISCUSSION

6.1 Summary of major findings

The results of Experiment 1 indicate that vermiteas derived from kitchen waste vermicompost could have increased the rooting success of selected cuttings, but had no positive effects on germination. The production methods used, however, require attention as the high levels of electrical conductivity observed might have been the limiting factor behind germination. Increased sample sizes for both seeds and cuttings might provide more significant results in future studies.

The results of Experiment 2 indicate that organic amendments have the potential to increase the survival of seedlings and cuttings in the medium compared with fertiliser. Plants treated with fertiliser, however, had significantly higher biomass averages compared with the two organic treatments. The vermiteas provided very little overall nitrogen, and this might have resulted in the low biomass averages observed. A number of authors have highlighted the need to incorporate vermiteas with other nutrient sources for increased biomass production. Better quality compost might have served this purpose.

6.2 Cost effectiveness of propagation methods

A summary of the costs observed per treatment over two growing seasons are compared in Table 6.1. Costs per treatment vary due to the nature of the different input materials required and the production/transport costs of these materials. These costs are based on the actual costs accrued during the experiment, and these include the development of the campus's worm farm (including earthworm outlay) and construction of the vermitea brewing system.

For comparative purposes these costs have been approximated to indicate the costs which could possibly be accrued from a project twenty times the size of this study (large) (Appendix 6). The overall recurring costs expected per treatment over two seasons of growth have been included where applicable. The approximate cost effectiveness of this study over a five year period (small and large) has been calculated to indicate the potential income which could be generated per treatment.

Table 6.1: A summary comparing the actual costs of the three treatments used in this study over two seasons of growth. Irrigation, nursery design and construction costs are not included as they were the same for all treatments.

Expenses over 2 season growth period		**Treatment**		
		Fertiliser	**Compost**	**Vermitea**
Growing media	Sand	R 150	R 120	R 120
	Bark	R 150	R 120	R 120
	Compost	-	R 60	R 60
	Transport costs	R 50	R 50	R 50
	Pots	R 378	R 378	R 378
Equipment	Worm farm outlay	-	-	*R 1 500
	Earthworms	-	-	*R 1 000
	Brewing equipment	-	-	*R 530
	Sprayer equipment	-	-	*R 282
Amendments	Additives	R 0	R 0	R 29
	NPK fertiliser	R 500	R 0	R 0
Total		**R 1 228**	**R 728**	**R 4 069**

*Once off construction/outlay costs/expenses that would not be repeated in consecutive years.

Extra costs have been included to express the conventional practices of disease and pest management (source: Tuinroute Agri, George). The possible income generation has been calculated from actual plant survival scores observed per treatment – calculated at R 25 plant^{-1} – and also includes the approximate income derived (decreased cost of input materials) from the production of vermicompost/vermiteas and the growth of earthworms. These values are approximations only and will vary from system to system.

6.2.1 Compost treatment

In the short term, the compost treatment accrued the lowest costs compared with both other treatments. The ability to produce compost onsite would further lower these costs, however, the production of high quality compost is a technical process, often requiring expensive machinery (Goyal *et al.* 2005). Compost production will ultimately depend on the availability of suitable organic materials, and the quality will depend on the parent material and the process used (Edwards 2011). Organic plant production has political support in Africa, and organic fertilisers have been shown to be economical, due to the high costs and availability of fertilisers (Auerbach 2013). The ability to produce high quality compost onsite will have economic potential in the long run. Many organic growers are turning to compost teas and vermiteas for biofertility management, as the application of these is more economical than the solid forms (Salter & Edwards 2011).

6.2.2 Fertiliser treatment

No pesticides or fungicides were used in this study and subsequently the compost and vermitea treatments provided higher survival scores in the pot experiment than the fertiliser treatment. With higher production, the fertiliser treatment shows good economic potential in the short term. To maintain plant production, however, the fertiliser treatment would require ongoing inputs of fertiliser, and added inputs of fungicide and pesticide for improved plant survival. These costs have shown the fertiliser treatment to be far less cost effective in the long term, than both the organic treatments (Appendix 4).

