# YIELD AND QUALITY RESPONSE OF HYDROPONICALLY GROWN ROSE GERANIUM (*Pelargonium* SP.) TO CHANGES IN THE NUTRIENT SOLUTION AND SHADING

by

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# DECLARATION

I **Moosa Mahmood Sedibe** declare that the thesis hereby handed in for the qualification **Philosophiae Doctor (Agronomy)** degree at the University of the Free State, is my own independent work and that I have not previously submitted the same work for a qualification in another university/faculty for a degree either in its entity or in part.

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## ABSTRACT

This study was undertaken to determine the effect of different concentrations of phosphate, ammonium, nitrate, and sulphate as well as that of shading and moisture stress on oil yield and quality of hydroponically grown rose geranium. Five separate trials were conducted during the 2009 and 2010 growing seasons. Different concentrations of phosphate, ammonium, nitrate and sulphate were used in the first four trials, while the last study focused on the effects of shading and moisture stress on rose geranium.

The phosphate, nitrate, ammonium and sulphate trials were conducted in a greenhouse at the west campus of the University of the Free State in Bloemfontein, South Africa. Plants were grown for four months using a randomized complete block experimental design. The concentrations of phosphorus evaluated were 0.10, 0.80, 1.50 and 2.20 meq $L^{-1}$. Ammonium concentrations were 0.00, 0.50, 1.00 and 1.50, nitrate concentrations were 8, 10, 12 and 14 and sulphate concentrations were 0.36, 1.90, 3.44 and 4.98 meq $L^{-1}$.

Foliar drymass and oil yields increased as P concentrations were increased to 2.20 meq $L^{-1}$. Both, the guaia-6,9-diene content and the citronellol:geraniol (C:G) ratio were better at the high level of phosphate indicating that the best quality oil, as required by the perfume industry is obtained with relatively high phosphate concentrations.

Plant growth as measured by the number of branches and biomass production, peaked at 10 to 12 meq $L^{-1}$ nitrate concentrations. The highest chlorophyll content in the foliage was found at the nitrate concentrations of 10 and 12 meq $L^{-1}$, where the best oil yield was also produced. At this nitrate level the citronellol:geraniol (C:G) ratio was slightly higher than the upper limit required for good oil quality but the geraniol and citronellylformate contents were within range for top quality oil. Height, biomass, oil yield and chlorophyll content of the leaves were not affected by ammonium, but the concentrations of plant tissue sulphur and nitrogen increased linearly with increasing concentrations of applied ammonium. Rose geranium needs to be grown at a

relatively high nitrate concentration (10 to12 meq $L^{-1}$) to ensure high oil yield. This application falls within the range that is used for most vegetable and ornamental crops under soilless conditions. Ammonium concentrations of up to 1.00 meq $L^{-1}$ can be used without affecting yield or oil quality of rose geranium.

A significant effect of sulphate on branches, height and branch:height (B:H) ratio and foliar dry mass (DM) was observed. The four sulphate concentrations showed a statistically non significant trend on yield. Based on the standards used by the perfume industry the oil of rose geranium was not of a good quality in this trial probably due to the autumn planting time.

Shading and moisture stress were used as treatments in a study conducted at the University of the Free State experimental farm during spring and summer. A split plot experimental layout was assigned using 0%, 20%, 40%, 60% and 80% shade treatments allocated to the main plots. The subplots were exposed to moisture stress levels at 0 and -0.15 MPa of osmotic pressure.

Rose geranium grew well under a shading of 40%, where plant growth parameters such as foliar fresh mass (FM), foliar dry mass (DM) and the branch:height ratio were increased. Subsequently the best oil yield was obtained at this level. Proline content was high due to excessive solar radiation at 0% shade as well as where moisture stress was induced, however, oil quality was not affected. The number of oil glands $cm^{-2}$ of leaf area was not significantly affected by shading, but tended to be lower at shading levels higher than 60%. Fresh mass, DM, the ratio of branches to height and oil yield were affected by shading. Proline content gave a clear indication of stress conditions of plants at full radiation as well as moisture stress. Growers are advised to use 40% shading to grow geraniums in summer at radiation levels similar to those found in this study.

