GROWTH, MINERAL CONTENT AND ESSENTIAL OIL QUALITY OF BUCHU (*AGATHOSMA BETULINA*) IN RESPONSE TO pH UNDER CONTROLLED CONDITIONS IN COMPARISON WITH PLANTS FROM ITS NATURAL HABITAT

by

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Declaration

I, the undersigned, hereby declare that the work contained in this thesis is my own original work and has not previously been submitted in its entirety or in part at any University for a degree.

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ABSTRACT

The Cape Floristic Region is a highly distinctive phytogeographical unit which is recognized as a floral Kingdom on its own. Buchu (*Agathosma betulina*) plants fall under this important Kingdom. Buchu is one of the traditional medicinal plants originating in the Western Cape province of South Africa and the essential oil derived from the leaves is exported in large volumes. Due to high demand, under supply, restrictions of wild harvesting and high prices for Buchu essential oil, growers have started to introduce and commercialize this species as a crop. This commercialization of Buchu necessitated agronomic research to optimize production techniques. The objective of this study was to determine the optimum pH range for the cultivation of high yielding Buchu with acceptable essential oil quality under controlled conditions and compare this with the conditions in the natural habitat. Plant, soil and climatic data were gathered from eleven sites in the natural habitat of Buchu (*A. betulina)* in the Cederberg Mountains. At all sites most rainfall occurred from May to September, while high temperatures were recorded in summer. Soil analyses indicated low levels of nutrients and low soil pH, ranging from 3.7 to 5.3 at all the sites studied. Low levels of nutrients were also obtained from foliar analysis collected from plants at each of the different sites. Chemical analyses of the essential oil indicated that the plants were from a high quality diosphenol chemotype.

In the greenhouse experiment, five different pH levels (pH 33.99, 4-4.99, 5-5.99, 6-6.99 and 7-7.99) were evaluated to determine the effect on growth, yield and quality of *A. betulina*. Complete nutrient solutions were used to irrigate the plants grown in pots filled with a sand and coco peat mixture. Although the plants subjected to the pH treatment of 4-4.99 tended to have the highest growth rate and yield, this did not differ significantly (P=0.05) from plants subjected to pH values between 3 and 6.99.

In contrast, the pH 7-7.99 treatment lead to reduced growth and lower vegetative yields. Levels of nutrients obtained from the leaf mineral analysis differed significantly with different pH treatments. High pH levels resulted in high nitrogen, phosphorus, sodium, manganese and boron contents, but lower contents of copper. Nitrogen, phosphorus, calcium and zinc were higher than those recorded for plants from their natural habitat, but still within the norm reported for most plants. Levels of manganese, sodium, magnesium and copper were found to be more or less similar to the values obtained in plants from the natural habitat. No significant differences were found in essential oil quality in response to the pH treatments. However, high pulegone levels (10.8 to 13.2 %) were obtained from all the treatments in the greenhouse experiment. The high levels of this essential oil constituent could have a negative effect on the marketability of the oil and this aspect may need some attention in future studies.

UITTREKSEL

Die Kaapse Fynbos bioom is ‘n hoogs unieke plantegroeistreek en word as ‘n afsonderlike planteryk beskou. Boegoe (*Agathosma betulina*) vorm deel van hierdie planteryk en is een van die tradisionele medisinale plante wat hul oorsprong in die WesKaap Provinsie van Suid-Afrika het. Weens die hoë aanvraag, gevolglike ondervoorsiening, beperking op die oes van plante in hul natuurlike omgewing en hoë pryse is daar begin om boegoe kommersieël te verbou. Hierdie kommersialisering het egter die behoefte na agronomiese navorsing laat ontstaan om optimale produksietegnieke te ontwikkel. Die doel van hierdie studie was om die optimum pH peile vir hoog produserende boegoe plante met ‘n goeie essensiële olie-inhoud onder gekontroleerde groeitoestande te bepaal en te vergelyk met toestande wat in die natuurlike groeiomgewing ondervind word. Plant, grond en klimaatsdata wat versamel is van elf lokaliteite in die Cederberge waar boegoe natuurlik voorkom, het getoon dat hierdie gebied meestal reën kry in die maande Mei tot September en dat hoë temperature in die somer voorkom. Grondontledings van die verskillende lokaliteite toon dat die grond baie arm aan voedingstowwe is en die grond pH tussen pH (KCl) 3.7 – 5.3 wissel. Lae vlakke van plantvoedingstowwe is ook gevind in die plantmonsters wat ontleed is. Chemiese ontledings het getoon dat die plante van die hoë kwaliteit diosfenol chemotipe is.

In die kweekhuis eksperiment is die invloed van vyf verskillende pH vlakke (pH 3-7.99) op die groei, opbrengs en kwaliteit van *A. betulina* ondersoek. Volledige voedingsmengsels is gebruik om die plante wat in potte gevul met ‘n sand en klapperhaar mengsel gegroei het, te besproei. Hoewel die plante wat by ‘n pH van 4-4.99 gegroei het, geneig het om die vinnigste te groei en die hoogste opbrengs te lewer, was dit nie statisties (P0.05) beter as plante wat by pH vlakke van 3-6.99 gegroei is. Boegoe plante wat by ‘n pH vlak van 7-7.99 gegroei is, het egter betekenisvol

stadiger gegroei en laer vegetatiewe massas geproduseer. Die chemiese samestelling van die plantmonsters is ook deur die pH van die besproeingswater beïnvloed. Hoë pH vlakke het die stikstof-, fosfor-, natrium-, mangaan- en boor inhoude van die plante verhoog, maar laer koper inhoude tot gevolg gehad. Die stikstof-, fosfor-, kalsium- en sink inhoude was hoër as wat gevind is in plante in hul natuurlike omgewing, maar was steeds binne die norme wat in die algemeen vir plante gestel word. Die mangaan-, natrium-, magnesium- en koper inhoude van die plante in die kweekhuiseksperiment was ongeveer dieselfde as vir plante in hul natuurlike omgewing. Hoewel die olie-kwaliteit nie deur die pH behandelings beïnvloed is nie, het alle plante hoë pulegoon inhoude tot gevolg gehad. Dit kan die markwaarde nadelig beïnvloed en hierdie aspek sal vir opvolg studies ondersoek moet word.

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CHAPTER 1

INTRODUCTION

Buchu (*Agathosma betulina*) falls under the protected Kingdom Flora of the Cape Floristic Region (CFR) and Cape Nature Conservation exercises very strict control over the harvesting of wild plants in order to prevent exploitation and destruction of the wild Buchu stands. It is one of the traditional medicinal plants in the Western Cape Province of South Africa and the essential oil derived from the leaves is exported in large volumes. The essential oil is not only used for medicinal purposes but also as a flavour fixative in the food industry (Coetzee, Jefthas & Reinten, 1999).

Dry Buchu was first introduced into the pharmaceutical industry in the United Kingdom in 1821 (Pillans, 1910). The Cape Colonists of South Africa exported dry Buchu leaves to Reece & Company, based in United Kingdom (Simpson, 1998; Rust, 2003). In 1873, the total exports from the Cape Colony to Reece & Company were about 181,440 kg of dried leaves, of which nearly 27,216 kg were exported to the United States of America (Simpson, 1998). Between 1910 and 1914 an average of 92,600 kg of leaves was exported annually, which at the time earned South Africa approximately R60800 in foreign revenue (Pillans, 1910). At present, 1 kg of fresh material earns between R40 and R60 and the price of 1 kg of dry leaves is about R320. The essential oil is obtained from the fresh material through distillation, with a yield of 1 - 2% (Schneider & Viljoen, 2002), and sells for R5500 - R6000 per kilogram. It is expected that the international demand for natural flavourants and fragrances will rise by more than 7% per annum in the next decade (WESGRO, 2000). Currently, most of the Buchu material harvested in South Africa is distilled and sold as essential oil (Coetzee *et al*., 1999). According to WESGRO (2002), 600 tons of Buchu oil were being sold annually and it was estimated that the Cape industry was earning R45 million from these sales. However, Cape Nature (2004) reported that the Buchu industry was earning about R120 million per annum. Approximately 65% of the harvest comes from wild populations. About 95% of the total production is exported, with Europe being the most important export destination. Currently the price of *A. betulina* oil is ranging between R2500 to R3000 per litre while the fresh material ranges between R15 to R30 per kilogram (Dr. M. Esterhuysen, 2007, pers. comm., Puris Natural Aroma Chemicals (Pty) Ltd, P. O. Box 12127, Die Boord 7613, South Africa

Due to the high demand, under supply and price for Buchu oil, growers have started to commercialize Buchu and vast fields have been established. The commercialization of Buchu necessitated agronomic research in order to optimize production practices. To date, very little research has been done on the production of Buchu. However, funds were heavily invested in research focusing on the essential oil and its constituents, and also medical research. The consequence is that very little published literature is available on the production of Buchu.

The main objective of this study was to determine the ideal pH range for the cultivation of high yielding, early maturing Buchu with acceptable essential oil quality. This was achieved by conducting a field survey of the natural habitat of Buchu, whereby climatic data and soil mineral content was studied. Plant vigor, leaf mineral content and essential oil constituents was determined from samples harvested in the natural habitat. Secondly, a greenhouse experiment was conducted to determine the application of hydroponic techniques to determine the ideal pH range for the production of Buchu under protected conditions. Biomass accumulation, leaf mineral composition and essential oil constituents was studied.

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CHAPTER 2

LITERATURE REVIEW

1 Historical background

Buchu (*Agathosma betulina*) is indigenous to South Africa and grows wild in the mountains of the Western Cape (Von Wielligh, 1913). It is part of the cultural heritage of the Khoikhoi and San people, who used the dried and powdered leaves mixed with sheep fat to anoint their bodies (Spreeth, 1976). Khoikhoi and San people prepared brandy by distilling the leaves with wine, which was then used as an efficient remedy for all infections of the stomach, bowels and bladder (Pillans, 1910; Watt & Breyer-Brandwijk, 1962; Roberts, 1990; Bruneton, 1995). They also applied a decoction of the leaves to wounds (Simpson, 1998). Buchu leaves may be steeped in vinegar to make a medicine for chest problems and cleansing wounds (Farnsworth & Soejarto, 1991; Arkcoll, 1997; Blomerus, Coetzee & Reinten, 2001).

As the European demand for the product increased, the value and marketability increased (Van Wyk, Van Oudtshoorn & Gericke, 1997). Buchu was first exported as a medicine in the early 1800's and it is still exported today in the form of packaged dry leaves and essential oil (Pillans, 1910; Roberts, 1990; Simpson, 1998). The increase in demand lead to poor harvesting procedures, over-harvesting and harvesting during the wrong time of the year which prevented Buchu from producing seeds for the next generation (Blomerus, 2002). Pests and disease problems and increased commercial agriculture were destroying the natural habitat of Buchu, leaving it with less space to grow.

The commercial value and demand of Buchu products has lead to commercial cultivation of this plant (Lubbe, Denman &

Lamprecht, 2003). Due to the commercial interest in the crop, research was initiated on propagation techniques and essential oil quality. However, little is known about the crop, and agronomic research is required (Coetzee, 2001). Past research concentrated largely on the chemical characterization of the Buchu oil, the impact of different Buchu species on essential oil composition and the medical and food applications of this indigenous plant and plant product. Buchu has a very high commercial value and used today largely in the food and flavour industries as a flavor enhancer (Blomerus *et al.*, 2001).

2 Botanical description

Buchu species belong to the economically important family Rutaceae, which includes citrus (Spreeth, 1976; Handforth, 1998). Most plants under the Rutaceae family are trees, shrubs, rarely herbs and frequently aromatic (Dyer, 1975).

Buchu is a shrub that grows up to two meters in height. The leaves are about 20 mm long, wedge-shaped towards the petiole, with a rounded apex which curves backwards (Pillans, 1950; Van Wyk & Wink, 2004). This odiferous, multi-stemmed bush has twiggy, angular branches of a purplish-brown color (Spreeth, 1976). The conspicuous oil glands are present along the margins and lower surface of the leaves (Van Wyk *et al.*, 1997; Rust, 2003). The leaves have a characteristic smell when crushed. The flowers are small, star-shaped and white or pale purple in color (Van Wyk & Wink, 2004). The fruit comprises of five upright carpels, each containing a single, oblong and shining black pyriform seed (Van Wyk & Gericke, 2000; Cloete, 2005).

3 Geographic distribution

Buchu populations are mainly concentrated in the south-western Cape and extend to Kwazulu-Natal, including about 135 species. However, the natural habitat of the commercially important species *Agathosma betulina* is mainly in the Western Cape Mountains (Figure 1). Buchu is limited to the higher mountain slopes of Piketberg, Ceres, Tulbagh, Citrusdal, Clanwilliam and Calvinia (Spreeth, 1976). According to Feldma & Youngken (1944) the natural distribution of Buchu is frequent on the Schurfdebergen, the Olifants River Mountains and Cederberg Mountains.

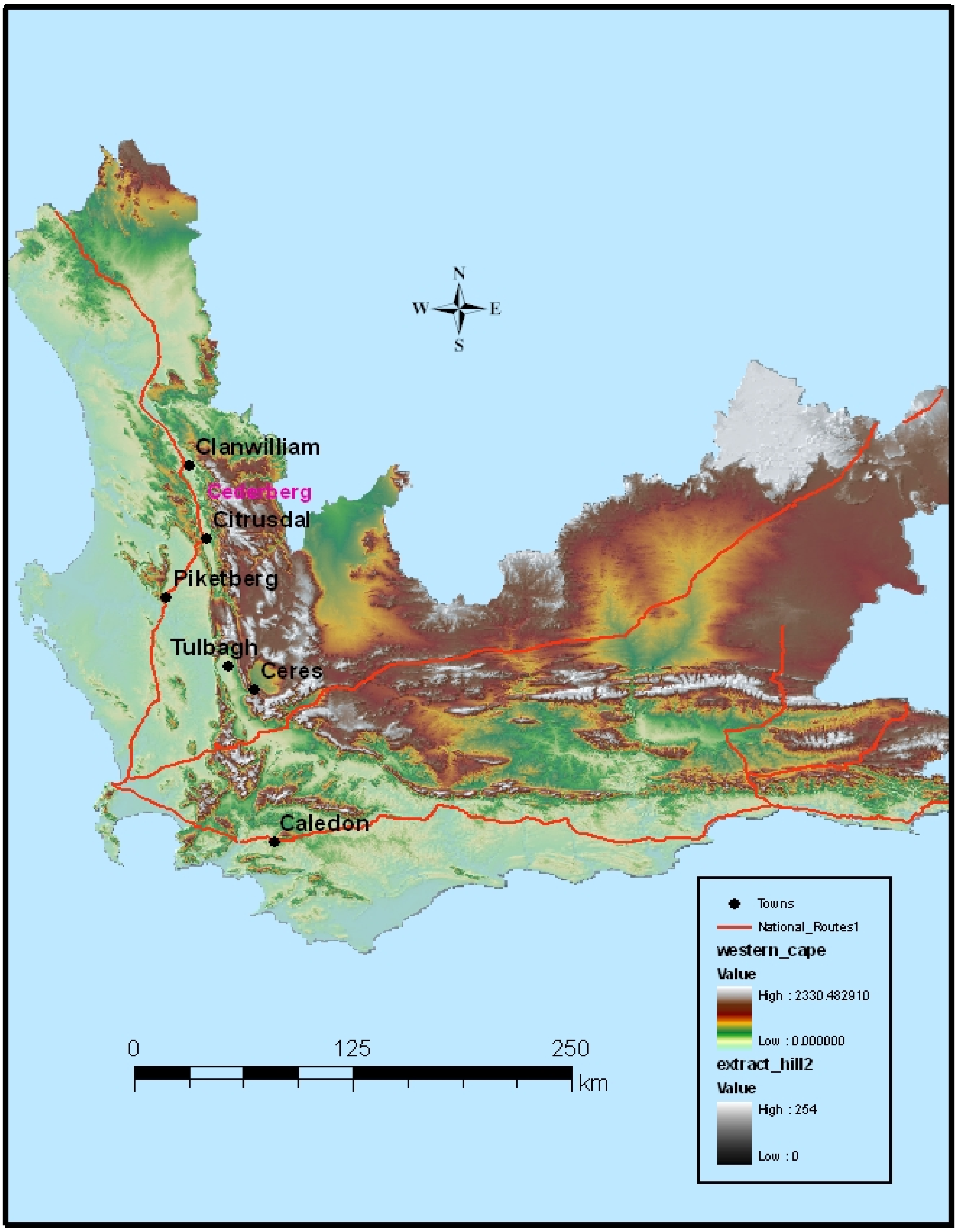

Figure 1 Geographic distribution of *Agathosma betulina* in the Western Cape, South Africa

4 Growth requirements

Buchu is a minor member of fynbos vegetation and adheres to the fynbos fire-prone life span of between 10 to 15 years becoming woody as they age. They perform best when planted in the full sun. Experiments to determine whether Buchu can be cultivated commercially were conducted at Kirstenbosch National Botanical Garden in 1920. The experiments yielded positive results and the farmers started to cultivate Buchu commercially since then (Werner, 1949).

Even though the experiments started many years ago, the information on the plants' growth requirements is quite limited especially in terms of soil, water and nutritional needs and adaptability. Many Buchu species are restricted to certain soils (acidic), altitudes, aspects and climatic conditions. Buchu is fairly specific as to where it grows naturally, that is, at a certain altitude ranging from 1 737 to 2 028 m above sea level on south-west facing slopes (Blommaert, 1972a).