6.2.3 Vermitea treatment

The vermitea treatment accrued the highest overall costs in the short term; this might be explained by the once off instalment/construction of the worm farm and the compost tea brewing equipment. The university earthworm farms were constructed on site, using old animal feed troughs. Low cost materials and equipment were used. The sale of earthworms is a commercial enterprise (Jensen *et al.* 2011) and the earthworms breed rapidly, doubling in mass approximately every two months (Dominguez & Edwards 2011b). The initial input costs for the earthworms would in time generate earthworm biomass, which would reduce the costs of stocking a larger system. As their numbers increase, the earthworms progressively consume more waste.

On-site kitchen waste and paper waste were used as the primary feedstock, reducing transport costs of input materials. The market value of vermicomposts is on the rise (Jensen *et al.* 2011), and the production of vermicompost onsite will lower the need for expensive input materials such as fertiliser (Zandonadi & Busato 2012). Vermicomposting has been shown to produce high quality compost, and there are a wide range of designs available for worm farming, ranging from low (labour - intensive but low - cost) to high technology (fully - automated and high - cost) (Jensen *et al.* 2011).

Low technology was used for producing all vermiteas on site; however, an electricity supply was required for production. Development of a larger vermitea brewing system would be a once off expense, and the application could be incorporated into existing irrigation/fertigation equipment (Salter & Edwards 2011). No new and expensive equipment would be required for vermitea applications. The use of vermiteas as soil drenches and foliar sprays are an attractive option for the organic industry; namely, for use in pest and disease management practices (Scheuerell & Mahaffee 2002; 2006). For these reasons, many growers have been able to reduce inputs of expensive pesticides and fungicides (Zandonadi & Busato 2012).

Over a five year period the vermitea treatment could potentially provide the highest profitability compared with the other two treatments. This is based on three factors; namely increased plant survival, the production of onsite vermicompost and thus vermiteas and the growth of earthworms.

6.3 Assumptions and limitations of study

This study focused on the selected plant species only, and results might differ between other species. This study was carried out from late spring 2012 through to autumn 2013, focussing on the initial four months of growth for each species. Results might differ over a more extensive period. This project was carried out under semi-controlled shade house conditions, and the results are relevant to the environmental conditions of the specified period only. Furthermore, this study was carried out in 12 cm pots, which might have imposed limitations on rooting ability. The use of 100% coconut coir as the initial rooting medium aimed to eliminate variability, and it is assumed that rooting and germination potential were equal across treatments.

All laboratory analyses were carried out at the Western Cape Department of Agriculture (Elsenburg), and it must be assumed that all methods were standardised for accurate results. Standard methods were maintained for each batch of vermitea produced, and it is assumed that any variability observed was due to the parent material (vermicompost) used. Laboratory analyses did not provide for total nitrogen for any of the soil or compost/vermicompost samples. This lack of total nitrogen prevented accurate calculation of the actual nitrogen provided per treatment as well as the establishment of accurate C:N ratios. Without a measurement for total N it was impossible to compare treatment effects accurately, as well as the overall NPK delivered per treatment. All pots under the fertiliser treatment received an equal portion of the specified growing media and slow-release fertiliser. All pots under the compost and vermitea treatment received an equal portion of compost in the initial media. It can be assumed that all pots per treatment received a comparable initial dose of nutrients.

Due to the complexity of testing the microbiology of composts and compost teas (Scheuerell & Mahaffee 2002; Pant *et al.* 2009), such analyses fell beyond the scope of this study. Furthermore, this study did not assess the actual presence or concentrations of plant growth regulators/hormones in the compost or the vermitea. There is extensive literature documenting the high diversity of beneficial microorganisms (Scheuerell & Mahaffee 2004; Siddiqui *et al.* 2009; Siddiqui *et al.* 2011) and the presence of plant growth regulators/hormones and their roles in plant growth and development (Atiyeh *et al.* 2002a; Arancon *et al.* 2003b; Arancon *et*

*al.*2006b; Zandonadi & Busato 2012). It follows that germination and rooting potential should be improved with vermitea treatment versus no treatment.