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# CHAPTER 1

## INTRODUCTION AND BACKGROUND TO THE STUDY

## 1.1 Rational and motivation

Rose geranium *(Pelargonium graveolens* L.) is an aromatic plant that has increased in popularity to become one of the most important essential oil crops in South Africa's agricultural industry. Currently, the crop is grown in open fields, not always ideal for successful production of high value aromatic oil crops. Exposure of these crops to extreme weather conditions alters the quality of oil and also lowers oil yield (Rao *et al.,* 1996). Plants that are grown under unprotected conditions may also take a protracted period to become established (Rao *et al.,* 1996). To avoid the effects of adverse weather conditions, growers in South Africa are considering growing rose geraniums under protection, i.e. in greenhouses, tunnels or shade structures.

Greenhouse crops are produced under controlled environmental conditions, where light intensity, temperature and humidity are controlled. Besides, plants that are cultivated under protection can also be grown on soilless substrates to be fertigated with a well balanced nutrient solution. These plants may establish more rapidly. Two or more planting seasons can be achieved per year and more plants can be cultivated per unit area (Bone & Waldron, 1999).

The main problem that has been detected with some greenhouse produced essential oil plants is the reduction in oil quality, even though the oil yield may be high. This may subsequently result in a reduced market price as was reported by Johnson *et al.* (1999) on sweet basil (*Ocimum basilica* L.). A preliminary study showed that plants grown under moisture stress conditions produced oil of good quality (Brown *at al.,* 2008).

Kaul *et al.* (1997) reported that oil yield of rose geranium cultivars was reduced when shaded by other crops in an intercrop system. This partial shading also reduced oil quality in all the cultivars that were tested but had no effect on the organoleptic characteristics (colour, specific gravity, refractive index and optical rotation).

The successful production of crops under soilless conditions depends on the water quality used for the preparation of the nutrient solution. Salinity is a problem affecting water quality in some areas of South Africa. With high concentrations of bicarbonates, sodium and chloride in the water, irrigated plants experience this as a water stress (Combrink, 2005; Du Preez *et al.,* 2000). Currently, there is little information available on the impact of water stress, shading, and different nutrient ratios on oil yield and quality of hydroponically grown rose geranium.

Soilless crop production has gained popularity in recent years for its potential to optimize yields. It allows farmers to grow plants more efficiently and water can be saved when using a re-cycling production system (Bone & Waldron, 1999).

## 1.2 Hypotheses

It is hypothesized that nutrient status (anions and cations) and light intensity might affect oil yield and quality. Employing soilless culture to optimize nutrition and protecting a summer crop with shade net might enhance oil yield and quality of rose geranium.

## 1.3 Objectives

The main objectives of this study were to determine the effects of different nutrient solutions, shading and moisture stress on oil yield and quality of hydroponically grown rose geranium. The specific objectives were:

- To evaluate the effect of phosphate on oil yield and quality of rose geranium, as well as an attempt to set standards for concentrations to be used in the nutrient solution of hydroponically grown plants.
- To evaluate nitrate and ammonium as sources of nitrogen and to set standards for soilless production of rose geranium.
- To determine the effects of sulphate levels in nutrient solutions on yield and quality of hydroponically grown *Pelargonium graveolens* and to set standards of sulphate to be used in the nutrient solution.
- To evaluate the effect of shading and moisture stress on yields and oil quality of hydroponically grown rose geranium.

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# CHAPTER 2

## LITERATURE REVIEW

## 2.1 Introduction

*Pelargonium* species are indigenous to South Africa and are confined to the Limpopo and the Western Cape provinces of South Africa (Van Der Walt & Vorster, 1988). They reported that some species of *Pelargonium* have been found in countries such as Zimbabwe and Mozambique.

The leading geranium oil producing countries are China, Reunion, Algeria, France, Spain, Morocco, Madagascar, Congo and Russia (Lis-Balchin, 1996). United Nations (2004) showed that the global market of essential oils is estimated to be more than R6 billion. United States (40%), Western Europe (30%) and Japan (7%) are major consumers of this oil (Lis-Balchin, 1996).

International markets for South African produced rose geranium oil are said to be growing steadily year by year. These markets require an average of about 2.5 t $year^{-1}$ of locally produced geranium oil (NEDLAC, 2011; DAFF, 2009). It is a mammoth task for South African producers to meet the growing market demand and adding to the problem, poor oil quality is correlated to adverse weather conditions (Brown *et al.,* 2008). Rose geranium is cultivated mainly in the Mpumalanga (Lowveld), KwaZulu-Natal, Western Cape and Limpopo provinces of South Africa. Limited cultivation also occurs in Gauteng, North West, Eastern Cape and the Free State provinces (DAFF, 2009). The area planted to rose geranium in South Africa is very small, estimated to be just over 2000 ha. The required level of production should be met by increasing yields through the use of better production systems rather than by expanding the area under production.