4.1 Soil requirements

Buchu grows on similar soils as other fynbos plants. The soils are generally coarse graded, acidic, nutrient poor and infertile (Maitre & Midgley, 1992; Arkcoll, 1997). The ecology of the vegetation of the infertile sandy soil is fragile and species are endangered through disturbance and because of their limited ranges, (Bond & Goldblatt, 1984). Fynbos soil is infertile mainly because it is derived from rocks which are poor in nutrients (Goldblatt & Manning, 2000). The rocks which are associated with these nutrient poor soils are the quartzites and hard sandstones. Buchu is found growing naturally on mountains and most of these mountain soils are whitish, low in pH, sandy and both shallow and rocky (Cowling & Richardson, 1995).

In its natural environment, Buchu grows in sandy to sandy loam soils (Arkcoll, 1997). The experiments done at Kirstenbosch, however, indicated that Buchu grows well in deep, reddish, light to medium loam soils (Werner, 1949).

Buchu plants grow well in acidic soils with a pH range of 3.5 to 4.5 with a maximum pH of 5.5 (Gentry, 1961), while low phosphate contents (less than 20 ppm) are preferable. Buchu plants do not tolerate saline soils. The soil for Buchu cultivation should be well drained with a minimum depth of 600 mm. (Mr. A Harris, 2006, pers. comm., Buchu Moon, P. O. Box 39, Wellington, South Africa)

4.2 Climatic requirements

Buchu grows well in the Mediterranean type of climate of the Western Cape, where summers are dry, but with rain during the winter months of May to September (Simpson, 1998). Winters in this area are generally mild except at higher elevations where night temperatures may fall below freezing with occasional snowfalls on the mountain peaks that may remain for several weeks. The Cape fynbos region experiences a variable rainfall regime. The rainfall pattern is orographic so that precipitation increases with altitude and rain shadow effect are more than usually pronounced (Bond & Goldblatt, 1984). The annual winter rainfall in these areas varies between 400 mm and 700 mm (Blommaert, 1972a; Arkcoll, 1997). According to Handforth (1998), Buchu requires a moderate to high water supply.

4.3 Plant nutrition

The cultivation of Buchu and the research on its water and nutritional requirements is still in its infancy and little can be concluded. In order to encourage growth, well-balanced fertilization is recommended (Handforth, 1998) but these generic fertility recommendations do not provide commercial growers with real guidelines. Yet, low levels of nutrients are characteristic of soils of Mediterranean type climates, and even within these soils the levels of nutrients vary considerably (Grooves *et al*., 1980). According to Cowling & Richardson (1995), fynbos plants exist in soils which are notoriously deficient in all the nutrients essential for plant growth. The levels of nitrogen and phosphorus are therefore a fraction of the amounts required to sustain traditional agricultural crops.

The floristically diverse fynbos vegetation of the Cape Floristic Region has evolved in response to frequent stochastic fires and leached soils with a low nutrient status (Allsopp & Stock, 1993; Linder, 2003). Fire is the major mineralizing agent in fynbos, because it returns mineral elements held in the above ground biomass and litter to the soil (Brown & Mitchell, 1986). The incidence of frequent fires in a Mediterranean-type climate is therefore the key environmental factor that is coupled with nutrient paucity (Stock & Allsopp, 1992). High concentrations of tannins, resins and essential oils in the sclerophyllous leaves of many fynbos plants increase the flammability of the community during periods of water stress (Cloete, 2005). Stock & Allsopp (1992), described fire as a regular disturbance initiating successful changes, during which time alterations occur in the patterns of resource availability. These changes follow definite patterns, with the suggestion that availability of all resources such as water, light and nutrients are elevated at the soil surface shortly after the disturbance and that the availability of these resources diminishes with time (Tilman, 1986; Stock & Lewis, 1986). After fire, nutrient flushes become a characteristic feature of fynbos systems and the availability of nitrogen and phosphorus are increased (Musil & Midgley, 1990).

Research has been done on mineral nutrition of Proteaceae as Buchu falls under the same plant Kingdom, and therefore results from these studies will be discussed in the review. Proteas are usually found on highly leached, nutrient deficient and acidic soils and have low mineral requirements (Silber, Ganmore-Neumann & Ben-Jaacov, 1998; Fernandez-Falcon *et al*. 2003; Montarone & Allemand, 1995; Madakadze, Nyamangara & Mahenga, 2006) under natural conditions. Furthermore Claassens (1986) and Heinsohn & Pammenter (1986) reported that a relatively high concentration of nutrients does not appear to be a problem in culture medium. Problems may, however, occur with high levels of particular elements. Research for instance, showed that low phosphorus contents are necessary in leaves of some species for optimal photosynthesis (Barrow, 1977) and that high soil phosphorus contents may be detrimental to Proteaceous plants (Claassens, 1986; Handrek, 1991). In contrast, Prasad & Dennis (1986) and Silber *et al.* (1998) reported that low available phosphorus would impede the growth of *Leucadendron* “Safari Sunset”. This clearly indicates that there are differences in species in terms of fertilizer requirements. Sensitivity to phosphorus in Proteaceae in South Africa has been linked to genetic variation within species (Elgar, 1998). Claassens & Folscher (1980) also reported that phosphorus can reduce proteoid root production of *Leucospermum* grown on sand.

Nichols & Beardsell (1981) showed that high calcium content can worsen leaf necrosis due to high phosphorus content, while high nitrogen and potassium contents can reduce it. Montarone & Allemand (1995) and Montarone & Ziegler (1997) concluded that a 1:1 NH_4^+:NO_3^- ratio was ideal and a total salt content of 0.8 me.L^{-1} for growing Proteaceae in a soilless system. Nitrogen applied as ammonium appears to be a nutrient of importance in protea cultivation (Claassens, 1986). According to Heinsohn & Pammenter (1986), nitrate nitrogen is not an efficient source of N and at a concentration of 2.5 me.L^{-1}, it was found to be toxic for *Leucadendron salignum.* The different findings in Proteaceae fertilization research make it difficult to have a good understanding of nutritional requirements (Montarone, 2001). The study by Madakadze *et al.* (2006) showed that the application of fertilizer beneficiates proteas.

Soil pH measures the concentration of hydrogen ions in soil water. It is an important factor in production through its control on nutrient availability (Haynes & Swift, 1985; Killham, 1994). At alkaline reactions, insoluble compounds of nutrients are formed, thus creating nutrient deficiencies in plants. Most obvious are the less soluble carbonates and phosphates of calcium and magnesium (Stout & Overstreet, 1985). Under strongly acidic conditions, aluminum becomes increasingly soluble and becomes toxic to plants (Killham, 1994). In acidic soils other indirect effects are induced tending to alter the availability of mineral nutrients (Stout & Overstreet, 1985).

5 Plant propagation

Buchu seed is difficult to obtain and germinate and the rooting of cuttings has not been very successful (Blomerus, 2002). The major problems in establishing plantings are poor seed germination and survival of seedlings following transplanting in the field (Blommaert, 1972b).

5.1 Seed propagation

Cape fynbos is generally distinguished from other vegetation formations in the Southern Cape with regard to reproductive traits, such as seeds which are dispersed by ants or other insects. This is rare or absent in other vegetation formations on the subcontinent. The main reason for rare seeds is the fact that fynbos occurs on nutrient poor soils and under a climate which ranges from winter to bimodal rainfall, a combination which is not found elsewhere in Southern Africa (Maitre & Midgley, 1992). However, Buchu is not an exception to this group of plants since seed availability is one of the limitations in the propagation of this plant.

Buchu plants produce their seeds in a capsule from which it is expelled on ripening (Werner, 1949). This phenomenon is called ballistic dispersal. The seeds that are collected from fully ripe capsules show a higher germination percentage (Blommaert, 1972a). Findings of the research done by Blommaert (1972b) indicate that poor seed germination can be caused by the collection of unripe seeds containing abortive embryos. In order to obtain a percentage of viable seeds, attempts should be made to harvest at frequent intervals after the first capsules start to shed their seeds. This is based on the fact that Buchu plants normally flower over an extended period and seed capsules in different stages of development are to be found on the same plant when maturation commences. The timing of seed harvesting is critical and must coincide with the time of maximum seed viability (Littlejohn, 2000). To enhance germination, smoke treatment should be applied in a covered area with good light and air circulation and the medium should be kept moist. This is not surprising since fynbos is fire-prone vegetation (Maitre & Miggley, 1992). Periodic fires are a natural phenomenon in fynbos and fire-stimulated seed germination has been reported for a number of fynbos species (Brown, 1993; Brown & Duncan, 2006).

5.2 Vegetative propagation

Buchu can be propagated from cuttings (Karsen, 2003). Cuttings have the advantage of producing a larger flowering plant quicker than seedlings. Semi-hardwood cuttings are taken 50 to 70 mm from the current year’s growth. The cuttings are prepared by making a clean cut below a node and removing the lower third of the foliage. In order to enhance rooting, cut ends are dipped in a rooting hormone. The cutting trays should be placed in a well aerated propagation unit with a bottom heat of 24°C with intermittent misting. Rooting occurs in 9 to 11 weeks (Handforth, 1998). The rooting percentage obtained by Karsen (2003) was between 40- 45% when 500-1000 ppm indole butyric acid (IBA) was applied and concluded that rooting success of 40% is commercially plausible. According to Malan (1992), a minimum rooting percentage of 80% is regarded as ideal for effective nursery propagation. Karsen (2003) also argued that Buchu cuttings are inherently slow to root, therefore, poor rooting results are related to the ability of the cutting to survive long enough for rooting to take place. The research done by Blomerus, *et al*. (2001) indicates that the time of taking the cutting is crucial. According to Vogts (1982), a plant grown from a cutting is rarely as strong or as well formed as one grown from the seed. The other factor which may contribute to suboptimal rooting of cuttings is the inability to control diseases despite stringent sanitation (Malan, 1992). However, vegetative propagation also has its advantages over reproductive propagation. These include the fact that the resulting plant will have the same set of genes as the mother. This will have positive impact on growth rate or vigor if the mother plant was superior (Radke, 2005).

6 Harvesting

When the leaves are harvested in an inappropriate season of the year, their value deteriorates due to poor quality and this practice will also shorten the lifespan of the plant (Von Wielligh, 1913). Buchu should be harvested during the months of January through March (Von Wielligh, 1913 and Phillips, 1917). According to Werner (1949), the best time for harvesting is in February. This is based on the fact that the pulegone content is low during this time of the year. Most species of Buchu flower between September and February and according to Cape Nature (2004), harvesting during this time prevents seed production. For seed production, harvesting standards are required to ensure that Buchu is not harvested during the flowering season that a sufficient proportion of each plant is not picked, to allow sufficient re-growth and that plants are not harvested more than once in two years (Mathews, 1919).

Table 1 Seasonal variations in pulegone levels of *Agathosma betulina* essential oil as percentage of total fractions measured (Endenburg, 1972)

Month	Pulegone
January	4,8 ± 1,3
February	1,5 ± 0,2
March	1,8 ± 0,0
April	1,2 ± 0,1
May	1,7 ± 0,0
June	1,2 ± 0,3
July	1,3 ± 0,1
August	3,5 ± 0,8
September	4,0 ± 0,2
October	11,5 ± 3,7
November	10,9 ± 1,4
December	3,8 ± 0,6

7 Essential oil quality parameters

Essential oil quality is one of the aspects that need to be considered when planning to produce Buchu. According to Tadmor *et al.* (2002), producers should establish what the market and consumer demands are first and then develop methods of production to meet those product quality demands. The improvement in education, increasing consumer concern about personal health, concerns about environmental safety, sustainable harvesting and loss of genetic diversity has led to a demand for high quality, safe, effective and clean natural plant products. It is also important to note that international markets require a good quality product. Therefore, quality control in Buchu production is very important since the main markets of Buchu are international. However, the leaves can contain about 1 to 2 % of essential oil, mucilage and flavonoids (Bruneton, 1995; Schneider & Viljoen, 2002).

7.1 Volatile oils

The essential oil of Buchu has a peppermint like aroma and is slightly antiseptic and weakly diuretic (Watt & Breyer-Brandwijk, 1962). The volatile oil accumulates and is contained in large circular cells just beneath the epidermis of the under surface of the leaf; with *A. betulina* yielding a higher percentage than *A. crenulata*. The essential oil consists mainly of monoterpenes including: diosphenol, *Ψ*- diosphenol, limonene, pulegone as well as menthone as its' major constituents (Schneider & Viljoen, 2002). The essential oil of *A. betulina* is identified by a pulegone content of 2.4% to 4.5%, while the hybrids have about 7.6% to 27.8% and *A. crenulata* reported to have 31.6% to 73.2% of pulegone. The urinary tract antiseptic actions of Buchu are thought to be due to the volatile component, monoterpene diosphenol. *A. crenulata* is not suitable for medicinal use due to their lower diosphenol content and higher pulegone content (Collins *et al.*, 1996). *A. betulina* oil has a 53:47 equilibrium mixture of diosphenol and *Ψ*-diosphenol as well as limonene, pulegone and menthone as major constituents (Schneider & Viljoen, 2002). In the analysis done by Kaiser, Lamparsky & Schudel (1975), the occurrence of six major constituents and ten minor constituents was verified.

Table 2 Composition of essential oil of *Agathosma betulina* (diosphenol chemotype)

Component	Relative Percentage (%)	Reference
Diosphenol	15-30	Watt & Breyer-Brandwijk, 1962
Ψ-Diosphenol	10.28-23-27	Collins *et al*, 1996
Limonene	10-25	Endenburg, 1972
Pulegone	<5	Klein & Rojahn, 1967
Isopulegone	0.4-4.55	Collins *et al*. 1996
Menthone	<9.6	Collins *et al*, 1996
Isomenthone	4.57-29.07	Collins *et al*, 1996
Cis-trans-8-mercapto-p-menthan-3-one	2-3.5	Collins *et al*, 1996

There are two chemotypes of *A. betulina* that have been identified: an isomenthone chemotype and a diosphenol chemotype (Collins *et al*., 1996). The diosphenol chemotype is believed to contain a high amount of *Ψ*- diosphenol and diosphenol content (>05 and >12%, respectively) and a low content of isomenthone and menthone (<29 and <9.6%, respectively) However, the isomenthone chemotype is the reverse of diosphenol chemotype with high isomenthone of more than 31%; menthone of 27% and low diosphenol of less than 0.14% concentrations. Even though the diosphenol chemotype would be preferred due to high diosphenol content, both chemotypes produce highly fragrant essential oil, which contain less than 5% pulegone (Blommaert & Bartel, 1976).

These two chemotypes differ in their geographic distribution. The isomenthone chemotype is mainly found in parts of the Piketberg Mountains on northern and western slopes, whereas the distribution of the diosphenol chemotype is mainly in the mountains surrounding Citrusdal (Dr. M. Esterhuysen, 2006, pers. comm., Puris Natural Aroma Chemicals (Pty) Ltd, P. O. Box 12127, Die Boord 7613, South Africa). However, Collins *et al.* (1996), found no significant correlation between the oil composition and geographic origin of the chemotypes. They concluded that the genetic makeup is more important than the environmental factors. The conclusion was based on the fact that all the *A. betulina* plants with low diosphenol isomers were found on the Piketberg mountain range, but not all the plants from the Piketberg had low diosphenol content.

The important aromatic volatile oil chemical compounds in these chemotypes are:

Diosphenol is the main constituent of Buchu oil. According to Phillips (1917) and Arkcoll (1997), diosphenol constitutes about 20 to 30% of the oil distilled from the leaves of *A. betulina,* whereas Van der Riet (1933) stated that diosphenol constitutes about 8% of fresh Buchu oil as opposed to Watt & Breyer-Brandwijk (1962), who concluded that diosphenol occurs to an extent of 15 to 30%. However, Collins, *et al*. (1996), argued that *A. betulina* oil consists about 15.67% of diosphenol. According to Guenther & Althausen (1949), some of the oils partly crystallize at room temperature, the crystal being the diosphenol. The antiseptic and strong diuretic properties are evident due to this active ingredient (Watt & Breyer-Brandwijk, 1962). Diosphenol possesses a peculiar mint-like odor and it is readily attacked by oxidizing agents (Guenther & Althausen, 1949).

***Ψ*- diosphenol** is the crystalline component soluble in cold water. It comprises about 45 % of the total diosphenols in the oil (Fluck, Mitchell & Perry, 1961).

Menthone / Isomenthone The component which was found to be closely related to menthone was isomenthone. It is the colourless liquid bearing a strong menthone colour as identified by Endenburg (1972) and Klein & Rojahn (1967). These components are found in higher amounts in the isomenthone chemotype of *A. betulina* and in lower amounts in the diosphenol chemotype (Collins *et al*. 1996). Menthone in diosphenol chemotype is <9,6% while isomenthone is <29% (Collins *et al.* 1996)

Limonene is the colourless liquid with a strong lemon odour. It constitutes about 10 to 25% of the Buchu oil (Endenburg, 1972).

Pulegone is a toxic compound mostly found in *A. crenulata* and makes it unsuitable for medicinal use. This oval leaf Buchu is believed to contain approximately 65% of this pale yellowish liquid (Endenburg, 1972). A. *betulina* contains trace amounts of pulegone (Fluck, *et al*. 1961). Less than 5% of Pulegone is found in the essential oil of *A. betulina*, which makes it suitable as an ingredient in formulations intended for human consumption (Klein, & Rojahn, 1967).

7.2 Flavonoids

Buch also contains many other bioactive compounds apart from the aromatic volatile oil constituents which make up the essential oils. For example, Buchu contains the flavonoids diosmin, rutin, quercitin and hesperidin. These flavonoids exhibit anti-oxidant activity by preventing cell damage caused by unstable oxygen molecules. The anti-inflammatory effects of Buchu are thought to be due to the flavonoids. These flavonids are diosmin, rutin, quercitin and hesperidin (Bruneton, 1995).

8 Conclusions

The economic importance of the crop, environmental concerns and the lack of published agronomic information has lead to the initiation of this study to determine the optimum pH range for the production of Buchu. The literature survey revealed that previously, research on Buchu was mainly directed towards the extraction, chemical constituents, quality and commercial application of the essential oil. Propagation of the crop, both reproductive and vegetative, has been dealt with by many researchers. Furthermore the survey revealed that no published literature were available on the soil and plant nutritional requirements of Buchu.