Pest and disease management, assessment and identification did not form part of this study. Organic materials have been well documented in the suppression of plant disease and pest incidence (Zhang *et al.* 1996; Scheuerell & Mahaffee 2002; Scheurell & Mahaffee 2004; Siddiqui *et al.* 2008; Siddiqui *et al.* 2009; Siddiqui *et al.* 2011). The researcher assumed that compost and vermitea should provide some positive effects on plant performance. No rhizobial bacteria inoculations were carried out for either of the legume species used in this study, and no checks were carried out as to whether rhizobium was present before or after seedlings/cuttings were propagated. This project did not assess the medicinal or phenolic properties of the different species under different treatments, but rather focussed on comparisons of plant survival and growth.

6.4 Recommendations

6.4.1 Experiment 1

Due to very poor overall germination, future studies should assess the effect of vermiteas produced from different vermicomposts at different concentrations (5–40%). Differences in processes and feedstock have shown marked difference in produced vermicomposts (Edwards *et al.* 2011b). These studies should monitor the levels of electrical conductivity and pH of the different vermiteas during brewing, and production methods should be altered if unacceptable levels are observed.

Future studies should increase sample sizes for both seeds (> 900) and cuttings (> 150) so as to provide more robust results across treatments than were observed here. Numerous studies have observed species-specific responses to different concentrations and rates of vermiteas, and therefore finding the optimal treatment for a target species would be ideal. Laboratory analysis should be undertaken to assess the actual concentrations and diversity of the biological properties (microbial community & plant growth regulators/hormones) found in various vermiteas derived from different vermicompost sources. Future work should test these biological properties where possible, between the ranges of vermiteas produced, so as to quantify the effects of these properties on species specific plant performance.

6.4.2 Experiment 2

A more accurate understanding of the soil dynamics with regard to nitrogen availability and uptake will be useful in future studies. Looking at the biomass production, total nitrogen might have been a limiting factor under the organic treatments. Laboratory analyses provided very low overall values for nitrogen in the vermiteas. Future studies should examine nitrogen boosting amendments such as humic acids, kelp powder, rock dust and fish emulsions (Salter & Edwards 2011). These additives can be included into the brewing process with other essential elements so as to economise on spray applications. This study was carried out on a small scale with the use of a knapsack sprayer. However, vermiteas can be incorporated into a more comprehensive fertigation/irrigation system for ease of application as a soil drench or foliar spray (Scheuerell & Mahaffee 2006). Future work should incorporate the specified inoculations used for each legume species so as to increase N_2 fixation potential, and thus increase the biomass potential of these species.

The brewing time for vermiteas used in this study was 34 hours (Scheuerell & Mahaffee 2002; 2004); however, work done at the Soil Ecology Laboratory at Ohio State University recommended brewing time around 24 hours (Edwards *et al.* 2011c). This longer brewing time might have been the reason for the very high EC values observed in the vermiteas. For this reason, ongoing monitoring of the electrical conductivity and pH will be necessary in future studies. For comparison, future studies should assess the EC of teas produced over a range of brewing times between 12 and 36 hours. These studies should compare changes in the chemical and biological properties across this range.

Aeration is essential for extracting and increasing the microorganism diversity and biomass in vermiteas. Although constant aeration was provided for vermiteas, laboratory analyses did not provide for dissolved oxygen content or for microbial-N biomass. No comparisons could therefore be made between samples to assess if adequate aeration was provided or to assess changes in microbial-N. Dehydrogenase activity has been shown to correlate positively with microbial biomass, and can be used as a good indicator of total microbial activity (Edwards *et al.* 2011c). Monitoring of the chemical and biological characteristics of vermiteas can help to establish optimal brewing times.