The oil market in South Africa is handled by local and international buyers. This includes marketing agents and companies from the chemical and pharmaceutical industries, as well as the food and flavouring industries. Large quantities of oil are exported to the international industries for flavour and fragrance, cosmetics and personal health care, aromatherapy as well as food manufacturers (DAFF, 2009).

## 2.2 An overview analysis of rose geranium

### 2.2.1 Origin and distribution

There are over 250 species of *Pelargonium* in the Geraniaceae family and hundreds of hybrids amounting to thousands of cultivars being available. South Africa is the centre of origin of this genus, with most of the known species commonly found in the Western Cape region of South Africa. Some of these species have scented leaves that produce essential oils, such as *P. capitatum, P. graveolens, P. odoratissium* and *P. radens* (Leistner, 2000). Apart from being indigenous to South Africa, rose geranium is widely cultivated in Egypt, India and China. To a lesser extent, it is also grown in Central Africa, Madagascar, Japan, Central America and Europe (Leistner, 2000).

It is believed that William van der Stel imported *P. peltatum* and *P. zonale* into the Netherlands in 1700. *Pelargonium* was introduced to England in 1701 and 1710 (Weiss, 1997). *Pelargonium* become popular and was exported to almost every European colony. Commercial cultivation of geranium for oil extraction began in the early nineteenth century around Grasse in France. Plants of *P. graveolens* were sent from Grasse to Algeria in 1874 and to the Reunion in the 1880s to establish local geranium oil plantations. The first commercial farms of rose geranium were established in Mitidja around Blinda and Boufaric (Weiss, 1997).

Rose geranium became an important oil crop, subsequent to its introduction to Réunion in 1870. The first oil produced in Réunion was from a species of unknown origin which later became a distinct local cultivar (Rose/ Bourbon/ Reunion). This cultivar is from a hybrid originating from a cross between *P. capitatum* and *P. radens* (Weiss, 1997; Lis-Balchin, 1996). The basic chromosome number of *Pelargonium* is $x$=11 and the somatic number for *P. graveolens* is $2n$=88. The Réunion cultivar is heptaploid ($2n$=77) suffering to some degree of male sterility (Weiss, 1997).

Rose geranium is characterized by large, bushy, upright and branching shrubs that grow to a height of 1.3 m (Demarne, 2002). It has a green to grey green (soft) stem, turning darker and woody with age. The leaf has 5-7 palmate lobes with opposite branches. The base is cordate, apex obtuse with revolute and toothed margins. The size of the leaves is variable, being approximately 5 cm long with long petioles (30 mm). The shape of the stipules is an asymmetrical triangular. The inflorescence is terminal and has a long (15-60 mm) peduncle (Fig. 2.1). It has a shallow to moderate root system that develops to a depth of 30 cm. Some stems are covered by long and fine bristles while others have short and scarcely visible bristles (Miller, 2002; Weiss, 1997).

*Pelargonium* is the only member of the Geraniaceae family with zygomorphic flowers, with a superior ovary, lobed five times and each lobe has a single pendulous with a short and hairy style. The inflorescence of *Pelargonium* is axillary with small umbels of 3-7 pink, rosy-purple flowers. Most *Pelargonium* does not produce seeds, only few species are capable of producing seeds. The seeds are small, oblong-ovoid and brownish and the testa is hard (Weiss, 1997).