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CHAPTER 3

THE NATURAL ENVIRONMENT OF BUCHU (*AGATHOSMA BETULINA)*

Abstract

The study was conducted in the Cederberg Mountains, which are situated about 200 km north of Cape Town at eleven sites during March 2006 to gain some information on climatic and soil characteristics of natural habitats and determine possible variation in Buchu oil quality at different sites. Plant samples were collected and plant height recorded for eleven populations of Buchu plants at these sites. Soil samples were taken as close as possible to the sampled plants, while the direction of the slope was also recorded. Climatic data such as temperature, wind, radiation, relative humidity and rainfall, were also gathered from nearby weather stations, and indicated a typical Mediterranean type of climate with hot and dry summers and rain during winter months. Large differences not only occurred between sites, but also between different years at each site. The soil analyses indicated that the Cederberg soils are mostly sandy soils with low levels of nutrients. The pH of the soil was found to be very low, varying between pH (KCl) 3.7 and 5.3. Plant heights varied with age and between sites and the analysis of the leaves indicated low levels of most of the mineral elements. Essential oil from the sites indicated slight variations, but generally low pulegone levels, high levels of diosphenol and absence of measurable amounts of *cis*-*trans*-acetylthio-p-menthan-3-one isomers indicated that all the material originates from pure *A. betulina* genetic material. High levels of diosphenol also indicated that the material was from the diosphenol chemotype.

Key words: Cederberg, nutrition, pH, soil type, and essential oil

Introduction

The Cederberg area lies east of the towns Clanwilliam and Citrusdal in the Olifants River valley of the Western Cape Province, between latitudes 32° 00’ and 32° 45’ S and longitudes 18° 50’ and 19° 25’ E (Taylor, Bands & Scheepers, 1996). The area is about 71 000 ha with very mountainous terrain (Fernqvist & Florberger, 2003). The vegetation is mostly mountain fynbos (Acocks, 1988). The mountain fynbos of the Cape Floristic Region has rich flora and a high degree of endemism (Campbell, 1983; Bohnem, 1986) and is the home to many useful herbs and medicinal plants such as Buchu (Campbell. 1986). The Rutaceae owes its prominence mainly to the genus *Agathosma* which accounts for nearly 60% of Rutaceae species in the Cederberg area. The genus *Agathosma* is the third largest in the Rutaceae family (Williams, 1982). *Agathosma* species seem to favor rugged rocky outcrops and well drained sands (Williams, 1981).

The fynbos climate comprises a complex of climates, ranging from typical Mediterranean in the west to humid temperatures, uniform rainfall types in the east and from sub-arid to humid (Grooves *et al*., 1983; Goldblatt & Manning, 2000). This range reflects the effects of normal weather systems on the character and arrangement of the major landforms and of the Algulhas and Benguela Currents (Kruger, 1979). The Cederberg area is part of the Western Cape Province which has a Mediterranean climate. Climate has direct effects on weathering processes, and therefore on the release of nutrients in the soil and on the structure and functioning of plants (Raven, 1973). Greater seasonal temperature ranges and higher annual pan evaporation are recorded in the west during summer (Campbell, 1983), while local gradients in precipitation increase from low valleys to the high peaks and decrease from the coast-facing to the interior sides of the mountains. Pronounced differences in radiation between north and south facing slopes are also found with the north receiving much more energy than the equivalent south facing slopes, especially in winter (Goldblatt & Manning 2000). All these factors account for greater variations in the local climates in the Cederberg.

The main objectives of this study were to gather climatic and geographic data of the natural habitat of Buchu in the Cederberg Mountains within the Cederberg Nature Reserve by visiting Buchu plant populations within the reserve to measure the height of the plants; determine the mineral composition and oil quality of the Buchu mineral analysis of the leaves and stems sampled, and establish the mineral composition of the soils where these sampled populations grow.

Materials and methods

The survey was conducted during the first week of March 2006 in the Cederberg Nature Reserve where *Agathosma betulina* grows naturally. The surveyed area is situated between 32°19’39.89” and 32°27’19.7” South and 19°01’21.56” and 19°10’11.2” East. Climatic data for the period 2002-2005 from the weather stations closest to the study area (Citrusdal at approximately 24.54 km and Stagmanskop at approximately 22.22 km from the sampling areas) were used as an indication of weather conditions during which the plant material was produced. Citrusdal represented the following sites: Eikeboom 1, Eikeboom 2, Driekhoek Kruis, Uitkyk 1, Uitkyk 2, Tielmanroos 1 and Tielmanroos 2, while the other sites (Jamaka 1, Jamaka 2, Jamaka 3 and Middelburg) were represented by Stagmanskop.

As plant populations are found in distinct areas, we decided to select sampling sites accordingly. Eleven plant populations were identified and sampled in the described area, namely: Eikeboom

1, Eikeboom 2, Driekhoek Kruis, Uitkyk Suid 1, Uitkyk Suid 2, Tielmanroos 1, Tielmanroos 2, Middelburg, Jamaka 1, Jamaka 2, and Jamaka 3. Time since the last natural occurring fire, geographic information and plant height was recorded at each site. Plant and soil samples were collected at each site. Plant material was sampled from all the plants in each population by topping the plants to a depth of 20-30 cm. The height of between 5 and 7 plants in each population were measured using a measuring tape. All plant material was divided into two equal quantities (sub-samples) to be analyzed for mineral and essential oil content. The sub-sample to be used to determine the mineral content was dried in an oven for 48 hours at 80°C and 100 g of dried material was used in the analyses. The other sub-sample of fresh plant material from each site was sent to Puris Natural Aroma Chemicals (Pty) Ltd to determine the concentration and composition of essential oil. The oil was extracted by direct solvent extraction (acetone) of the plant material. Oil analyses were carried out by means of gas chromatography method (Clevenger, 1928). The results are reported in terms of relative percentages of the integrated peak-areas on the chromatogram.

Soil samples were collected at each site to determine the mineral content. A standard soil sampling technique was used to sample (Crepin & Johnson, 1993), but only the 0-15 cm profile was sampled because deeper layers were too stony. Soil analyses and mineral analyses of the leaves were done at the Bemlab laboratory. The soil was analyzed for pH (1.0 M KCl, P and K (Bray II), exchangeable cations, namely, K, Ca, Mg and Na (extracted with 0.2 M ammonium acetate) soil resistance and micronutrients (The Non-affiliated Soil Analysis Work Committee, 1990).

Results and Discussion

General

Buchu plants grow naturally on steep slopes. Plant populations sampled in this study were mostly found on south and south west facing slopes. On the east facing slopes the plants were growing close to or between large rocks, which shaded the plants. This helps to create a cool environment for the plants, or keep them out of direct sunlight. However, most of the sites where Buchu patches were found in this study were cooler and on the higher parts of the mountain. The altitude of these areas ranged from 1 737 m to 2 028 m above sea level.

Climate

The climatic data from two weather stations (Stagmanskop and Citrusdal) nearest to the area of study is presented in Tables 1, 2, 3 and 4.

Rainfall The rainfall data indicated that the area receives most rain in the months May to August. The total precipitation and distribution of the rain however differed between years and sites. Total annual rainfall at Citrusdal varied between 220.6 mm in 2003 and 323.2 mm in 2002. At Stagmanskop, the highest total annual rainfall of 521.0 mm was also recorded during 2002 and the lowest (305.0 mm) during 2003, but at Citrusdal for example, highest monthly rainfall during 2002 was recorded in July, while the month of May received the highest rainfall at Stagmanskop in the same year. During 2003 and 2004, highest rainfall at both localities was received during the months of August and June respectively. In its natural habitat, Buchu plants start to grow in August when days started to become longer and temperatures increased after the winter. From the rainfall data, it appears that this is also the period when rainfall start to decrease very rapidly. The lack of rainfall (soil moisture) during the most active growing period of Buchu at the study sites would therefore be a limiting factor. This is also true for other plant species growing in the Cape region (Goldblatt & Manning, 2000). Campbell (1985) however, found that although the Cederberg is among the westerly and northerly of fynbos Mountains, it receives at least 80% of its rain during the three winter months (June, July and August) and the annual total is less than in most other Cape Mountains. Buchu will, therefore, most probably experience more drought stress during its active growth period in this area compared to other fynbos areas.

Rainfall is strongly affected by the orographic nature of the landscape (Van Rooyen & Steyn, 1999), especially in mountainous areas. Mountain slopes facing prevailing winds receive considerable more precipitation than those that face away from the winds. Actual rainfalls for the different populations sampled could therefore, differ considerably from the rainfall data presented in Tables 1-4.

Temperature Mean daily maximum and minimum temperatures for different months clearly showed that maximum temperatures at Citrusdal were higher during summer months, but minimum temperatures were lower compared to Stagmanskop for the period 2002 to 2004 (Tables 1-4). Temperatures at Stagmanskop were therefore more moderate compared to the more extreme temperatures experienced at Citrusdal. The highest mean daily temperatures at both localities were recorded during February. At Citrusdal a mean daily maximum of 36.11°C was recorded during 2002. In contrast to Citrusdal where temperatures of above 30°C are frequently recorded during November to April, temperatures at Stagmanskop rarely reach 30°C which happens mainly during February. It is, therefore, clear that plants sampled in this study were subjected to very high temperatures of about 30°C or even more than 30°C within the month prior to sampling, which may have had an effect on oil quality. It must, however, be remembered that the Cederberg is a mountainous area and temperatures vary considerably with changes in topography and altitude (Fuggle & Ashton, 1979).

Although climatic conditions at the actual sampling sites might differ from the data summarized in Tables 1- 4, for the reasons already mentioned, all sampling sites experienced typical Mediterranean type of climatic conditions, with the sites closer to Citrusdal weather station were subjected to more extreme conditions (greater water stress and both higher and lower temperatures) compared to the sites closer to Stagmanskop weather station.

Table 1 Climatic data from Citrusdal and Stagmanskop weather stations (2002)

	Citrusdal			Stagmanskop		
Month	Temperature (°C)		Rainfall (mm)	Temperature (°C)		Rainfall (mm)
	Max	Min		Max	Min	
Jan	32.19	13.97	13	26.34	13.7	41
Feb	36.11	15.66	13.2	30.74	17.71	24.4
Mar	35.27	14.71	0.4	29.55	16.64	2.4
Apr	30.36	9.69	12.6	24.54	13.77	23.2
May	23.36	6.98	86.2	18.64	10.27	130.6
Jun	19.54	3.75	19	15.56	8.1	33.8
Jul	19.1	3.81	99.2	15.24	7.84	123.4
Aug	21.45	4.17	53.8	17.02	9.02	67
Sept	27.09	7.6	17.8	21.97	12.28	31.4
Oct	29.08	8.32	***	22.6	10.02	18.1
Nov	31.74	9.72	6.4	25.72	12.29	19.2
Dec	34.65	16.34	1.6	29.05	15.38	6.5
Total			323.2			521

*** Data is not available

Table 2 Climatic data from Citrusdal and Stagmanskop weather stations (2003)

Month	Citrusdal			Stagmanskop		
	Temperature (°C)		Rainfall (mm)	Temperature (°C)		Rainfall (mm)
	Max	Min		Max	Min	
Jan	35.20	14.98	10.60	29.15	15.47	7.50
Feb	35.84	15.96	4.40	29.55	16.10	5.70
Mar	31.56	14.37	17.20	26.61	15.09	23.20
Apr	31.21	12.90	15.00	25.34	14.90	11.80
May	26.45	7.41	11.80	Citrusdal	11.62	11.90
Jun	24.54	1.87	1.40	Rainfall	9.75	5.90
Jul	23.89	2.58	13.20	(mm)	9.09	26.90
Aug	20.40	4.69	97.40	14.88	6.77	118.60
Sept	23.97	7.78	21.20	17.75	9.06	34.60
Oct	28.44	10.63	15.40	22.82	11.75	39.10
Nov	32.62	12.34	0	26.48	12.63	0
Dec	31.01	12.11	13.00	25.66	13.09	19.80
Total			220.60			305.00

Table 3 Climatic data from Citrusdal and Stagmanskop weather stations (2004)

Month	Citrusdal			Stagmanskop		
	Temperature (°C)		Rainfall (mm)	Temperature (°C)		Rainfall (mm)
	Max	Min		Max	Min	
Jan	34.43	15.36	14.40	29.54	16.27	13.40
Feb	35.13	15.44	0	30.16	16.59	0
Mar	31.04	11.16	5.80	26.19	13.35	5.90
Apr	28.74	9.71	46.20	24.17	14.17	57.20
May	27.71	7.01	1.60	23.24	13.55	5.30
Jun	21.00	4.70	49.20	17.31	10.06	69.10
Jul	21.04	0.85	36.60	17.50	9.29	50.90
Aug	21.21	5.52	24.60	16.95	8.99	43.10
Sept	26.11	5.37	14.40	21.18	10.56	14.80
Oct	28.42	8.24	46.20	23.87	12.04	60.50
Nov	32.50	12.24	1.80	27.57	14.63	1.30
Dec	33.73	14.27	0	28.60	15.35	0
Total			240.80			321.50

Table 4 Climatic data from Citrusdal and Stagmanskop weather stations (2005)

Month	Citrusdal			Stagmanskop		
	Temperature (°C)		Rainfall (mm)	Temperature (°C)		Rainfall (mm)
	Max	Min		Max	Min	
Jan	34.86	16.17	0.20	29.94	16.94	0.50
Feb	35.94	15.02	0.20	31.09	17.54	1.20
Mar	33.24	14.49	5.80	28.19	16.69	3.80
Apr	27.04	10.19	45.20	23.10	14.12	52.90
May	22.50	8.02	31.40	18.78	10.91	48.80
Jun	19.01	5.34	64.20	14.98	8.86	63.20
Jul	23.23	4.57	22.40	20.00	11.90	68.10
Aug	18.77	4.48	84.40	14.73	7.47	85.70
Sept	24.65	6.11	24.20	19.82	9.69	31.50
Oct	27.90	8.04	10.20	22.48	11.08	10.70
Nov	30.85	12.38	14.20	25.86	13.51	8.40
Dec	***	***	0	28.49	14.26	0
Total			302.40			374.80

*** Data is not available.

Mineral analysis of soil

Low levels of nutrients are characteristic of Mediterranean soils, but even in these soils, the levels of nutrients may vary considerably. According to Grooves *et al.* (1983), plants are however more affected by the extent to which nutrients are available or accessible than by the total quantity of nutrients in the soil. As a result of a wide range of environmental conditions, a great variation of soil types and associations of soils is a characteristic of the fynbos Biome (Lambrechts, 1979). Soils of the western mountain contain more coarse material than those of the eastern parts of fynbos Biome. The Cederberg soils are often yellow brown to brown in colour (Campbell, 1983). Soils in the study area mostly derived from the parent material of sandstone and shale (Lambrechts, 1979). Soils which derived from sandstone are in general coarse in texture, acidic and low in base status and nutrient reserve (Buol *et al*., 1997). Results from the soil analysis done on the soil samples from different sites are summarized in Tables 5 & 6.

pH

Results indicated that the natural environment of Buchu has a low pH. The lowest pH was found to be 3.7 at the Uitkyk Suid 1 site, while the highest pH of 5.3 was recorded at the Jamaka 3 site. The average pH of all the sites sampled was found to be 4.2, which clearly indicates that Buchu plants are well adapted to low pH conditions. More acidic soil conditions can be attributed to higher rainfall which accelerates the leaching of basic cations, such as calcium and magnesium. The leached basic cations are replaced by acidic cations such as aluminium and hydrogen resulting in lowering of soil pH (Madakadze, Nyamangare & Mahenga, 2006).

Soil resistance

Soil resistance varied between 2 780 Ohms at Jamaka 3 and 9 550 Ohms at Uitkyk Suid 2, indicating that soil salinity was very low. According to Von Wielligh (1913), Buchu is never found amongst limestone, brackish or barren sandy soils and stiff clays in its native state, but it is found on red or black sandy soils.

Phosphorus

Phosphorus content of the soil at most sites were below 8 $mg.kg^{-1}$ (Bray II), but at Eikeboom I and Eikeboom 2, the phosphorus was found to be 14 and 16 $mg.kg^{-1}$ respectively. Mattingly (1965) also found that acidic sandy soils usually have a low phosphate adsorption capacity and in extreme cases phosphate has been shown to leach from these soils (Sutton & Gunary, 1969). These results might indicate that Buchu may have a low phosphorus requirement, as it is the case for other fynbos species such as in many *Proteaceae* plants where high soil phosphorus has generally been accepted as detrimental (Claassens, 1981). However, findings by Prasad & Dennis (1986) contradicted the general view that members of *Proteaceae* are sensitive to phosphate concentration. The research findings indicated that low available soil phosphorus would impede growth of *Leucadendron* “Safari Sunset” and that concentrations of available phosphorus of more than 25 ppm are required. This leads to two hypothesis (1) Buchu does have a low phosphorus requirement; or that (2) Buchu may simply be adapted to growing in low P environments.

Potassium

The natural environment of Buchu was found to have moderate quantities of potassium in the soil as indicated in Table 5. The level of potassium was above 40 mg.kg^{-1} at most sites and only Uitkyk Suid 2 and Jamaka 2 indicated lower levels. At Tielmanroos 1, potassium was above 100 mg.kg^{-1}. Potassium is required to maintain the osmotic potential of cells which in guard cells governs the opening of stomata (Bennett, 1993), but although no data is available on the potassium requirements of Buchu, potassium requirements for other fynbos species such as *Leucadendron Salignum* Berg are low (Heinsohn & Pammeter, 1986).