Organically grown plants have shown increases in their phenolic content to which has been attributed to their pest resistance, and also to their potential in the medicinal plant and food industry (Theunissen *et al.* 2010). Future research should investigate the phenolic and nutrient physiology of target species, comparing wild grown stock vs. organically grown stock. Comparisons should be made across a variety of different organic amendments, with the aim of promoting biomass and phenolic/antioxidant properties of target species.

Vermitea and organic amendment applications and rates vary according to the species, cropping system, pest and disease pressures and existing conditions (St Martin & Brathwaite 2012). Collaborative studies should be carried out between researchers working in all aspects of this industry; from plant production, microbiological and biological aspects of the organic amendments, to those working with the phenolic/chemical/nutrient quality of the plants. Such collaboration will help growers to develop an understanding of the optimum organic amendments required per species per area.

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APPENDIX 1

The physiochemical properties of the two water sources used for this experiment, taken at six month intervals.

	December 2012		June 2013	
Variable	Municipal water	Rainwater	Municipal water	Rainwater
pH	7.0	5.0	7.2	5.0
EC (mS m^{-1})	17	3	21	4
Total dissolved solids mg L^{-1}	111	20	137	29
NH_4 mg L^{-1}	0.40	N/A	0.30	N/A
NO_3 mg L^{-1}	< 3.0	< 3.0	< 3.0	< 3.0
P mg L^{-1}	1	-	1	-
Ca mg L^{-1}	3	1	5	1
Mg mg L^{-1}	2	1	3	1
K mg L^{-1}	1	1	1	1
Na mg L^{-1}	32	3	34	2
Cl mg L^{-1}	35.50	7.10	46.17	6.90
SO_4 mg L^{-1}	3	1	3	1
Bicarbonate mg L^{-1}	40.00	5.00	35.00	5.00
Cu mg L^{-1}	0.1	0.03	0.23	0.05
Fe mg L^{-1}	0.04	0.02	0.05	0.01
Mn mg L^{-1}	0.01	0.01	0.01	0.01
Zn mg L^{-1}	1.20	-	1.30	-
B mg L^{-1}	0.02	0.01	0.02	0.01
Alkalinity mg L^{-1}	40	5	35	5
Cations(meq L^{-1})	1.73	0.29	2.07	0.25
Anions (meq L^{-1})	1.72	0.30	2.08	0.28
Hardness	16	7	25	7

APPENDIX 2

The physiochemical characteristics of the two growing media, the garden waste compost and the kitchen waste vermicompost used in this study prior to conversion.

Variable	Media		Pure compost (n = 1)	Pure vermicompost (mean ± standard error, n = 2)
	F (n = 1)	C (n = 1)		
pH (KCl)	6.60	7.40	5.40	6.8 ± 0.5
EC mS m^{-1}	2.00	2.00	3.00	100 ± 22
Ca (cmol (+) kg^{-1})	51.90	47.50	13.86	443 ± 115.8
Mg (cmol (+) kg^{-1})	2.94	2.92	4.09	285.9 ± 95
K (mg kg^{-1})	150.00	181.00	610.00	11400 ± 200
Na (mg kg^{-1})	102.00	101.00	81.00	4956 ± 2740
P (mg kg^{-1})	78.00	94.00	274.00	4950 ± 1450
Total cations (cmol (+) kg^{-1})	55.68	51.33	21.25	-
Cu (mg kg^{-1})	0.31	0.05	2.49	29 ± 2.19
Zn (mg kg^{-1})	5.47	0.15	26.47	239.2 ± 101
Mn (mg kg^{-1})	8.28	2.54	58.68	189.2 ± 31
B (mg kg^{-1})	0.30	0.23	1.10	18.6 ± 1.30
C (%)	1.54	1.99	5.50	24 ± 0.05
Fe (mg kg^{-1})	112.10	27.74	666.60	6624 ± 24
NH_4 (mg kg^{-1})	700.00	900.00	4500.00	17600 ± 3900

APPENDIX 3

The physiochemical characteristics of vermitea batches produced for the medicinal plant trials (mean ± standard error, n =18).