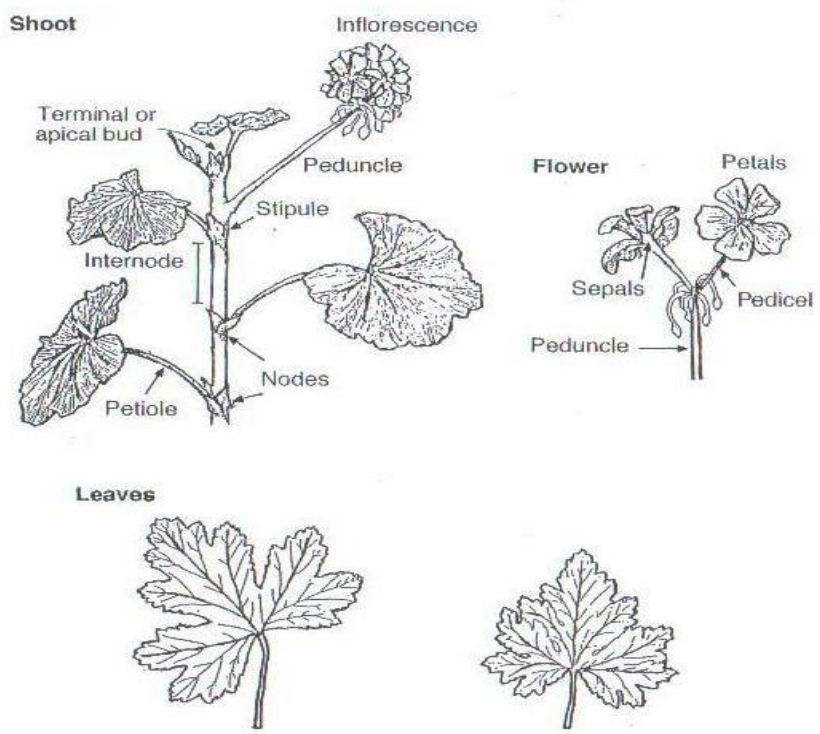


**Fig. 2.1** Schematic representation of a flower leaves and shoots of *Pelargonium* (Weiss, 1997).

The oil is contained in small and large trichomes distributed on green (leaves) parts of the plant. Their size and number govern the amount of oil produced by the plant. Leaf size and number are important factors affecting oil yield.

The pathway of oil biosynthesis and metabolism in rose geranium has been described by Motsa *et al.* (2006); Weiss (1997) and Ram and Kumar (1996). It varies with leaf age, with young leaves having more oil and greater geraniol content in the oil than older leaves (Weiss, 1997; Rao *et al.,* 1996).

### 2.2.2 Importance

#### 2.2.2.1 Uses

Rose geranium oil is used as flavouring agent in many major food categories, alcoholic beverages and soft drinks. Fresh rose geranium leaves can be used to impart a rose flavour to desserts and jellies (Gough, 2002). The oil is also used as an important ingredient in the perfume and cosmetic industries (Kulkarni *et al.,* 1997). Gomes *et al.* (2006) explained that in masculine perfumes, rose geranium oil is used as a heart note, adding a floral character to green and fougère compositions, as in Kouros by Yves Saint Laurent or in Polo Blue by Ralph Lauren. Rose geranium oil is used less in women's fragrances, although it constitutes a heart note in a classical chypres such as Aromatics Elixir by Clinique and Dioressence by Dior, and in florals, such as Paris by Yves Saint Laurent and Paul Smith Women by Paul Smith.

Rose geranium oil is used to treat colds, bronchitis, laryngitis, menopausal problems and has an important use in improving the quality of life used as a relaxant bath in aromatherapy to reduce nervous tension (Lis-Balchin, 1996). In the past, rose geranium oil was used to treat diarrhoea, dysentery, wounds (as astringent), abscesses, fever reduction, colic, nephritis, sore throats, gonorrhoea, stimulation of milk production and as well as a worm remedy (DAFF, 2009; Lis-Balchin, 1996). Rose geranium oil is also used commercially for extraction of rhodinol a mixture of linalool, citronellol and geraniol (Lis-Balchin, 1996).

#### 2.2.2.2 Production and imports

France is the major exporter of geranium oil to the USA when compared to other major exporting countries (Table 2.1). The chief importers of oil are the USA, France, United Kingdom, West Germany and Japan, importing 65, 95, 20, 15 and 20 metric tons year$^{-1}$, respectively. Market prices of geranium oil are not stable and will always fluctuate according to Chinese production and sales levels on the international market. Geranium oil from Réunion is of good quality and obtains a relatively high market price of \$162 kg$^{-1}$ oil (Lubbe & Verpoorte, 2011). The Egyptian and Chinese oil can be sold at \$65 and \$55 kg$^{-1}$ oil, respectively (ADC, 1998).

**Table 2.1** United States imports of rose geranium oil (ADC, 1998)

| | Year (metric tons) | | | | |
|---|---|---|---|---|---|
| **Supplier** | **1992** | **1993** | **1994** | **1995** | **1996** |
| France | 23 | 34 | 34 | 54 | 14 |
| China | 12 | 10 | 27 | 14 | 6 |
| Egypt | 12 | 13 | 15 | 29 | 3 |
| Others | 6 | 7 | 6 | 9 | 4 |
| **Total** | **53** | **64** | **82** | **106** | **27** |

Tables 2.1 and 2.2 further indicate that the world trade of the leading importers (US and Europe) of geranium oil is unstable and these countries will occasionally import oil from other countries (ADC, 1998).