Calcium

The calcium content from all the sites was low and the lowest value was found at sites with very low pH (Table 5). In sites where pH was found to be less than 4, the exchangeable calcium was found to be below 1 cmol(+).kg^{-1} and in sites where pH was above 4, the exchangeable calcium was above 1 cmol(+).kg^{-1}. The increased calcium content may reduce the phosphate content in the soil solution and hence the availability to the plant (Barber, 1985).

Magnesium and Sodium

Low levels of exchangeable cations of magnesium and sodium were obtained from the soil mineral analysis. In most sites, the levels were below 1 cmol(+).kg^{-1} with the exception of Jamaka 1 and Jamaka 3 where magnesium was above 1 cmol(+).kg^{-1}.

Micronutrients

In general, high levels of manganese were observed with levels of above 15 mg.kg^{-1} in most sites, but at Uitkyk Suid 2, Tielmanroos 1 and Tielmanroos 2 manganese was found to be only 4.00, 6.60, and 6.40 mg.kg^{-1} respectively (Table 6). Manganese levels ranging between 5 and 140 mg.L^{-1} (DTPA extraction method) are considered to be acceptable for plants (Sillanpaa, 1982). However, levels in the range of 2 to 5 mg.L^{-1} are considered deficient and marginal respectively (Fernandez-Falcon, Alvarez-Gonzalec & Rodriguez-Perez, 2003). Cecil *et al*. (1995), observed that *Leucadendron* grew well is soils containing around 10 mg.L^{-1} manganese.

Levels of copper were very low (<1 mg.kg^{-1}). According to Kishk *et al*. (1973), ranges between 1 and 10 mg.L^{-1} are considered to be normal in agricultural soils. Fernandez-Falcon *et al*. (2003), reported that values between 1 and 2 mg.L^{-1} may be marginal for some crops, while Jokinen & Tähtinem (1987) concluded that 1.5 to 2 mg.L^{-1}Cu are critical for oats crops. However, for *Leucadendron*, levels below 1 mg.L^{-1} are considered to be sufficient (Cecil *et al*., 1995).

Zinc and boron nutritional levels were very low and zinc is considered to be most soluble and mobile under acidic conditions (Fernandez-Falcon *et al*., 2003). In most sites, zinc levels were below 0.5 mg.L^{-1} except in Eikeboom 1, Eikeboom 2, Tielmanroos 1 and Jamaka 1 where levels were 0.9, 0.5, 0.7 and 0.7 mg.L^{-1} respectively. According to Singh, Karamanos & Stewart (1987), zinc levels below 0.5 mg.L^{-1} are considered to be deficient for most crops, including *Leucadendron* (Cecil *et al*., 1995), while levels of 0.5 to 1.0 mg.L^{-1} were found to be potentially deficient or marginal (Sakal, Sinch & Sinch, 1988).

Carbon percentage varied among the sites, with some sites having a percentage below 1.0 (Uitkyk Suid 2, Middelburg and Jamaka 3), some between 1.0 and 2.0 (Eikeboom 1, Driekhoek Kruis, Tielmanroos 1, Tielmanroos 2, Jamaka 1 and Jamaka 2) while in some sites it was above 2.0 (Eikeboom 2 and Uitkyk Suid 1). Percentage carbon of less than 1.0% is considered to be low, between 1.0 and 2.0 % is moderate or normal while above 2.0 % is considered to be high (Cecil *et al*., 1995).

In general, we concluded that although differences in soil chemical properties were found for different localities, soil pH as well as most of the other macro- and micro- elements in the soil were low compare to the requirements set for crop production in literature and in comparison to values found in fields used for winter field crop and pasture production in the Western Cape (De Villiers, Lamprecht & Agenbag, 2006; Labuschagne & Agenbag, 2006). If Buchu is grown commercially on these soils, fertilizer requirements would be very low.

Table 5 Soil mineral analysis: macronutrients

Site Name	pH	Resist.	P (Bray II)	K	Na	K	Ca	Mg
	(KCl)	(Ohm)	mg.kg^{-1}		Exchangeable cations (cmol(+)kg^{-1})			
Eikeboom 1	4.3	5810	14	69	0.15	0.18	1.61	0.58
Eikeboom 2	4.3	4150	16	70	0.10	0.18	1.60	0.63
Driehoek Kruis	3.8	4710	6	53	0.06	0.14	0.67	0.41
Uitkyk Suid 1	3.7	4090	8	93	0.08	0.24	0.61	0.45
Uitkyk Suid 2	4.1	9550	3	33	0.03	0.08	0.14	0.07
Tielmanroos 1	4.3	4880	5	109	0.08	0.28	0.98	0.60
Tielmanroos 2	3.8	7850	5	48	0.05	0.12	0.45	0.29
Middelburg	3.9	6500	3	92	0.05	0.24	0.14	0.18
Jamaka 1	5.0	5110	4	63	0.08	0.16	2.03	1.20
Jamaka 2	3.8	6700	6	37	0.05	0.10	0.53	0.29
Jamaka 3	5.3	2780	4	98	0.09	0.25	1.93	1.30
Average	*4.2*	*5648*	*6.73*	*70*	*0.07*	*0.18*	*0.97*	*0.55*

Table 6 Soil mineral analysis: micronutrients

Site Name	Cu	Zn	Mn	B	C
	mg.kg^{-1}				(%)
Eikeboom 1	0.19	0.90	22.80	0.16	1.80
Eikeboom 2	0.25	0.50	29.70	0.10	2.09
Driekhoek Kruis	0.15	0.30	18.70	0.10	1.67
Uitkyk Suid 1	0.40	0.40	28.00	0.13	2.35
Uitkyk Suid 2	0.38	0.20	4.00	0.14	0.68
Tielmanroos 1	0.23	0.70	6.60	0.09	1.60
Tielmanroos 2	0.13	0.20	6.40	0.09	1.23
Middelburg	0.27	0.30	29.10	0.08	0.83
Jamaka 1	0.08	0.70	18.10	0.26	1.13
Jamaka 2	0.09	0.30	11.20	0.10	1.02
Jamaka 3	0.13	0.30	29.80	0.16	0.22
Average	*0.21*	*0.44*	*18.58*	*0.13*	*1.33*

Plant height

The height of the plants differed largely between sites due to the age (determined by the date of the last fire), slope and environmental conditions at each site (Table 7). The tallest plants were found at Jamaka 1 (average height of 130 cm) where the last natural fire occurred five years ago, followed by Driekhoek Kruis (average height of 116 cm) where the last natural occurring fire took place eight years ago. At Eikeboom where the last fire occurred only a year ago average plant heights were found to be between 64.0 and 64.2 cm, but similar average heights were found at Tielmanroos 1 and Tielmanroos 2 where the last fire occurred four years ago and at Uitkyk Suid 1 & 2 where the last fire occurred even eight years ago. These results indicated that environmental conditions as determined by soil, climate and slope may be more important. At the Jamaka 1, 2 & 3 sites which were all burned five years ago, average heights of 130 cm, 90 cm and 71 cm were measured. Differences between Jamaka 1 and Jamaka 2 must be due to differences in soil conditions because both populations were found on a south western slope, while differences between these sites and Jamaka 3 may be the result of either soil conditions or slope.

Table 7 Average plant height, slope and years since the last fire in surveyed sites

Site Name	Slope	Average Plant Height (cm)	Years since last fire
Eikeboom 1	South	64.2	1
Eikeboom 2	South	64	1
Driekhoek kruis	South West	116	8
Uitkyk Suid 1	West	59.6	8
Uitkyk Suid 2	East	62	8
Tielmanroos 1	South West	62.8	4
Tielmanroos 2	South West	63	4
Middelburg	North west	97	4
Jamaka 1	South West	130	5
Jamaka 2	South West	90	5
Jamaka 3	East	71	5

Mineral analysis of leaves

Differences in elements between the sites were found (Table 8), but in general the mineral content of most elements in the leaves was low, corresponding with the mineral contents in the soil.

Nitrogen

The levels of nitrogen (ranging from 0.46 to 0.7%) in leaf samples were very low compared to the norm of 1.5 to 2.1% reported for *Leucadendron* “Safari Sunset” (Ran *et al*., 2001). Thompson & Troeh (1978) also reported that the average chemical composition of a large number of plants is 1.52%. In citrus crops, the range of sufficient N (% Dry Mass of leaves) is between 2.3 to 2.9 % while the contents range of 2 to 2.2% is considered to be low and levels above 3.3% are considered high (Barker & Bryson, 2007).

Phosphorus

Levels of phosphorus in the leaves varied between 0.02 and 0.08%, which correlates with levels of 0.04% found in *Protea* cultivar "Silver Tips" by Madakadze *et al*. (2006). Cresswell (1991) suggested the following standards for interpretation of the phosphorus status in recently matured leaves of *Leucadendron* cultivars:

<0.15% = low or deficient
0.15 - 0.42% = desirable
0.42 - 0.45% = high
>0.57% = supra optimal.

According to Thompson & Troeh (1978) average chemical composition of a large number of plants is about 0.22%. For most ornamental crops like asparagus fern and geranium plants, levels between 0.2 – 0.3 and 0.4 – 0.67% respectively, are considered to be sufficient (Charles, 2007)

Potassium

Compared to the value of 1.0% generally found to be sufficient in plants (Madakadze *et al*., 2006), levels of potassium in the surveyed Buchu populations were found to be low. Levels of potassium in the leaves from all the sites ranged from 0.23 to 0.63 %. Potassium values of about 0.3% were, however, also recorded in *Leucadendron* "Coniferum" (Madakadze *et al*., 2006), despite of high levels of potassium in the soil. Low levels of potassium in the leaves of Proteas which did not correlate with levels in the nutrient solution were also reported by Silber *et al*., (1997). This lead to the conclusion that some Protea cultivars have low potassium consumptions. It would appear as though Buchu also has a low consumption of potassium.

Calcium and magnesium

Levels of these two elements were also low and varied among the sites with calcium levels between 0.25 to 1.0%, while magnesium levels were between 0.15 to 0.3%. Similar values were reported by Madakadze *et al*. (2006) for Proteas. For calcium, levels between 1.4 to 2.0% are considered to be low, while 1.48% is considered to be adequate in citrus (Pilbeam & Morley, 2007). Sufficient levels of magnesium in citrus leaves are in a range of 0.17 to 1.0% (Merhaut, 2007). For most plants, the average calcium content is 0.77% and 0.3% for magnesium. Low levels were associated with low pH levels which enhance the leaching of these cations from the top soil. Low levels of calcium in the leaves may also be due to the reduced ability of plant roots to absorb calcium at pH levels of less than 5 (Combrink, 2005).

Sodium

Sodium content was found to range between 920 and 3 931 $mg.kg^{-1}$ in sampled plants. Chiy (1992) as quoted by Chiy & Phillips (1995) reported Na concentrations in forages as ranging between 30 and 2030 $mg.kg^{-1}$ DM. However, Thompson & Troeh (1978) reported 0.37 % (equivalent to 3 700 $mg.kg^{-1}$) to be the average Na concentration of a large number of plants. Great variation in levels of Na between the sites was found. According to Gorham (1995), a number of factors can influence the Na content of plants including nutrition, other stresses, tissue type and age. The age of the analyzed plants differed from 1 to 8 years (Table 7). The highest Na content in this report was obtained at Eikeboom 2, where the plants were one year old (3 231 $mg.kg^{-1}$) and Jamaka 3, where the plants were five years old (3 931 $mg.kg^{-1}$). Bogemans, Stasssert & Neirinckx (1990) concluded that young leaves have much lower Na concentration than mature leaves although the steepness of the gradient differs considerable between species.

Micronutrients

The manganese content in the leaves varied between 159 mg.kg^{-1} at Jamaka 3 to 1 065 mg.kg^{-1} at Middelburg with an average of 402 mg.kg^{-1}. These values are higher than the reported values of 95 and 65 mg.kg^{-1} respectively in *Leucadendron* cultivars “Safari Sunset” and “Silvan Red” (Ran *et al*., 2001). In cotton, the range considered to be adequate is between 50 and 350 mg.kg^{-1} (Humphries, Stangoulis & Graham, 2007). The critical manganese levels however vary with different plant species and age of the plant. In maize, for instance, the critical level of manganese ranges between 2 500 to 6 500 mg.kg^{-1} (Yost, 2000). Results indicated a large variation (77 to 245 mg.kg^{-1}) in levels of iron in the leaves from different sites. In the leaves of lucerne, 119 mg.kg^{-1} is considered adequate while 87 mg.kg^{-1} is regarded as deficient (Gupta, 1990). In oats, 50 to 80 mg.kg^{-1} is considered to be adequate range (Brown, 1979). Iron availability depends to a large extent on soil pH and redox potential. Proper soil management may reduce soil pH and redox potential and thereby increase iron uptake (Moraghan & Mascagni, 1991). Copper levels in the leaves from different sites ranged between 2 to 3 mg.kg^{-1} which is similar to values recorded by Cecil *et al*. (1995). However, at the Middelburg site a much higher leaf copper value of 5 mg.kg^{-1} was recorded.

Also the levels of Zinc in this study were found to be similar to these of Cecil *et al*. (1995), but the levels of Boron (21-50 mg.kg^{-1}) in this study were slightly higher than that reported by Cecil *et al*. (1995). Values of Boron were however lower than the critical levels of 80-120 mg.kg^{-1} reported for spring barley (Kluge & Podlesak, 1985).

Table 8 Mineral analysis of leaves

Site Name	N	P	K	Ca	Mg	Na	Mn	Fe	Cu	Zn	B
	%					Mg.kg^{-1}					
Eikeboom 1	0.60	0.04	0.62	0.42	0.18	2924	179	92	3	8	27
Eikeboom 2	0.68	0.04	0.55	0.38	0.16	3231	183	77	3	7	21
Driehoek Kruis	0.52	0.04	0.63	0.68	0.20	1448	438	130	3	5	29
Uitkyk Suid 1	0.50	0.08	0.51	1.00	0.24	1074	759	145	3	10	31
Uitkyk Suid 2	0.48	0.03	0.49	0.83	0.19	2957	279	245	2	8	31
Tielmanroos 1	0.52	0.02	0.42	0.74	0.24	2005	182	88	2	5	33
Tielmanroos 2	0.64	0.02	0.61	0.93	0.20	1522	212	121	2	6	50
Middelburg	0.46	0.02	0.62	0.56	0.15	920	1065	93	5	20	21
Jamaka 1	0.72	0.03	0.23	0.39	0.30	2748	534	81	3	5	32
Jamaka 2	0.56	0.02	0.45	0.29	0.28	1244	427	78	3	5	39
Jamaka 3	0.63	0.02	0.43	0.25	0.22	3931	159	123	3	8	27
Average	0.57	0.03	0.51	0.59	0.21	2182	402	116	2.9	7.9	31

Essential oil analysis

The results (Table 9) of the essential oil analysis are reported in terms of relative percentages of the integrated peak areas relative to the total integrated peak-areas on the chromatogram. The results indicate slight differences in levels of essential oil constituents because the gas chromatogram of all sites was similar and a representative chromatogram is presented in Figure 1. The findings show very low levels of pulegone, high levels of diosphenol and the absence of any measurable amounts of *cis*- and *trans*-acetylthio-p-menthan-3-one isomers in the analysed samples indicate that it originates from pure *A. betulina* genetic material. The high levels of diosphenol in the oil also indicated that the plant material represents the diosphenol chemotype of *A. betulina*.