Variable	Vermitea
pH (KCl)	6.3 ± 1
EC mS m^{-1}	350 ± 48
NO_3 mg L^{-1}	>3.0
NH_4 L^{-1}	0.59 ± 0.1
P mg L^{-1}	31.39 ± 3
K mg L^{-1}	654.17 ± 20
Ca mg L^{-1}	125.1 ± 6
Mg mg L^{-1}	80.9 ± 6
Na mg L^{-1}	103.8 ± 6
Mn mg L^{-1}	0.65 ± 0.07
Fe mg L^{-1}	1.62 ± 0.26
Cu mg L^{-1}	0.04 ± 0.01
B mg L^{-1}	0.21 ± 0.01
Zn mg L^{-1}	0.24 ± 0.03

APPENDIX 4

Growth measurements observed for the two species of medicinal plant seedlings tested under three experimental treatments (mean ± standard error). Survival rate was quantified and thus samples sizes varied between treatments. (DRM = dry root mass [g plant $^{-1}$]; DRM = dry root mass [g plant $^{-1}$]; RSR = dry root: shoot ratio). Values with the same letter in the same row do not differ significantly ($p < 0.05$).

Variable	Species	Treatment			Statistic
		Fertiliser	Compost	Vermitea	
DRM	*C. genistoides*	0.13 ± 0.01^{a}	0.02 ± 0.00^{b}	0.02 ± 0.00^{b}	$F = 69.63$; df = 2, 53; $p < 0.001$
DSM	*C. genistoides*	0.21 ± 0.02^{a}	0.01 ± 0.00^{b}	0.01 ± 0.00^{b}	$F = 281.90$, df = 2, 53; $p < 0.001$
RSR	*C. genistoides*	0.61 ± 0.04^{a}	1.97 ± 0.18^{b}	2.04 ± 0.15^{b}	$F = 29.52$; df = 2, 53; $p < 0.001$
DRM	*P. citronellum*	2.46 ± 0.96^{a}	0.05 ± 0.01^{b}	0.04 ± 0.01^{b}	$F = 88.67$; df = 2, 68; $p < 0.001$
DSM	*P. citronellum*	2.83 ± 0.25^{a}	0.07 ± 0.01^{b}	0.06 ± 0.01^{b}	$F = 119.13$, $df = 2, 68$; $p < 0.001$
RSR	*P. citronellum*	0.87 ± 0.05^{b}	0.71 ± 0.03^{a}	0.67 ± 0.06^{ab}	$F = 3.991$; df = 2, 53; $p = 0.023$

APPENDIX 5

Growth measurements observed for the three species of rooted cuttings under three treatments (mean ± standard error). Survival rate was quantified and thus sample sizes varied between treatments. (DSM = dry shoot mass [g plant $^{-1}$]; DRM = dry root mass [g plant $^{-1}$]; RSR = dry root: shoot ratio). Values with the same letter in the same row do not differ significantly ($p < 0.05$).