**Table 2.2** European Union imports of rose geranium oil (ADC, 1998)

| **Supplier** | **France** | **Germany** | **UK** | **Other EU** | **Total** |
|---|---|---|---|---|---|
| France | 0 | 16 | 22 | 11 | 49 |
| Germany | 0 | 0 | 16 | 1 | 17 |
| UK | 3 | 0 | 0 | 2 | 5 |
| Spain | 0 | 0 | 2 | 14 | 16 |
| Other EU | 1 | 0 | 0 | 3 | 4 |
| Egypt | 18 | 2 | 10 | 1 | 36 |
| Réunion | 6 | 0 | 0 | 0 | 6 |
| USA | 3 | 14 | 2 | 4 | 23 |
| China | 28 | 4 | 5 | 15 | 52 |
| Other non EU | 7 | 0 | 1 | 2 | 10 |
| **Total** | **66** | **36** | **59** | **52** | **213** |

### 2.2.3 Chemical composition

The chemical composition of rose geranium oil is complex in nature and comprises a wide array of compounds. The composition of these chemical compounds varies among geraniums that originate from different countries (Table 2.3). Given this large variation in oil composition it can be seen that oil quality is difficult to define (Rana *et al.,* 2002).

Rana *et al.* (2002) identified thirty compounds that contribute up to 99.1% of the oil that is produced by rose geranium. For commercial purpose only six compounds are determined; linalool, citronellol, geraniol, citronellylformate, geranylformate, guaia-6,9-diene. The relative proportion of these compounds determines the odour quality of the oil. The citronellol:geraniol (C:G) ratio is used by the perfume industry to determine oil quality. A C:G ratio greater than 3 signifies oil of low odour quality. In contrast, a C:G ratio ranging from 1 to 3 is associated with a better odour quality of the oil (Saxena *et al.,* 2000).

**Table 2.3** Comparative chemical composition (%) of geranium oil from different countries (Lawrence, 1996)

| **Compound** | **Réunion** | **Moroccan** | **Egyptian** | **Chinese** | **Australian** |
|---|---|---|---|---|---|
| α-pinene | 0.8 | 9.9 | 0.8 | 0.4 | 4.6 |
| Linalool | 9.9 | 9.9 | 6.5 | 3.9 | 4.6 |
| *cis*-Roseoxide | 0.6 | 0.8 | 0.9 | 1.4 | 0.4 |
| *trans*-Roseoxide | 0.3 | 0.3 | 0.4 | 0.6 | 0.2 |
| Menthone | 1.0 | 2.1 | 0.5 | 2.4 | 0.2 |
| Isomenthone | 9.5 | 4.2 | 5.7 | 5.4 | 7.6 |
| α-terpineol | 0.8 | 1 | 0.5 | 0.3 | 0.5 |
| Citronellol | 20.6 | 28 | 27.7 | 36.5 | 31.7 |
| Nerol | 0.8 | 0.6 | 0.4 | 0.2 | 0.5 |
| Geraniol | 18.1 | 20.6 | 18 | 8.7 | 9.8 |
| Citronellylformate | 7.4 | 6.5 | 6.5 | 10.1 | 12.8 |
| Citronellylacetate | 0.9 | 0.9 | 0.7 | 0.7 | 0.8 |
| Geranylformate | 5.6 | 4.1 | 3.7 | 2.1 | 3.4 |
| β-caryophyllene | 1.3 | 0.5 | 1.3 | 1.2 | 1.3 |
| Guaia-6,9-diene | 5.8 | 0.5 | 0.3 | 6.5 | 4.6 |
| Germacrene D | 0.3 | 0.2 | 0.3 | 0.4 | 0.2 |
| Geranyl proplonate | 1.2 | 0.7 | 1.1 | 0.9 | 1.1 |
| Citronellyl butyrate | 0.5 | 0.4 | 0.6 | 0.9 | 0.6 |
| δ-cadinene | 0.5 | 0.6 | 0.9 | 0.7 | 0.8 |
| Geranyl butyrate | 1.0 | 0.4 | 1.5 | 0.6 | 0.6 |
| 2-phenethyl tiglate | 0.6 | 0.4 | - | 0.6 | 0.7 |
| Citronellyl tiglate | 0.3 | 0.3 | 0.5 | 1.0 | 1.2 |
| 10-epi-γ-eudesmol | - | 2.5 | 5.5 | - | - |
| Geranyl tiglate | 1.2 | 1.1 | 1.9 | 1.3 | 1.6 |
| Citronellol:Geraniol ratio | >1 | >1 | >1 | >4 | >3 |