The concentration of the important *cis-trans*-8-mercapto-p-menthan-3-one isomers in the analysed plant material is slightly below the normal average for cultivated Buchu (2.0 to 3.5 %, sum of isomers), but is still within the normal range for plant material that occur naturally (Collins *et al*., 1996). In general, the concentration of 8-mercapto-p-menthan-3-one is higher in young plants (<3 years), or in plants that contain fresh growth (i.e. plants that are harvested annually). It is therefore unlikely for older (more mature) plants that have not been harvested often to contain low levels of this compound (Dr. M. Esterhuysen, 2006, per. Comm., Puris Natural Aroma Chemicals (Pty) ltd, P. O. Box 12127, Die Boord, 7613, South Africa.). However, one exception to this trend is Jamaka 2 which contains about 3% 8-mercapto-p-menthan-3-one (sum of isomers). The levels of all other determined chemical constituents in these samples fall within the ranges set for *A. betulina* as described by Collins *et al*. (1996)

Table 9 Chemical composition (relative % of fresh) of *Agathosma betulina* oil obtained from plant material of surveyed sites

	Essential Oil Constituents (As Rel. % of total oil)									
Site	Limonene	Menthone	Isomenthone	*Cis-/trans-*Isopulegone	Pulegone	Ψ-Diosphenol	Diosphenol	*trans*-8-Mercapto-p-menthon-3-one	*cis*-8-Mercapto-p-menthan-3-one	Sum of constituents
Eikeboom 1	25.8	3.4	12.2	4.1	3.2	17.9	20.8	0.2	1.4	89
Eikeboom 2	24.6	2.7	9.1	2.8	2.2	19.6	23.3	0.2	1	85.5
Driekhoek kruis	30.2	3.4	11	2.6	1.5	16.2	19.5	0.2	1.2	85.8
Uitkyk Suid 1	30.7	4.2	13.1	1.7	0.6	16.7	20.2	0.2	1.5	88.9
Uitkyk Suid 2	25.8	3.4	12.8	2.4	1.3	20	22.9	0.1	1.8	90.5
Tielmanroos 1	33.5	3.6	11.4	2	0.7	15.2	18.5	0.1	0.9	85.9
Tielmanroos 2	30.1	4.2	11.7	2.8	3.3	15.3	19.4	0.2	1.6	88.6
Middelburg	29.6	5.3	15.5	1.9	1.9	13.9	17.8	0.2	1.8	87.9
Jamaka 1	33.1	5.5	14.2	2.7	1.5	12.5	15.5	0.2	1.4	86.6
Jamaka 2	24.9	6.8	19.8	2.2	0.5	14.9	18.4	0.3	2.7	90.5
Jamaka 3	27.4	3.8	11.7	4	1.4	17.1	20.1	0.2	1.6	87.3

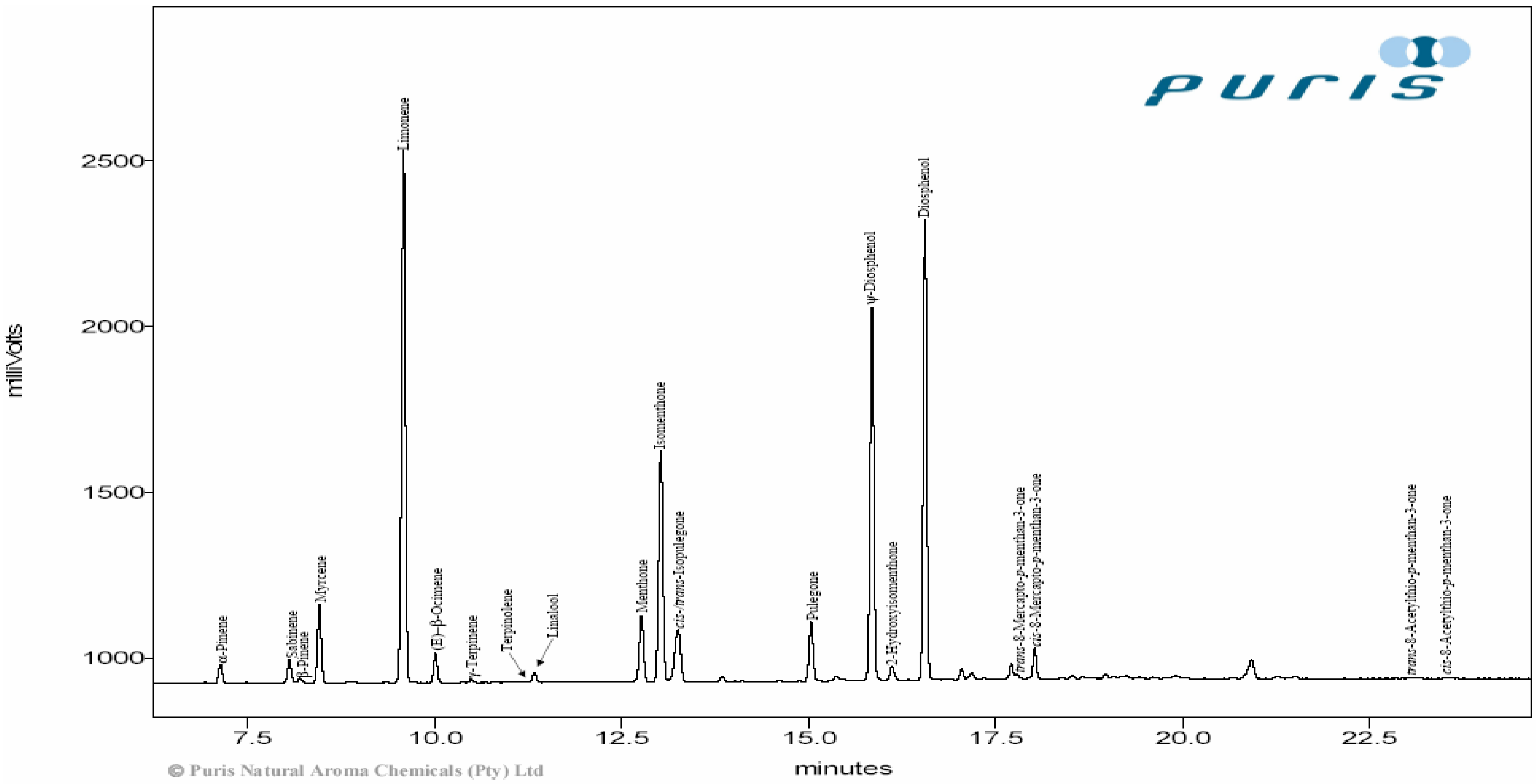

Figure 1 A Representative Gas Chromatogram of Buchu (*Agathosma betulina*) oil obtained from plant material sample Eikeboom 1 (Puris Natural Aroma Chemicals (Pty) Ltd)

CONCLUSIONS

Low levels of nutrients were found from the soil chemical analyses. In addition, soil pH was also found to be very low in the range of 3.7 – 5.3.This gives an indication that Buchu can grow well under conditions of low pH and nutrients, but whether growth, yield and quality of oil can be improved when grown under conditions of higher pH and nutrition is unknown and more research on this aspect is necessary. Therefore, future research should focus on nutrition and ideal pH range for the production of high yielding and good quality oil composition.

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CHAPTER 4

EFFECT OF pH ON GROWTH YIELD AND QUALITY OF BUCHU (*Agathosma betulina*)

Abstract

Agathosma betulina seedlings propagated from seed were transplanted into 9.5 litre pots filled with a 1:1 mixture of graded, washed sand and coco peat after six months of growth and placed in a plastic greenhouse where the temperature was controlled using an evaporative cooling system. Five pH treatments (3-3.99, 4-4.99, 5-5.99, 6-6.99 and 7-7.99), replicated eight times, were applied using a hydroponic system to determine the optimum pH range for the production of high yielding Buchu with acceptable essential oil quality. The electrical conductivity (EC) was kept between 0.8 and 1.1 $mS.cm^{-1}$ in all the nutrient solutions used. The pH was adjusted using phosphoric acid and sodium hydroxide to the required levels after every mixing and monitored throughout the experiment. Plant height was measured at 14 day intervals from nine weeks after transplanting for a period of 17 weeks, while biomass production, oil production and plant chemical analysis were determined during the final harvest after seven months of growth. Plant height displayed an exponential increase with time, but plants from the pH 7-7.99 treatment grew less vigorously compared to other treatments. Highest biomass production was achieved in the pH 4-4.99 treatment. High levels of diosphenol and absence of any measurable amounts of *cis*- and *trans*-acetylthio-p-menthan-3-one isomers were obtained from oil analyses from all the pH treatments indicating that it originates from pure *A. betulina*. Pulegone levels were also high, and there were no significant differences in all the pH treatments with regard to the quality of the oil.

Key words: EC, pH, nutrition, growth, yields and essential oil

Introduction

Buchu (*Agathosma betulina*) is a member of the Rutaceae family and an indigenous species of the Cape Floristic Region. The high commercial value of the product has lead to its domestication and commercial cultivation. Propagation and post harvest research has been initiated both by the private and public sector on how to successfully grow the plant. Although plants grow well in nutrient solution within relatively wide limits of nutrition, the critical limits of the various factors may vary for different plant species (Ellis & Swaney, 1947). Some plant species are, for example, particularly well adapted to growth at either more acidic or more alkaline pH ranges (Troeh & Thompson, 2005).

The pH of the nutrient solution is an extremely important factor in plant growth in conventional and hydroponics farming systems. Plant roots absorb water and nutrients at optimum rates within specific pH ranges of the nutrient solution (Troeh & Thompson, 2005). In hydroponics, the aim is to have a medium in which the solution pH can be controlled between 5.8 and 6.5. In conventional planting, the soil acts as a buffer on the pH. A pH (KCl) of 6.5 is the best in soil for availability of nutrients to plants (Bridgewood, 2003).

Most nitrogen and sulphur mineral compounds, except calcium sulphate, are soluble at any pH and are subject to leaching (Bohn, McNeal & O'Connor, 2001). A pH between 6.5 and 7.5 is usually the best for phosphorus availability. Iron and aluminium phosphates precipitate at low pH and calcium phosphates precipitate at high pH (Thompson & Troeh, 1978). Potassium is soluble at any pH, but is removed from solution by sorption. Some of it is exchangeable, but some is held more tightly. As a soil or growing medium is leached and becomes acidic, the total potassium declines, and the amount available also declines (Troeh & Thompson, 2005).

All iron, manganese, copper and zinc form metallic cations and precipitate in low-solubility compounds at high pH. Deficiencies of these elements often limit plant growth in high alkaline mediums. Iron availability increases as the pH is lowered. Boron may be leached at very low pH, and its solubility is low at very high pH. Since boron requirements are small and boron is usually plentiful in alkaline environments, the low solubility is not often a problem. Molybdenum is precipitated by iron and aluminium at low pH values (Troeh & Thompson, 2005). Zinc availability to plants from fertilizers and other sources depends to a greater extent on pH which governs the solubility of zinc and greatly influences zinc adsorption. Wu & Aasen (1994) concluded that zinc availability was most affected above a pH value of 6.5.

Previous experiments have been conducted on the mineral nutrition of Proteas. The range of different research findings makes it difficult to obtain a good understanding of nutritional requirements of Proteas (Montarone, 2001). However, Madakadze, Nyamangara & Mahenga (2006) concluded that Proteas grow on nutrient deficient and acidic soils and have low mineral requirements. In contrast, Brown & Duncan (2006) concluded that artificial fertilizers must be avoided especially those containing phosphorus and potassium as they are absorbed in excessive quantity by the proteoid roots resulting in collapse and death of plants. Similar conclusions with regard to the sensitivity of proteoid roots may not be drawn for Buchu since it has different types of roots, although it is also found growing on nutrient poor soils. Heinsohn & Pammeter (1986) reported that a relatively high concentration of nutrients in culture medium did not appear to be a problem, but a high level of a particular element may be a problem. Very little is known about the agronomic aspects of Buchu and the purpose of this paper is to evaluate the influence of pH on biomass accumulation and essential oil quality of Buchu.

Materials and Methods

Locality and climate

The experiment was conducted in a plastic greenhouse at Stellenbosch in the Western Cape Province of South Africa between the months of October 2005 and May 2006. The average monthly maximum and minimum temperatures outside the greenhouse are presented in Figure 1. Monthly average maximum and minimum temperatures increased during the growing season and peaked in February 2006, thereafter, it decreased during autumn (Fig. 1). The greenhouse was, however, fitted with a wet wall and fan system, to keep maximum daily temperatures below 25 ˚C. All temperatures below this range were for this reason almost similar to outside temperatures.

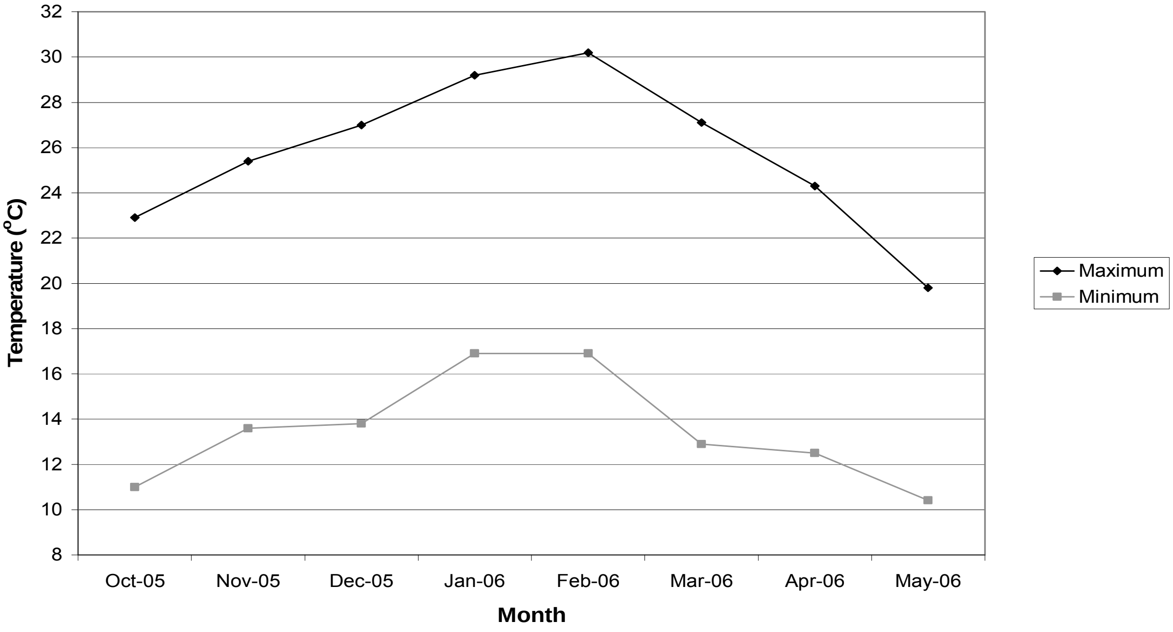

Figure 1 Average monthly minimum and maximum temperature during the growing period

Cultivation practices

Buchu seeds were sown in April 2005, using germination techniques as described by Brown (1993) and Brown *et al.* (2003). The seedlings of *A. betulina* as confirmed by Mr. T. Trinder-Smith (Bolus herbarium, Botany Department, University of Cape Town, Private Bag X3, Rondebosch, 7701, Cape Town) were produced in a growing medium that consisted of peat moss, vermiculite and polystyrene balls. Seedlings were watered with a 0.8 to 1.1 mS.cm^{-1} nutrient solution and a pH of about 6.2 (Table 1) and transplanted into a greenhouse on 25 October 2005. One seedling was transplanted per 9.5 liter white plastic container filled with a substrate which consisted of a 1:1 mixture of graded, washed sand and coco peat. The coco peat was treated with calcitic lime ($CaCO_3$) at a rate of 5 kg.m-^{3}. The $CaCO_3$ was used to increase the buffering capacity of the medium. Crude coco peat is rich in Na and Cl, which may damage the plants. Ca is usually added to facilitate Na removal and to provide nutrients (Diels *et al.*, 2002). Irrigation was accomplished using a computerized drip irrigation system. Drainage holes were made in the bottoms of the containers to allow free drainage of excess water. Pots were arranged in four double rows and the spacing between rows (from centre of the pot) was 40 cm and within the rows was 30 cm. Spacing between each double row was 120 cm. Standard cultural practices for the production of greenhouse plants were applied. A forced ventilated greenhouse was used. To induce a more bushy plant, the growing points of seedlings were manually pinched when the seedlings were about 8 cm tall.

Treatments

Five pH treatments (pH 3 – 3.99; 4 – 4.99; 5 – 5.99; 6 – 6.99; 7 – 7.99) were evaluated. In order to adjust the nutrient solution pH, phosphoric acid (H_3PO_4) was used to acidify the nutrient solution and sodium hydroxide (NaOH) was used to increase the nutrient solution pH. To keep the phosphorus content at the same level in each treatment solution, the water soluble phosphorus fertilizer application was reduced when H_3PO_4 was used to acidify the nutrient solution. The irrigated nutrient solution used is presented in Table 1.

Table 1 Composition of nutrient solution used in all treatments

K^+	Ca^{++}	Mg^{++}	NH_4^+	NO_3^-	$H_2PO_4^-$	$SO_4^=$	Cl^-
			me.L^{-1}				
3.3	2	1.4	0.9	5.5	0.59	1.4	0.12

The nutrient solution was sampled during weeks 5, 10, 15, 20 and 25 after transplanting. The EC of the five different samplings varied between 0.97 and 1.04mS.cm^{-1} and the pH for each treatment was within the required range (Table 2).

Table 2 Electric Conductivity (EC) and pH of nutrient solutions used for irrigation of the different pH treatments as sampled at weeks 5(W5), 10 (W10), 15 (W15), 20 (W20) and 25 (W25) of the growth period

Factor	Weeks after transplanting				
	pH 3-3.99				
	W5	W10	W15	W20	W25
pH	3.85	3.80	3.60	3.75	3.70
EC (mS.cm^{-1})	1.06	1.04	1.10	0.98	0.93
	pH 4-4.99				
pH	4.55	4.3	4.2	4.6	4.6
EC (mS.cm^{-1})	0.96	1.09	1.14	0.95	0.89
	pH 5- 5.99				
pH	5.7	6.05	5.5	5.6	5.8
EC (mS.cm^{-1})	1.00	1.07	1.20	0.94	0.62
	pH 6-6.99				
pH	6.90	6.60	6.55	6.10	6.60
EC (mS.cm^{-1})	0.95	1.05	0.97	0.96	0.92
	pH 7-7.99				
pH	7.30	7.40	7.27	7.35	7.25
EC (mS.cm^{-1})	1.05	1.10	1.15	0.97	0.92

Nutrient solutions for the pH treatments were mixed and stored in 1500 litre plastic tanks. Netafim drippers (pressure compensated, non-leakage), with a capacity of 2 L.hr^{-1} were used to irrigate each pot individually. The system was designed so that each treatment had its own separate irrigation system to prevent mixing of nutrient solution. The plants were irrigated six times daily. The total volume per treatment per day was the same for all the treatments. The first and last irrigation took place within 1 hour of sunrise and sunset respectively. Water application ranged from 300 ml per plant per day after transplanting to 430 ml per day before harvesting (7 months after transplanting). Irrigation volumes were gradually increased during the growing period and included an over irrigation of 10 to 15%. The EC, pH and mineral content of the municipal water used in the experiment was also measured regularly (Table 3).