Variable	Species	Treatment			Statistic
		Fertiliser	Compost	Vermitea	
DRM	*C. genistoides*	0.45 ± 0.03^{a}	0.27 ± 0.02^{b}	0.26 ± 0.01^{b}	F = 35.517, df = 2, 126; $p < 0.001$
DSM	*C. genistoides*	0.52 ± 0.04^{a}	0.29 ± 0.02^{b}	0.20 ± 0.01^{b}	F = 45.859, df = 2, 126; $p < 0.001$
RSR	*C. genistoides*	0.87 ± 0.04^{b}	0.93 ± 0.05^{b}	1.30 ± 0.12^{a}	F = 23.554, df = 2, 126; $p < 0.001$
DRM	*P. citronellum*	0.81 ± 0.10^{a}	0.21 ± 0.03^{b}	0.20 ± 0.02^{b}	F = 43.536, df = 2, 82; $p < 0.001$
DSM	*P. citronellum*	5.32 ± 0.59^{a}	0.61 ± 0.08^{b}	0.64 ± 0.06^{b}	F = 86.688, df = 2, 82; $p < 0.001$
RSR	*P. citronellum*	0.15 ± 0.01^{a}	0.34 ± 0.04^{b}	0.31 ± 0.03^{b}	F = 11.808, df = 2, 82; $p < 0.001$
DRM	*A. afra*	1.03 ± 0.41^{a}	0.14 ± 0.03^{b}	0.12 ± 0.03^{b}	F = 14.049, df = 2, 37; $p < 0.001$
DSM	*A. afra*	2.18 ± 0.63^{a}	0.27 ± 0.06^{b}	0.20 ± 0.05^{b}	F = 15.413, df = 2, 37; $p < 0.001$
RSR	*A. afra*	0.47 ± 0.06^{c}	0.52 ± 0.07^{c}	0.60 ± 0.06^{c}	F = 0.812, df = 2, 37; p = 0.451

APPENDIX 6

Comparisons of the cost effectiveness of the three treatments used in this study over two seasons of growth (small). These costs have been calculated to indicate the approximate costs a grower might encounter on the expansion of the project by up to twenty times the original size (large). Overall calculations have been included to indicate the approximate profitability (income – expenses) that might be expected (both small & large scale) over a 5 year period.

		Treatment					
Initial outlay		Fertiliser		Compost		Vermitea	
Expenses over 2 season growth period		Small	Large	Small	Large	Small	Large
Growing media	Sand	R 150	R 3 000	R 120	R 2 400	R 120	R 2 400
	Bark	R 150	R 3 000	R 120	R 2 400	R 120	R 2 400
	Compost			R 60	R 1 200	R 60	R 1 200
	Transport costs	R 50	R 1 000	R 50	R 1 000	R 50	R 1 000
Equipment	Worm farm outlay					*R 1 500	*R 50 000
	Earthworms					*R 1 000	*R 18 000
	Brewing equipment					*R 530	*R 12 000
	Sprayer equipment	*R 282				*R 282	
Pest/disease control	Fungicides	R 80	R 1 600				
	Pesticides	R 60	R 1 200				
Amendments	Additives	R 0		R 0		R 29	R 580
	NPK fertiliser	R 500	R 10 000	R 0	R 0	R 0	
Propagation materials	Rooting hormone	R 294	R 5 880	n/a	n/a	R 0	R 0
Subtotal		**R 1 566**	**R 25 680**	**R 350**	**R 7 000**	**R 3 691**	**R 87 580**

Table continued

Recurring costs over additional 2 season growth period		R 1 284	R 25 680	R 350	R 7 000	R 379	R 7 580
Income over 2 season growth period							
Plant survival	Seedlings (R 25 plant^{-1})	R 1 050	R 21 000	R 1 100	R 22 000	R 1 025	R 20 500
	Cuttings (R 25 plant^{-1})	R 1 725	R 34 500	R 2 125	R 42 500	R 2 325	R 46 500
Vermiculture	Vermicompost (R 8 kg^{-1})					R 320	R 6 400
	Earthworm growth (R 1 g^{-1})					R 2 500	R 45 000
Subtotal		R 2 775	R 55 500	R 3 225	R 64 500	R 6 170	R 118 400
Total profitability (Income - expenses)		**R 1 209**	R 29 820	R 2 875	R 57 500	R 2 479	R 30 820
5 year profitability over 10 growth seasons		**R 14 346**	**R 298 200**	**R 28 750**	**R 575 000**	**R 51 286**	**R 948 200**

* Once off construction/outlay costs/expenses that would not be repeated in consecutive years. The scale of worm farm design ranges from R15 000 - R100 000 depending on the availability of organic waste material (Source: Jensen *et al*. 2011). Commercial compost tea brewing equipment are available ranging from R 5 000 - R28 000 depending on the scale of production (Source: www.growingsolutions.com).