## 2.3 Cultivation of rose geranium

### 2.3.1 Environmental requirements

*Rainfall and temperature*

For dryland production of rose geranium 700 to 1 500 mm of precipitation is needed per year, uniformly distributed throughout the season. In low rainfall areas rose geranium can be grown with supplementary irrigation. Rose geranium prefers warm temperate to subtropical climates with a long growing season without extreme weather conditions. It grows well at temperatures ranging from 10 to 33°C, but the optimum temperature range is between 20 and 25°C. At temperatures as low as 6°C growth is inhibited. The plant is sensitive to cold weather and cannot withstand frost. This is the reason why it is mostly grown in spring as it becomes dormant in winter. Enough sunshine is needed for the development of oil (DAFF, 2009).

### 2.3.2 Agronomic aspects

#### 2.3.2.1 Propagation and cultivation

Rose geranium is a flowering plant that suffers from some degree of male sterility (Lawless, 1995) making it difficult to propagate through seeds. Male sterile genes inhibit the development of viable pollen and prevent normal self-fertilization, resulting in infertile seeds. As a result these plants are mainly propagated by stem cuttings, but root cuttings and suckers are equally effective, although they require more time to produce. The application of micro propagation is possible, though more expensive than the current methods (Saxena *et al*., 2008; Satyakala *et al*., 1995). The Bourbon cultivar of rose geranium is used by farmers in South Africa (and across the world) and is also available in most local nurseries (ADC, 1998). Cuttings are made from strong and healthy plants and rooting hormones is used to encourage rooting. Cuttings of 10 to 15 cm in length are obtained from young top shoots and propagated in trays or seedbeds. A mixture of 30% fine compost and 70% sand is used and on the onset of roots as from 2 to 6 weeks, the plants can be replanted in a prepared seedbed. Winter cuttings should have more leaf material than summer cuttings. Cuttings can also be made from older stems. Strong and healthy cuttings of 15 to 30 cm in length can be planted directly. Cuttings of rose geranium are susceptible to fungus attack such as damping off and have to be treated with a suitable fungicide (DAFF, 2009). In high rainfall areas, a plant population of 50 000 to 80 000 plants $ha^{-1}$ is recommended. The recommended plant density for low rainfall areas is between 20 000 and 30 000 plants $ha^{-1}$ (DAFF, 2009).

The ADC (1998) recommends that inorganic fertilizer together with manure should be applied before planting and thereafter N should be applied after each harvest. The annual standard basal dressing is 30 kg N $ha^{-1}$, 35 kg P $ha^{-1}$ and 25 kg K $ha^{-1}$ and an addition of eight equal top dressings of 25 kg N $ha^{-1}$ each are required annually at the following intervals; two applications before the first harvest, one application immediately after the first harvest, one application two weeks after the first harvest, one application after the second harvest, one application two weeks after the second harvest, one application immediately after the third harvest and one application two weeks later.

DAFF (2009) recommend 2 or 3 weeding during the growing season. If weeding is not done correctly it might lower oil yield and oil quality of rose geranium. Weeding can be done by hand or by hoe or it can be carried out mechanically with a tractor-drawn cultivator. Mulching with compost or grass is recommended as it inhibits weed growth and retains soil moisture.

#### 2.3.2.2 Water requirement and irrigation

Good harvests are obtained with an evenly distributed annual rainfall ranging between 700 to 1 500 mm in summer rainfall regions. Weiss (1997) reported low herbage yield with high oil concentrations in Kenya after a three month dry period, compared to a three month wet period. Reports indicated that geranium can tolerate drought but growth can be severely retarded, also changing the oil characteristics and reducing oil yield (Brown *et al*., 2008; Weiss, 1997; Swamy *et al.*, 1960).

Limited water supply has a negative effect on the development of plants. However, water stressed essential oil plants appear to have improved biosynthesis of secondary metabolites and the formation and accumulation of essential oil in medicinal plants such as *Officinal* sp. (dandelion) are inclined to increase under dry conditions (Murtagh, 1996; Yaniv & Palevitch, 1982). Langenheim *et al.* (1979) found that moisture stress had no significant effect on oil composition and oil yield of *Hymenaea courbaril* (guapinol). Similar results were found on *P. graveolens* by Brown *et al.* (2008)

in a preliminary study that was conducted under moisture stress conditions in KwaZulu-Natal.