Table 3 Chemical analysis of the municipal water used during the experiment

	EC		Na^+	K^+	Ca^{2+}	Mg^{2+}	NH_4^+	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	Cl^-	HCO_3^-
Date	mS.cm^{-1}	pH	me.L^{1-}									
27 Oct ‘06	0.06	6.60	0.21	0.02	0.35	0.05	0	0	0	0.02	0.24	0.42
23 Jan ‘06	0.08	6.10	0.18	0.03	0.28	0.06	0	0	0	0.04	0.44	0.45
06 Apr ‘06	0.10	7.30	0.31	0.02	0.41	0.14	0	0.01	0	0.17	0.66	0.55

Drainage water was also sampled during the experimental period at week 8, 12, 16 and 24 after transplanting to measure pH and EC values as well as Ca and bicarbonate contents for different pH treatments. EC of drainage water varied between 1.07 (week 16 of pH treatment 3-3.99) and 1.73 (week 12 of pH 5-5.99) (Table 4). With the lower pH treatments (3-3.99; 4-4.99 and 5-5.99) EC tended to reach a maximum in week 12, while in the pH 7-7.99 treatment, EC reached a maximum in week 24. The pH of all treatments was higher in the drainage water (Table 5) than in the irrigation water. In general pH tended to increase during the growth period. According to Diels *et al.* (2002), increased buffer capacity slows down acidification from ammonium fertilizers and thereby reduces pH fluctuations. Calcium levels (Table 6) in the drainage water from the lower pH treatments also tended to reach a maximum in week 12, as it was the case with EC. The lowest Ca levels were however found in the drainage water of the highest pH treatment (pH 7-7.99) and with this treatment it reached a maximum in week 24. The bicarbonate content of the drainage water was also higher compared to the irrigation water (Table 7) and tended to reach a maximum in week 24. The highest levels were found in the pH 6-6.99 treatment. This increase during the experimental period might be the result of growing medium being treated with calcitic lime prior to planting. According to Small (1954), buffers are substances that control or regulate the concentration and therefore, the total activity of free hydrogen ions, when acids or alkalis are added to solution.

Table 4 EC of the drainage water for different pH treatments

Factor	EC ($mS.cm^{-1}$)			
	Weeks after transplanting			
pH	W 8	W 12	W 16	W 24
3-3.99	1.27	1.40	1.07	1.09
4-4.99	1.41	1.43	1.22	1.16
5-5.99	1.50	1.73	1.23	1.16
6-6.99	1.23	1.11	1.15	1.26
7-7.99	1.13	1.19	1.27	1.27

Table 5 pH of the drainage water for different pH treatments

Factor	pH			
	Weeks after transplanting			
pH	W8	W 12	W 16	W 24
3-3.99	6.80	5.65	6.85	6.90
4-4.99	6.80	6.80	7.05	6.95
5-5.99	6.90	6.90	7.15	7.20
6-6.99	7.00	7.25	7.35	7.25
7-7.99	7.00	7.15	7.30	7.30

Table 6 Calcium content of the drainage water for different pH treatments

Factor	Ca^{2+} (me.L^{-1})			
	Weeks after transplanting			
pH	W 8	W 12	W 16	W 24
3-3.99	3.98	4.73	3.15	3.34
4-4.99	4.32	4.63	3.6	3.67
5-5.99	4.25	5.48	3.30	3.28
6-6.99	3.21	3.01	2.47	3.10
7-7.99	2.17	2.37	2.4	2.79

Table 7 Bicarbonate content of the drainage water for different pH treatments

Factor	HCO_3^- (me.L^{-1})			
	Weeks after transplanting			
pH	W 8	W 12	W 16	W 24
3-3.99	1.52	1.74	1.88	2.36
4-4.99	1.75	2.44	2.73	2.13
5-5.99	1.82	2.58	3.04	3.95
6-6.99	2.25	3.05	4.06	4.57
7-7.99	2.20	3.21	3.57	3.67

Data collected

Plant height was measured every second week from 9 to 17 weeks after transplanting. Plant height was measured from the ground level up to the highest point on the plant. The entire plant was harvested on 22 May 2006 (after 28 weeks of growth). During harvesting, plants which were obvious outliers (unusual values), and thus not representing the growth pattern of that particular treatment, were discarded. These differences in plant growth for the same treatment may be due to genetic variation since the plants were propagated from seed collected in the natural habitat. After harvesting, the plant material was weighed to record the fresh mass. After weighing a sub-sample of approximately 100 g fresh material was taken to Puris Natural Aroma Chemicals (Pty) Ltd for oil extraction and analysis. Essential oil analyses were carried out by means of gas chromatographic methods and the results are reported in terms of relative percentages of the integral peak areas relative to the total integrated peak areas on the chromatogram (Clevenger, 1928). The remaining fresh material was weighed again, oven dried for 48 hours at 80°C and dry mass was recorded. The dried plant material samples were taken to Bemlab laboratory for the mineral analysis. The plant material was analyzed for N, P, K, Ca, Mg, Na, Mn, Fe, Cu, Zn and B (The Non-Affiliated Soil Analysis Work Committee, 1990). The dry material index was calculated to determine the percentage dry mass, using fresh and dry matter mass determined after the sub-samples had been taken. This dry material index was then used to calculate the total dry material taking into account the fresh mass of the sub-sample taken for oil extraction and analysis.

Statistical analysis

The experimental design was a randomized block design with 5 pH treatments (pH 3 – 3.99; 4 – 4.99; 5 – 5.99; 6 – 6.99; 7 – 7.99) and eight replicates. Ten plants represented an experimental unit. Analysis of variance (ANOVA) was performed using the General Linear Model (GLM) procedure of SAS statistical software version 9.1 (SAS, 2000). Lavene's test was performed to test for treatment homogeneity of variance (Levene, 1960). The Shapiro-Wilk test was performed to test for normality (Shapiro & Wilk, 1965). Student's t-least significant difference was calculated at the 5% level to compare treatment means (Ott, 1998).

Results and discussion

During the discussion of the results, values found in this study will be compared to those found in Buchu plants in their natural habitat (Chapter 3), or to values found for other "fynbos" species such a *Leucadendron* because very few studies have been done with Buchu in a hydroponic system.

Plant Height and growth

Over time, height displayed an exponential increase ($Height=A*e^{B*Week}$) (Fig. 2). Patterns could thus be linearised by Ln transforming height ($Ln(Height)=A+B*Week$). For each experimental unit, linear regression functions were fitted on average height change for the period 9-17 weeks after planting. Results from the linear regression analyses were subjected to analysis of variance to compare treatment regression parameters.

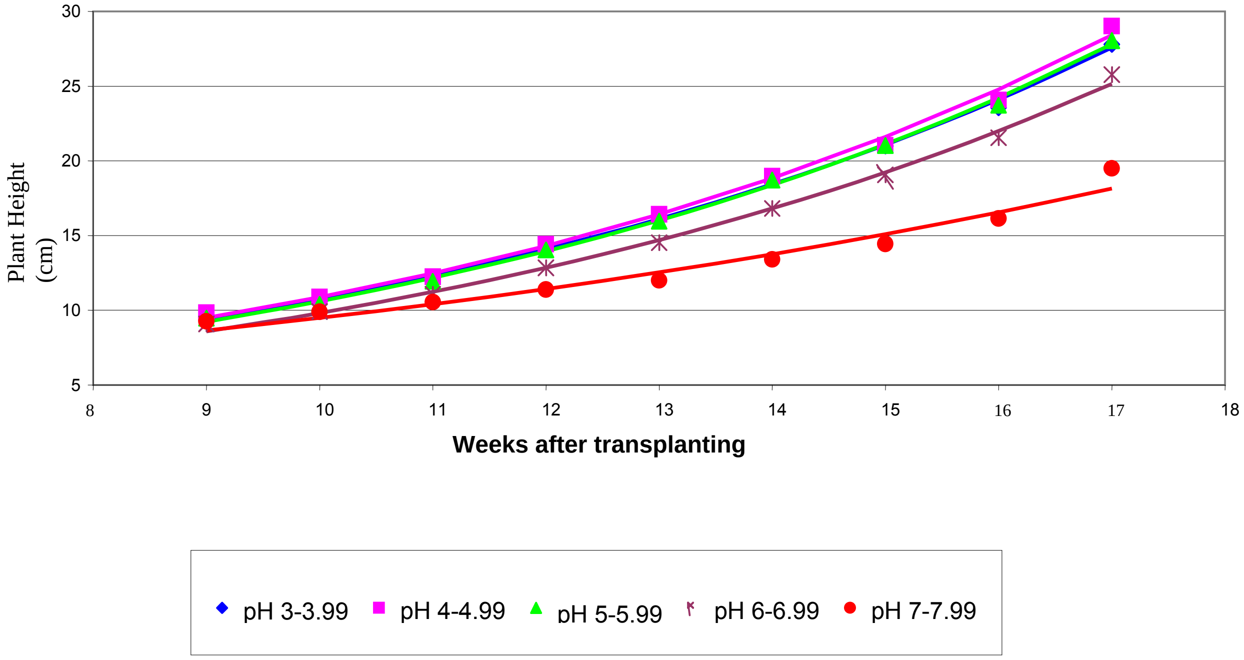

Figure 2 Plant Height measured throughout the growing period

During the first week, growth was slow and no significant differences were found between treatment means (Table 8). The height of plants from all treatments increased with time, but plants from the pH 6-6.99 and 7-7.99 treatments grew at a much slower rate. No differences in plant height were found for the pH 3-3.99, pH 4-4.99 and pH 5-5.99 treatments. Research findings of Canmore-Neumann *et al.* (1996), on *Leucadendron* ‘Safari Sunset’, which is also a “fynbos” specie like Buchu, showed that high pH values in the root environment, inhibited root growth and subsequently shoot growth. For adequate growth, pH values of less than 6 are required for *Leucadendron* ‘Safari Sunset’. Tang *et al.* (1992) and Yan, Sculbert & Mengle (1992) also showed that pH has direct effects on root elongation and plant growth.

Table 8 Average plant height as affected by pH treatments during the growing period

Factor	Height (cm)								
	Weeks after transplanting								
	W9	W10	W11	W12	W13	W14	W15	W16	W17
3-3.99	9.48	10.54	11.95	14.11	16.08	18.89	20.80	23.08	27.39
4-4.99	9.79	10.83	12.15	14.29	16.23	18.73	20.43	23.64	28.22
5-5.99	9.46	10.38	11.88	13.94	15.82	18.06	21.03	23.95	28.03
6-6.99	9.02	9.83	10.93	12.65	14.27	16.74	18.89	21.54	25.54
7-7.99	9.21	9.75	10.35	11.16	11.83	13.18	14.29	16.02	19.40

Biomass Yield

The pH of the nutrient solution applied had a significant effect on fresh mass, dry mass and dry matter index (Table 9).

Table 9 Analysis of variance (ANOVA) of fresh mass, dry mass and dry material index of Buchu

Source		Fresh Mass (g plant^{-1})		Dry Mass (g plant^{-1})		DMI (%)
	DF	*Pr > F*	DF	*Pr > F*	DF	*Pr > F*
Replicate	7	0.9864	7	0.9491	7	0.0384
pH	4	<.0001	4	<.0001	4	0.0003
Error	28		27		26	
CV (%)		29.32		28.64		5.56

Although the highest fresh and dry mass per plant was obtained with a pH of 4-4.99 in the nutrient solution, no significant difference due to pH treatments of 4-6.99 was obtained (Table 10). Fresh and dry mass per plant produced with a pH of 7-7.99 in the nutrient solution were significant less than that produced at lower pH values. Fresh and dry mass produced with a pH of 3-3.99 in the nutrient solution were also reduced compared to that with a pH of 4-4.99, but not significant less than that produced with pH values of 5-5.99 or 6-6.99. According to Canmore-Neumann *et al*. (1996), fresh mass of *Leucadendron* 'Safari Sunset' plants and roots were significantly higher when grown at a pH of 5.5 compared to a pH of 7.5.

Dry matter index ranged between 24.04 and 27.71% (Table 10) and no differences were found between pH treatments 3-3.99, 4-4.99, 5-5.99 and 6-6.99. The dry matter index obtained at the pH 7-7.99 treatment (24.04%) was, however, significantly lower than those of other treatments.

Table 10 Effect of pH on fresh mass, dry mass per plant and the dry matter index

Factor	Fresh Mass (g/plant)	Dry Mass (g/plant)	Dry matter index (DMI) (%)
pH			
3 - 3.99	307.26 b	84.74 b	27.71 a
4 - 4.99	409.96a	111.74 a	27.47 a
5 - 5.99	390.54 ab	105.86 ab	27.25 a
6 - 6.99	348.94 ab	95.81 ab	26.46 a
7 - 7.99	127.61c	30.34 c	24.04 b
LSD (P=0.05)	95.16	25.34	1.56
CV (%)	2.04	2.05	2.06

Means followed by the same letter do not differ significantly at P = 0.05 (LSD)

The results indicate that *A. betulina* is one of those plants which can be grown over a wide pH range, but pH values of above 7 seem to have negative effects on growth and biomass accumulation.

Mineral Analysis

With the exception of potassium, the pH of the nutrient solution applied had a significant effect on all the macro and micro elements measured in the Buchu plants during the final harvest after seven months of growth (Tables 11 and 12).

Table 11 Analysis of Variance (ANOVA) of the macro element content of Buchu plants as affected by the pH of the nutrient solution applied

		Percentage (%) of dry mass					
Factor		Nitrogen	Phosphorus	Potassium	Calcium	Magnesium	Sodium
	DF	*Pr > F*	*Pr > F*	*Pr > F*	*Pr > F*	*Pr > F*	*Pr > F*
Replicate	7	0.115	0.819	0.3205	0.6933	0.6416	0.4076
pH	4	0.0001	<.0001	0.188	0.0084	0.0154	<.0001
Error	28						
CV (%)		8.98	13.59	9.63	12.50	10.93	18.20

Table 12 Analysis of Variance (ANOVA) of the micro element content of Buchu plants as affected by the pH of the nutrient solution applied

Factor		mg.kg^{-1} dry mass				
		Manganese	Iron	Copper	Zinc	Boron
	DF	*Pr > F*	*Pr > F*	*Pr > F*	*Pr > F*	*Pr > F*
Replicate	7	0.2951	0.0795	0.7751	0.7973	0.1508
pH	4	<.0001	0.0005	0.0004	<.0001	0.0002
Error	28					
CV (%)		20.15	15.36	38.78	11.84	17.58

Nitrogen

The nitrogen content of Buchu plants irrigated with a nutrient solution with a pH of 7-7.99 was significantly higher than that of plants irrigated with nutrient solutions with lower pH values (Table 13). Within the pH range of 3-5.99 no significant differences with regard to the nitrogen content was found. The pH-treatment of 6-6.99 however resulted in nitrogen contents which were significantly lower than all other treatments with the exception of the pH 4-4.99 treatment. The nitrogen contents of between 1.48-1.91% found with all pH treatments were within the norm of 1.5-2.1% for *Leucadendron* 'Safari Sunset' set by Ran *et al*. (2001), but higher than average of 0.57% found in one to eight year old plants from their natural habitat (Chapter 3) or the range of 0.39-1.0%; 0.45-1.24% and 0.71-1.11% reported for *Leucadendron* 'Silvan red', *Leucadendron* 'Safari Sunset' and *Protea* 'Pink Ice' respectively (Cecil *et al*., 1995). Differences found for different pH treatments may be due to the effect of pH on the oxidation-reduction equilibrium and the solubility of micro elements as well as the ionic form of several elements, such as nitrogen and phosphorus (Hodgson, 1963).

Table 13 Influence of pH of the nutrient solution on the macro element composition of Buchu plants

Factor	Nitrogen	Phosphorus	Calcium	Magnesium	Sodium
	(%)				mg.kg^{-1}
pH					
3 – 3.99	1.64 b	0.42 b	0.83 a	0.17 a	939.3 bc
4 – 4.99	1.61 bc	0.44 b	0.83 a	0.15 b	763.1 c
5 – 5.99	1.69 b	0.41 b	0.84 a	0.15 b	804.4 c
6- 6.99	1.48c	0.40 b	0.72 b	0.15 b	1019.5 b
7 - 7.99	1.91a	0.68 a	0.93 a	0.16 ab	2017.3 a
LSD (P=0.05)	1.15	0.07	0.11	0.02	206.65
CV (%)	2.05	2.05	2.05	2.05	2.05

Means followed by the same letter do not differ significantly at P = 0.05 (LSD)

Phosphorus

In this study, phosphorus contents of Buchu plants varied between 0.40 and 0.68% (Table 13) which are about ten times higher than the average of 0.03% found in 1-8 year old plants sampled in their natural habitat (Chapter 3). No significant differences with regard to the phosphorus content as a result of the pH treatments between pH 3-6.99 were found, but the pH treatment 7-7.99 resulted in a significantly higher value of 0.68% (Table 13). Most studies on pH dependence of P uptake in higher plants have found that uptake rates are highest between pH 5.0 and 6.0 (Schachtman, Reid & Ayling, 1998). Research findings of Breeze, Edwards & Hopper (1987) indicate that phosphorus concentration from the shoots of white clover plants grown at pH 4 were up to 2.0 mg.g^{-1} plant dry mass lower than those of plants grown at pH 6, 6 and 7.

Calcium

Calcium contents of plants varied between 0.72 and 0.93% (Table 13) which were slightly higher than the average concentration of 0.59 % found in the natural habitat, although a calcium content of 0.93% was also found at the Tielmanroos 2 site in the natural habitat (Chapter 3). The concentration of 0.72% is within the range of 0.39-0.79% reported for *Protea* ‘Pink Ice’ (Maier *et al*., 1995), but lower values of 0.26-0.56 and 0.24-0.49% were reported for *Leucadendron* ‘Silvan Red’ and *Leucadendron* ‘Safari Sunset’ respectively (Cecil *et al*., 1995). In this study no significant differences due to the pH in the nutrient solution were found except the low value of 0.72% obtained with the pH 6-6.99 treatment. However, this lower value in pH treatment 6-6.99 maybe due to experimental error because no clear trend due to pH treatments were found.

Magnesium

Magnesium contents in plants varied between 0.15-0.17% due to the pH treatments applied (Table 13) and were within the range found in the natural habitat although slightly lower than the mean value of 0.21% (Chapter 3). Contents were also within the range found for the *Leucadendron* cultivars of ‘Silvan Red’ and ‘Safari Sunset’ (Cecil *et al*., 1995). In this study magnesium content for pH treatment 3-3.99 was significantly higher than that found in plants irrigated with a nutrient solution with pH values of 4-4.99, 5-5.99 and 6-6.99, but not significantly different from the pH 7-7.99 treatment.

Sodium

Levels of sodium in the Buchu plants were found to increase with the increase in pH in the nutrient solution from 3.0-7.99 (Table 13). However, the sodium contents of between 763.1 and 804.4 mg kg^{-1} of dry mass in pH 4-4.99 and 5-5.99 respectively were lower than the values obtained with plants from the natural habitat (Chapter 3) while other pH treatments were within the ranges found in the natural habitat. The concentrations obtained from pH 7-7.99 (0.2%) corresponded with the levels found in *Leucadendron* 'Silvan Red' (0.26-0.69%), *Leucadendron* 'Safari Sunset' (0.2-0.66%) while concentration in pH 6-6-99 (0.1%) was within the range obtained in *Protea* 'Pink Ice' (0.12-0.18%) by *Cecil et al*. (1995).

Manganese

Manganese content of Buchu plants did not indicate a clear trend as a result of the pH treatments in this study, except for a significantly higher value of 450.38 mg.kg^{-1} dry mass found with the pH 7-7.99 treatments compared to the lower pH treatments (Table 14). All values were however within the range of 159 and 1 065 mg.kg^{-1} found in the natural habitat (Chapter 3), but pH 7-7.99 was higher than the levels obtained by Cecil *et al*. (1995), on *Leucadendron* 'Safari Sunset' where a range of 105-313 mg.kg^{-1} was reported.

Iron

The mineral analysis of the Buchu plants during the final harvest indicated that pH treatments 3-3.99 and 7-7.99 resulted in significantly higher levels compared to the pH 4-4.99 and 6-6.99 treatments (Table 14), but no clear trend was found. The range of between 46.88 and 66.5 mg kg^{-1} was lower than the range of 77-245 mg.kg^{-1} found in the natural habitat (Chapter 3), but somewhat higher than the contents of 30-55 mg.kg^{-1} reported by Maier *et al.* (1995) on *Protea* 'Pink Ice'.

Table 14 Influence of pH on the micro element composition of Buchu biomass

Factor	Manganese	Iron	Copper	Zinc	Boron
pH	mg.kg^{-1}				
3 – 3.99	307.13 b	66.25 a	3.75 a	54.75 a	29.63 b
4 – 4.99	254.38 bc	49.75 c	2.38 b	33.13 b	28.88 b
5 - 5.99	279.63 bc	54.00 bc	2.37b	30.38 b	27.13 b
6 – 6.99	220.88 c	46.88 c	2.00 bc	31.75 b	26.63 b
7 – 7.99	450.38 a	61.25 ab	1.38 c	32.13 b	39.93 a
LSD (P=0.05)	62.43	8.75	0.94	4.42	5.47
CV (%)	2.05	2.05	2.05	2.05	2.05

Means followed by the same letter do not differ significantly at P = 0.05 (LSD)

Copper

Copper contents decreased from 3.75 mg kg^{-1} to 1.38 mg kg^{-1} with an increase in pH from 3-7.99 (Table 14). The levels of copper in 3-6.99 pH treatments were however within the range of 2 to 5 mg.kg^{-1} found in the natural habitat (Chapter 3), while research findings by Haynes & Swift (1985) on highbush blueberry also indicated that plant copper contents are significantly decreased by liming of the soil to increase the pH from 3.9 to 6.7.

Zinc

Zinc contents in this study were higher than the average of 7.91 mg.kg^{-1} found in the natural habitat (Chapter 3) and compared to the other pH treatments a significantly higher value of 54.75 mg kg^{-1} was found with the lowest pH treatment of 3-3.99 (Table

14). This was most probably due to a decrease in the solubility of inorganic zinc with increasing pH (Ehrlich, 1971; Marschner, 1986; Anderson & Christensen, 1988).

Boron

With the exception of a significant higher boron content of 39.93 mg kg^{-1}as a result of the pH 7-7.99 treatment, pH treatments did not have an effect on the boron content in this study (Table 14). On average, boron contents were within the range reported in Chapter 3 for plants found in the natural habitat. Boron absorption increases as pH increases above 4, reaching a maximum at pH 8-9 (Sims & Bingham, 1968; Moraghan & Mascagni, 1991). Bingham, Elseewi & Oerli (1973) measured the effect of solution pH on boron uptake by barley. They found a gradual decrease in boron uptake as pH was increased from 5 to 11.

Essential oil quality

Results are reported in terms of relative percentages of the integrated peak areas relative to the total integrated peak-areas on the chromatogram. The main factor pH however did not have a significant effect on the essential oil quality parameters measured in this study except for *cis*-8-Mercapto-p-menthan-3-one (Table 15).

Table 15 Analysis of Variance (ANOVA) of essential oil quality parameters

Factor		*Cis-/trans-* Isopulegone	Pulegone	*Ψ*- Diosphenol	Diosphenol	*trans*-8-Mercapto-p-menthan-3-one		Menthone	Isomenthone	*cis*-8-Mercapto-p-menthan-3-one		Limonene
		Relative Percentage (%)										
	DF	*Pr > F*	*Pr > F*	*Pr > F*	*Pr > F*	*Pr > F*	*DF*	*Pr > F*	*Pr > F*	*Pr > F*	DF	*Pr > F*
Replicate	7	0.2719	0.2425	0.2963	0.3664	0.2015	7	0.4535	0.2807	0.1955	7	0.0859
pH	4	0.7533	0.3599	0.3617	0.3434	0.4664	4	0.4179	0.0737	0.0001	4	0.1788
Error	*28*						28				28	
CV (%)		15.03	23.31	15.21	20.45	46.9		12.39	13.88	21.68		35.44

High levels of diosphenol and absence of any measurable amounts of *cis*- and *trans*-acetylthio-p-menthan-3-one isomers in the analyzed samples of most treatments indicate that it originates from very pure *A. betulina* genetic material (Dr. M., per. comm., P. 0. Box 12127, Die Boord 7613, South Africa). The high levels of diosphenol in the oil indicate that the plant material represents the diosphenol chemotype of *A. betulina*. Pulegone levels were very high in all the treatments. All other major constituents (Table 16) are also at higher than acceptable levels. The high pulegone levels maybe related to the time of harvesting. According to Von Wielligh (1913) and Phillips (1917), the best time for harvesting is from January to March. In our study, Buchu was harvested in May and this might be the reason of high pulegone levels. Endenburg (1972) concluded that harvesting in different times of the year resulted in differences in pulegone levels. The first harvest of Buchu is normally done after one year of transplanting and the trial was harvested after seven months of transplanting. Pulegone is hepatotoxic and it is a monoterpene that protects source plants against predators (Thomassen, Slattery & Nelson, 1990), as a result it is believed to be higher in younger plants (Dr. M. Esterhuysen, 2006, per. comm., Puris Natural Aroma Chemicals (Pty) Ltd, P. 0. Box 12127, Die Boord 7613, South Africa).

The levels of major essential oil quality parameters obtained in this study are similar to the levels obtained from the natural environment tabulated in Chapter 3 except for pulegone, *cis*-/*trans*-isopulegone, limonene and *trans*-8-mercapto-p-menthan-3-one. The pulegone levels from the natural environment ranged from as low as 0.5 to 3.3% while levels ranging from 10.8 to 13.3% were obtained from the greenhouse experiment. Age of the plants and time of the year during harvesting might have influence on pulegone levels (Endenburg, 1972; Thomassen *et al*., 1990). Levels of limonene ranged from 13.31 to 20.14% in the greenhouse experiment while higher levels ranging from 24.6 to 33.7% were obtained from the natural environment. The concentration of *cis* - *trans*-8-mercapto-p-menthan-3-one was higher in the greenhouse experiment compared to the natural environment and still higher than the average for cultivated Buchu (2.0 to 3.5 %, sum of isomers). However, the concentration of 8-mercapto-p-menthan-3-one isomers is higher in young plants (less than 3 years) or plants that contain fresh growth (Collins *et al*., 1996). This explains the differences in concentration of these isomers between the greenhouse experiment and plants from the natural environment. The gas chromatograms of different pH treatments are very similar with slight differences in levels of essential oil constituents (Figures 3, 4, 5, 6 and 7).

Table 16 Influence of pH on major essential oil quality parameters

Factor	Limonene	Menthone	Isomenthone	*Cis-/trans*-Isopulegone	Pulegone	Ψ-Diosphenol	Diosphenol	*trans*-8-Mercapto-p-menthan-3-one	*cis*-8-Mercapto-p-menthan-3-one
pH	Relative Percentage (%)								
3-3.99	14.888	3.8250	14.313	6.4000	10.800	16.075	20.788	2.0125	3.1625 b
4-4.99	16.388	3.9500	14.500	6.3625	13.063	13.988	17.000	2.3750	3.5750 b
5-5.99	13.313	4.1875	15.738	6.5625	12.513	15.513	19.813	2.1250	3.3750 b
6-6.99	20.138	3.8625	13.450	5.9375	11.413	14.800	19.188	1.7000	2.8126 b
7-7.99	14.863	4.2143	16.129	6.2875	13.288	14.325	18.025	1.6125	4.8857 a
LSD (P=0.05)	ns	ns	ns	ns	ns	ns	ns	ns	0.7957
CV (%)	2.05	2.05	2.05	2.05	2.05	2.05	2.05	2.05	2.05

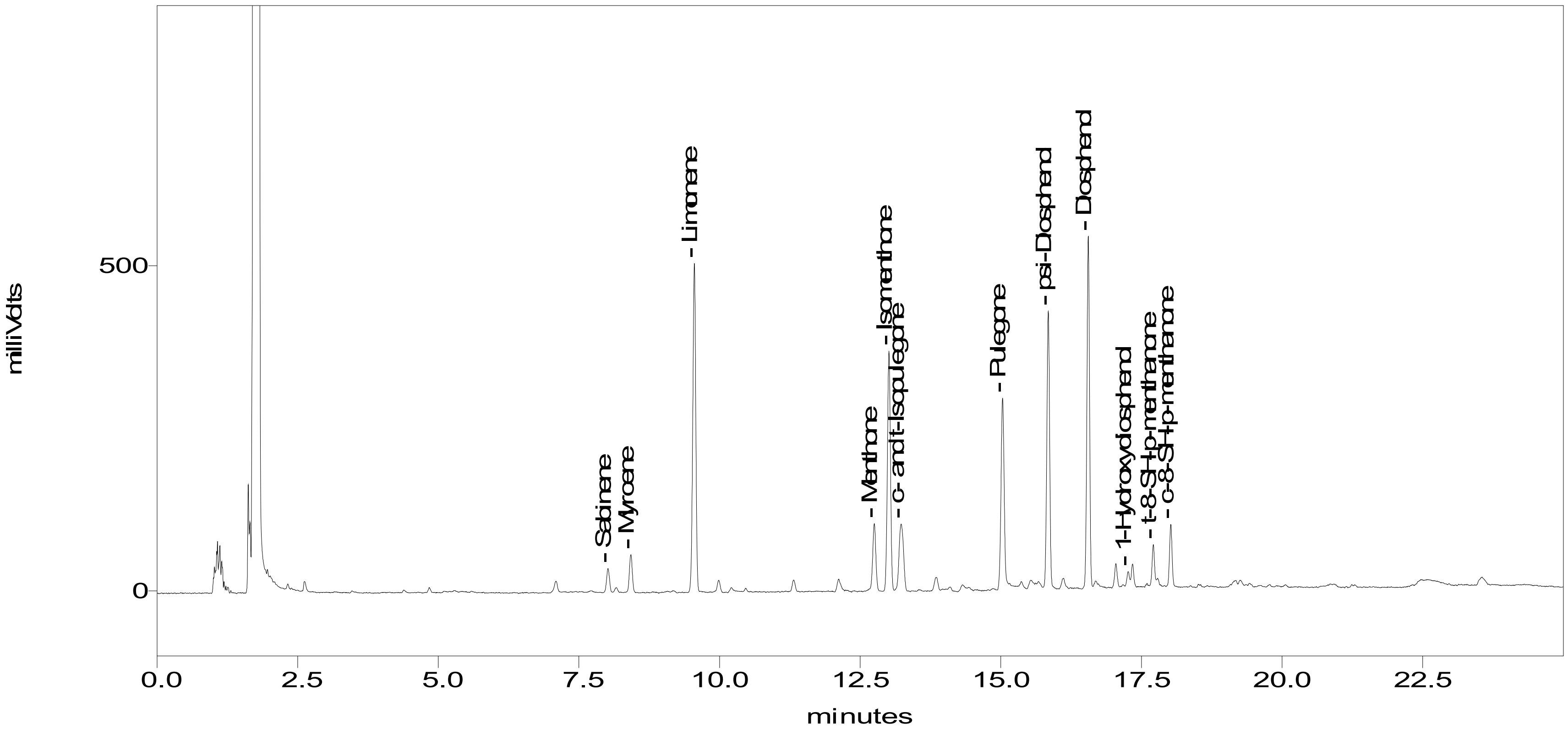

Figure 3 Gas Chromatogram of *A. betulina* oil obtained from a plant sample of the pH 3-3.99 treatment (Puris Natural Aroma Chemical (Pty) Ltd)

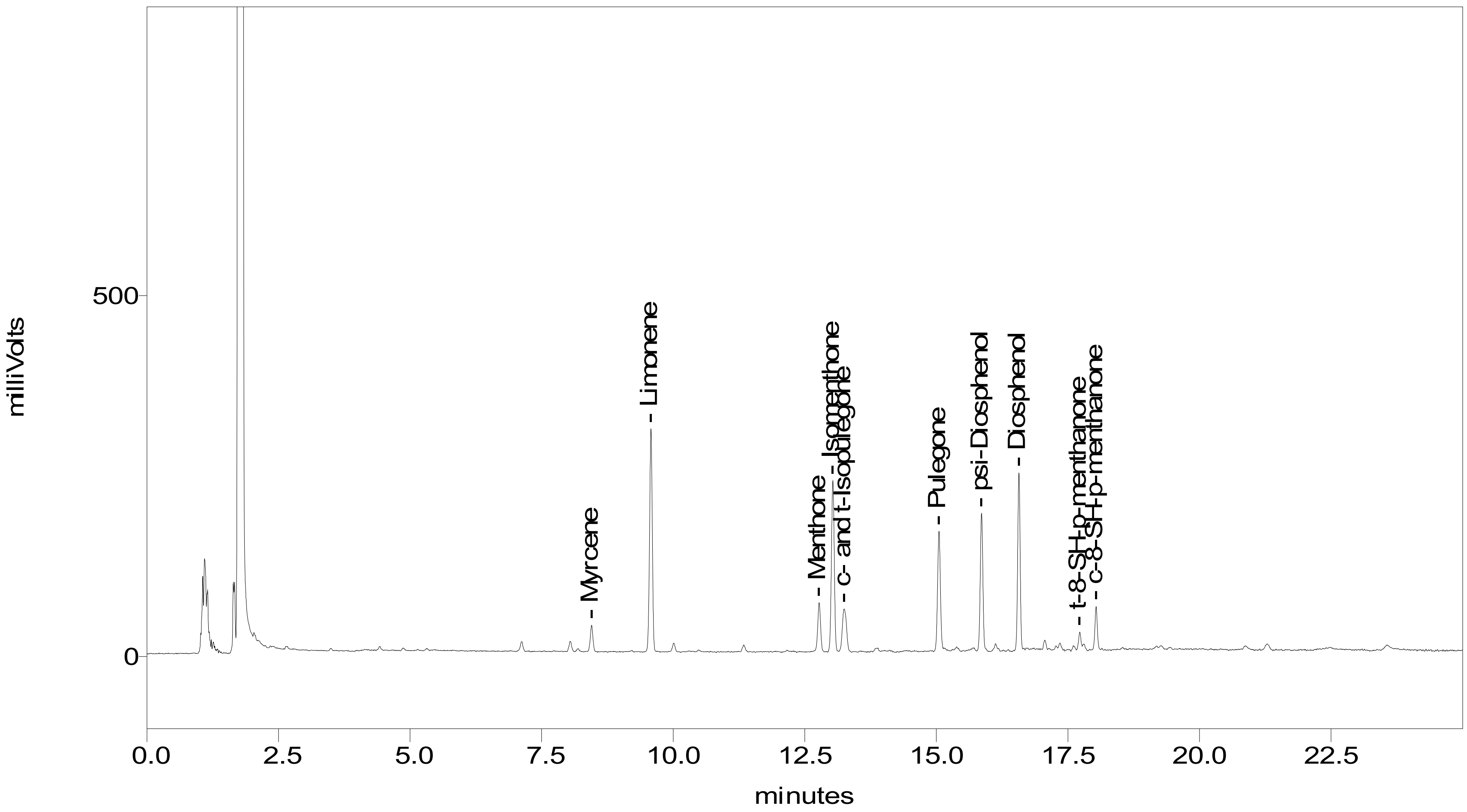

Figure 4 Gas Chromatogram of *A. betulina* oil obtained from a plant sample of the pH4-4.99 treatment (Puris Natural Aroma Chemical (Pty) Ltd)