Singh (1999) reported that rose geranium foliar and oil yield increased when the moisture regime was raised from 0.3 to 0.6 irrigation water: cumulative pan evaporation (IW:CPE) ratios on red sandy loam soils (alfisols). The foliage and oil yield obtained at these moisture regimes (0.3 to 0.6 IW:CPE) were 34 t $ha^{-1}$ and 102 kg $ha^{-1}$ compared to a herb yield of 27.7 t $ha^{-1}$ and an oil yield of only 72 kg $ha^{-1}$. Geraniol and citronellol were not affected by the changes in the moisture regime.

Singh *et al.* (1996) reported that 50 mm cumulative pan evaporation (CPE) applied at a depth of 30 mm optimised herbage and oil yield of rose geranium. The foliage and oil yield recorded was 23 t $ha^{-1}$ and 18.80 kg $ha^{-1}$ respectively. The data obtained was compared to the foliage yield of 19.50 and 17.30 t $ha^{-1}$ obtained at 75 and 100 mm CPE respectively. The oil yield at 75 and 100 mm CPE was 15.60 and 13.90 kg $ha^{-1}$, respectively.

### 2.3.3 Harvesting

In most production areas, rose geranium is harvested three to four times per season. Harvesting operations usually start approximately three months after transplanting, as determined by the stage of growth. Most of the essential oils are confined to the leaves and also young shoots of plants (ADC, 1998; Rao *et al.,* 1990). Harvesting is carried out during dry days and is done by hand picking. Motsa *et al.* (2006) reported that rose geranium has a higher yield when harvested in summer than harvested in autumn or winter. The authors observed that maximum herbage yields were attained three months after transplanting and that the ratio between citronellol and geraniol decreased during this period of plant growth. Plants harvested in spring or summer have a relatively high oil content, up to 0.088% compared to the 0.064% oil content obtained during autumn or winter harvests. Average rose geranium oil yield can range between 30 to 50 kg $ha^{-1}$ (ADC, 1998; Rao *et al.,* 1996).

Motsa (2006) reported that harvesting frequency has an impact on the leaf area index, herbage yield and oil yield. Closer harvesting intervals of two months produced a lower leaf area index, less herbage and a lower oil yield than longer frequencies of up to four months. Plants that were harvested after four months were better in all the parameters measured compared to plants that were harvested earlier.

### 2.3.4 Oil distillation

The extraction of essential oils from plant material can be achieved using a number of different methods, shown in Figs 2.2, 2.3 and 2.4. There are five main methods of extraction:

- Expression.
- Hydro- or water-distillation.
- Water and steam distillation.
- Steam distillation.
- Solvent extraction.

In each method there may be some variations and refinements and the extraction may be conducted under reduced pressure (vacuum), ambient pressure or excess pressure. The choice of extraction method depends on the nature of the material extracted, stability of the chemical components and specification of the targeted product.

Flowers are solvent-extracted not steam distilled, but with the exclusion of rose, ylang and orange flowers. In some applications an isolate is preferred to the total oil. Terpeneless oil (bay oil) and citrus oil are produced by removing unwanted compounds from the oil. This oil is fractionated to better its quality before use. Sometimes, fractionation is used to reduce undesirable notes. This is the case for antheole-containing essential oil from anise, star anise and fennel. Other processing steps may be applied to reduce instability of certain oil, lemon oil which is known to be unstable in soft drinks due to the level of citral. The production of some special oils, oleoresins, absolutes and concretes requires much greater technologically-advanced facilities, labour skills and safety systems.

Expression is used exclusively for the extraction of citrus oil from the fruit peel, because the chemical components of the oil are easily damaged by heat. Citrus oil production is now a major by-product process of the juice industry.

Water distillation is the simplest and cheapest of the three distillation methods. The plant material is mixed directly with water in a still pot. A perforated grid may be inserted above the base of the still pot to prevent the plant material settling on the bottom and coming in direct contact with the heated base of the still and charring

(Fig. 2.3). The quality of the oil can be modified due to the effects of direct heating and water contact.