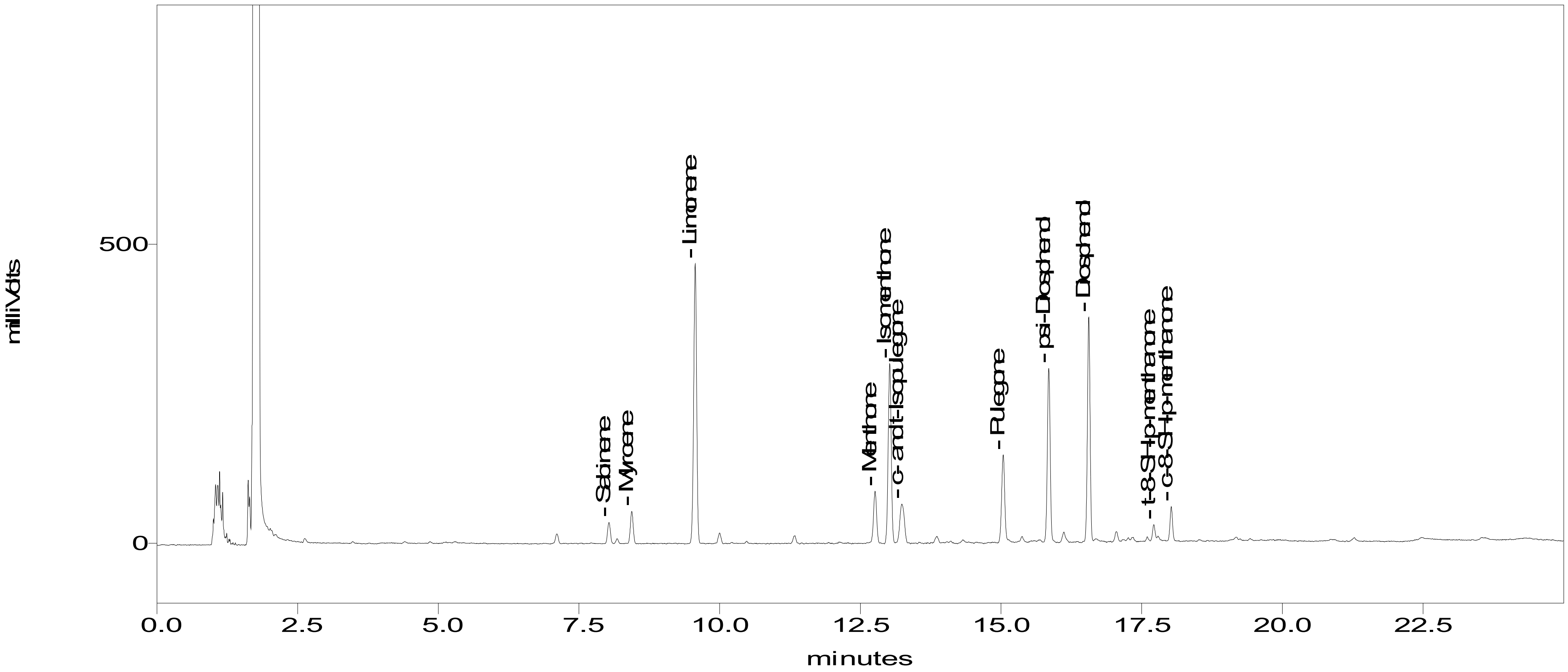

Figure 5 Gas Chromatogram of *A. betulina* oil obtained from a plant sample of the pH5-5.99 treatment (Puris Natural Aroma Chemical (Pty) Ltd)

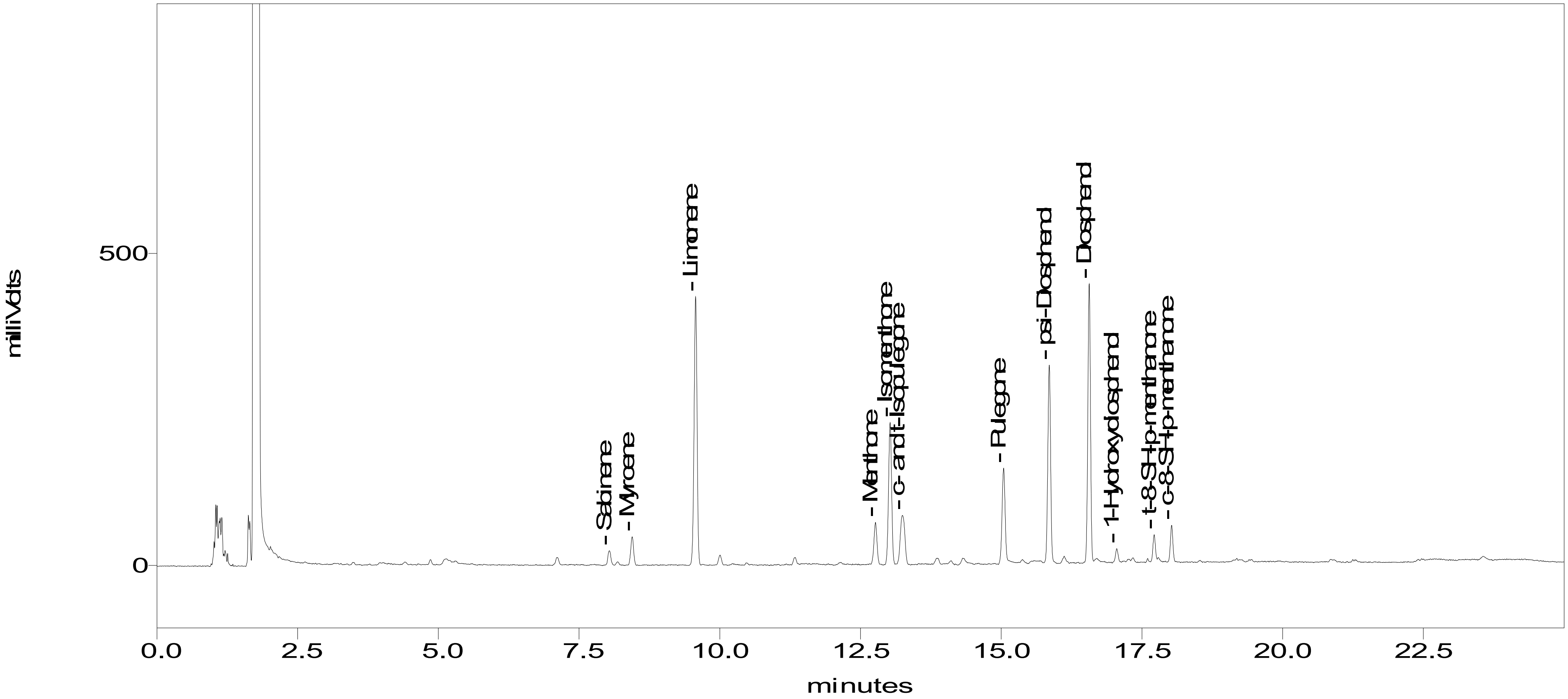

Figure 6 Gas Chromatogram of *A. betulina* oil obtained from a plant sample of the pH6-6.99 treatment (Puris Natural Aroma Chemical (Pty) Ltd)

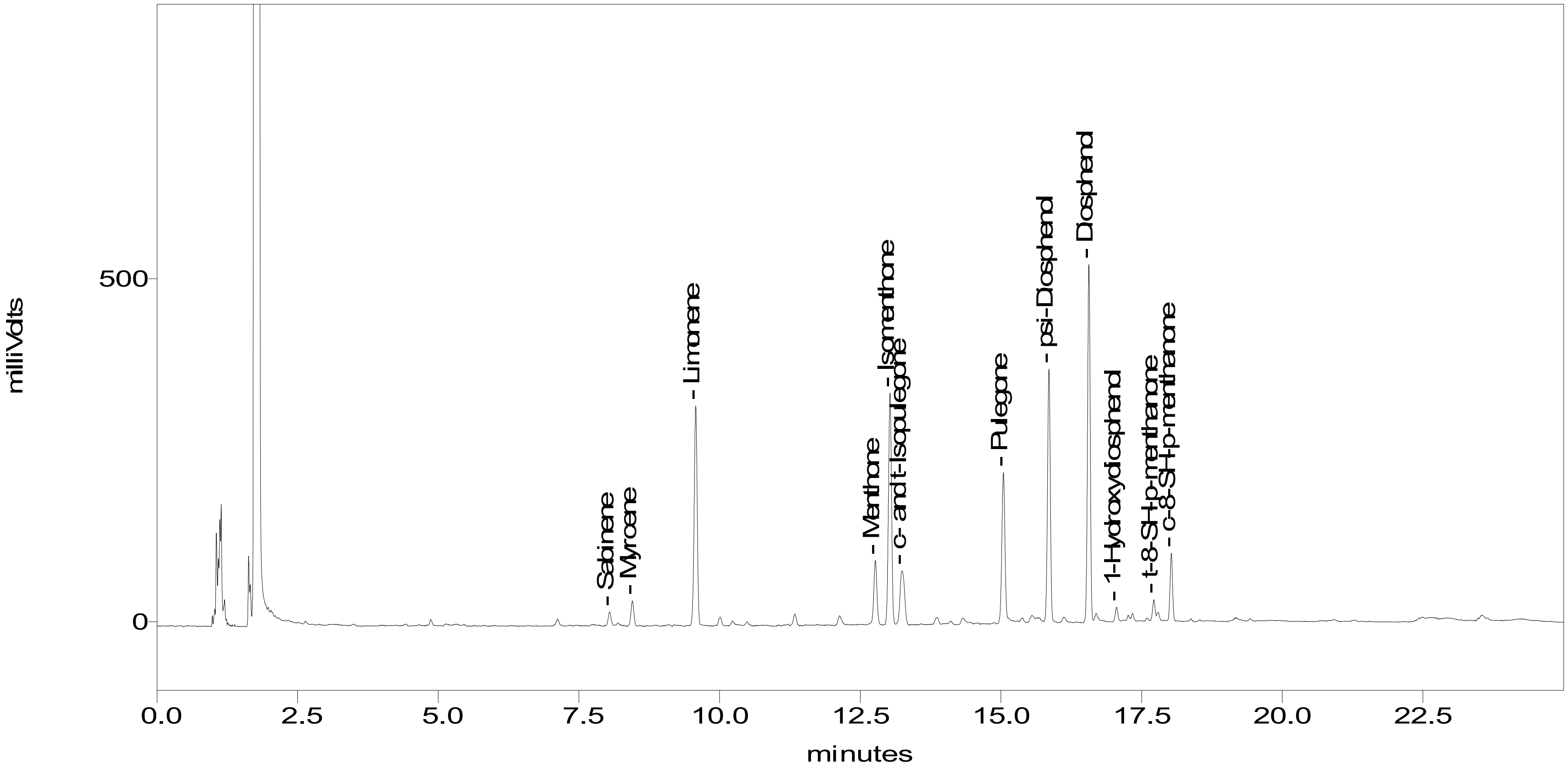

Figure 7 Gas Chromatogram of *A. betulina* oil obtained from a plant sample of the pH7-7.99 treatment (Puris Natural Aroma Chemical (Pty) Ltd)

Conclusions

Plants irrigated with a nutrient solution with a pH of 4-4.99 indicated the highest growth rate as measured by plant height and biomass accumulation, but were not significantly different from the pH treatments of 3-3.99, 5-5.99 and 6-6.99. This was expected since Buchu grows well in acidic conditions in its natural environment. In the natural environment, the soil pH ranges from 3.7 to 5.3. This also gives an indication that Buchu can grow well on a wide range of pH. For proper growth and high yields, pH in the range of 7-7.99 is not recommended because the plants become stunted and less bushy resulting in lower yields. The overall upper limit for proper growth is 6-6.99.

Both the macro and micro mineral contents of the plants during the harvest after seven months of exposure to different pH levels in the nutrient solution indicated significant differences. High pH levels resulted in high nitrogen, phosphorus, sodium, manganese and boron contents, but low copper contents. Nitrogen contents were within the norm of 1.5 to 2.1 % for *Leucadendron* ‘Safari Sunset” but higher than the average of 0.57 % found in one to eight year old plants from their natural habitat. Phosphorus was ten times higher than the average of 0.03 % found in the natural habitat. Sodium levels were within the norm reported for other plants, while values lower than the average of plants from the natural habitat were obtained with pH treatments 4-5.99. Manganese levels were within the range of 159 to 1 065 mg.kg^{-1} found in the natural habitat but higher than the levels reported for *Leucadendron* “Safari Sunset”. No clear trends were found with regard to calcium, magnesium, iron and zinc. There were no significant differences with regard to potassium. In this study values for nitrogen, phosphorus, calcium and zinc were higher than that recorded for plant from their natural habitat, but values for manganese, sodium, magnesium and copper were more or less similar. Boron and copper contents found in this study were somewhat lower than that recorded for plants from their natural habitat. Dry mass accumulation had a positive correlation with plant height. Less dry mass accumulation was obtained in pH 7-7.99 where the plant height grew at a much slower rate as compared to other treatments.

For essential oil quality, however, there were no significant differences between the major constituents in all pH treatments i.e. diosphenol, limonene, menthone, pulegone, isopulegone etc. This gives an indication that pH has little influence on quality of Buchu oil under the pH ranges studied and over the time frame evaluated. However, Buchu oil had high levels of pulegone content, higher than acceptable (state acceptable limits here). Therefore, the marketability of the oil would be negatively affected. There are many factors that might contribute to these high levels, such as age of the plants, time of harvesting and nutrition. Therefore, more research should focus on finding solutions on how to keep the pulegone concentration at minimum acceptable levels (o to 5%). In conclusion, this study has revealed that pH has an influence on Buchu biomass accumulation, while the quality of the essential oil is not affected.

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CHAPTER 5

GENERAL CONCLUSIONS

Buchu is one of the traditional medicinal plants in the Western Cape province of South Africa and the essential oil derived from the leaves is exported in large volumes. The essential oil is not only used for medicinal purposes, but also as a fixative in the food industry. Due to the high demand, under supply, restriction of wild harvesting and price for Buchu essential oil, growers have started to commercialize Buchu and established vast fields. The commercialization of Buchu necessitated the need for agronomic research. To date, very little research has been done on the production of Buchu both under conventional and hydroponic production techniques. However, funds were heavily invested in research focusing on essential oil and its constituents, as well as medical research. Therefore, the main objective of this study was to determine the optimum pH range for the cultivation of high yielding Buchu with exceptional oil quality grown under controlled environment and to compare it with conditions in its natural habitat.

Surveys of the natural habitat of Buchu were conducted whereby soil mineral content and climatic data were studied. Plant growth, leaf mineral content and essential oil constituents of Buchu plants from the eleven sites in the natural habitat were also determined. This study revealed that Buchu grows widely on sandy soils and under conditions of low nutrients. The soils are generally infertile with very low pH and low in base status and nutrient reserve. Levels of nutrients were found to be low compared to the general requirements set for crop production and in comparison with values found in fields used for winter field crops and pasture production in the Western Cape. Therefore, if Buchu is grown commercially on these soils, fertilizer requirement would be very low. pH results indicated that the natural environment of Buchu has a low pH. The pH ranged between 3.7 and 5.3 on all the sampled sites. Although differences between the sites were found, the mineral content of most elements in the leaves was low, corresponding with the mineral content of the soil. Levels of both macro and micro elements in the leaves were lower than the levels reported for most crops in literature. Results of essential oil analysis revealed no large differences in levels of major constituents because the gas chromatograms of all the sites were similar. Levels of all essential oil parameters were within the ranges set for *A. betulina* in the natural habitat. Very low levels of pulegone, high levels of diosphenol and the absence of any measurable amounts of *cis-trans*-acetylthio-p-menthon-3-one isomers indicate that the plants originates from a pure *A. betulina* genetic material. Also the high levels of diosphenol in the oil indicate that the plants represent the diosphenol chemotype.

The greenhouse experiment was conducted in order to evaluate the effect of pH on growth, yield and essential oil quality of *A. betulina*. A complete nutrient solution with five different pH adjustments was used in the experiment. To determine growth, height of the plants was measured, and results indicated no differences between pH treatments 3-5.99, but poor growth was obtained with the pH treatment 7-7.99. The highest fresh (409.96kg) and dry mass (111.74kg) per plant was obtained at a pH of 4-4.99 while the lowest fresh (127.61kg) and dry mass (30.34kg) per plant was obtained at the highest pH (7-7.99). However, there were no significant differences between pH treatments 3-6.99.

Levels of nutrients obtained from the leaf mineral analysis differed significantly with different pH treatments. High pH levels resulted in high nitrogen, phosphorus, sodium, manganese and boron contents, but lower contents of copper. No clear trends were found with regard to calcium, magnesium, iron and zinc. Nitrogen, phosphorus, calcium and zinc were higher than those recorded for plants from their natural habitat but still within the norm reported for most plants, while levels of manganese, sodium, magnesium and copper were more or less similar to the values obtained from the natural habitat.

The main factor pH had no significant difference in all the essential oil quality parameters. High levels of diosphenol and absence of measurable amounts of *cis-trans*-acetylthio-p-menthan-3-one isomers in the plant material indicate that it originates from pure *A. betulina* genetic material and diosphenol chemotype. These findings correlated with the results of the natural environment. Pulegone content was found to be very high, while other major essential oil parameters were at higher acceptable levels for *A. betulina* diosphenol chemotype.

The high pulegone levels in the greenhouse experiment may have a negative impact in the marketability of the essential oil. Factors which might contribute to these high levels of this component include time of harvesting, age of the plants, nutrition and other environmental conditions. In the natural environment, the plant material was collected in March during an inactive growth stage of the plants compared to May in the greenhouse experiment where the plants remain in an active growth phase throughout the growing period. February to March is recommended as the best time for harvesting Buchu under field and natural conditions. The plants in the natural environment were more than one year old, while the plants in the greenhouse experiment were harvested after seven months from transplanting. Differences in plant nutrition and environmental conditions between the natural habitat and greenhouse experiment might also contribute to differences in pulegone levels. For better comparison of natural habitat and greenhouse experiments, research under controlled environment should be extended to a period of more than one year. Future research should also be directed more towards obtaining exceptionally high quality essential oil with low pulegone levels. Therefore, research on maintaining the acceptable levels of pulegone through nutritional and environmental manipulation is recommended. Whether it is viable to plant Buchu under greenhouse conditions where the plant remains in the active stage throughout the growing period is still unknown. However, harvesting of Buchu plant material grown using hydroponic production system at different times of the year may be a solution. Therefore, future research should also incorporate harvesting at different times under hydroponic conditions. In conclusion, this study has revealed that pH has an influence on the biomass accumulation in Buchu, while the essential oil quality proved not to be affected by pH.