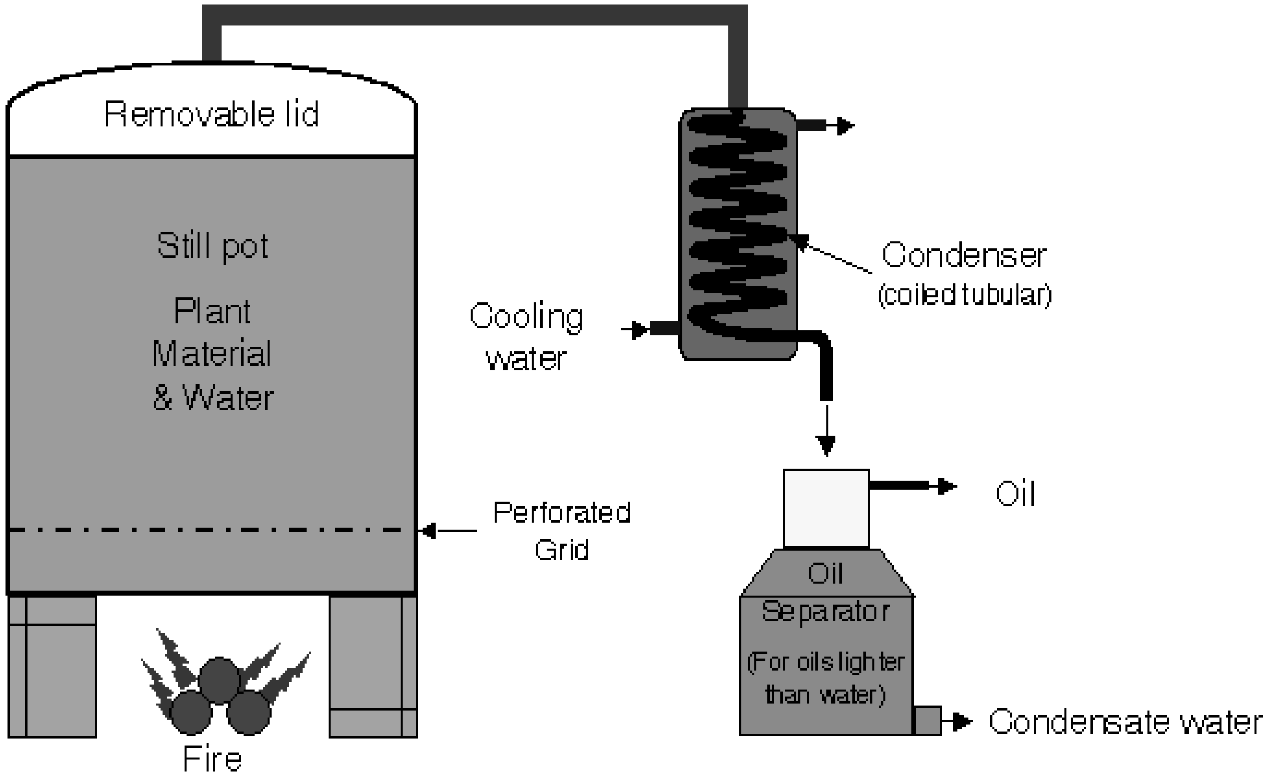


**Fig. 2.2** Schematic representation of water distillation unit where the plant material is suspended in the water (FAO, 2005).

In steam-and-water distillation the basic still design is very similar to that of water distillation (Fig. 2.3). The plant material is packed into the still pot placed on a grill or perforated plate above the boiling water. The capacity of the still pot volume is reduced but it may be possible to achieve a high packing density because the plant material is not suspended in the water. The advantages of steam and water distillation over water distillation are higher oil yield, oil components less susceptible to change due to wetness and thermal conductivity of the still from the heat source, the effect of refluxing is minimised, oil quality more reproducible and it is a faster process, so is more energy efficient. Steam distillation is the process of distilling plant material with the steam generated outside the still in a stand-alone boiler (Fig. 2.4).

As in the steam-and-water distillation (Figs 2.3 and 2.4) system the plant material is supported on a perforated grid above the steam inlet. The advantages and disadvantages of steam distillation are as follows:

- The amount of steam and the quality of the steam can be controlled.
- Lower risk of thermal degradation as the temperature generally does not rise above 100°C (Masango, 2005).
- It is the most widely used process for the extraction of essential oils on a large scale.
- Throughout the flavour and fragrance supply industry it is the standard method of oil extraction.
- There is a much higher capital requirement and with low-priced oils the payback period can be over 10 years.
- Requires a higher level of technical skill, and fabrication and repairs and maintenance require a higher level of skill.

Many variations of the process exist, e.g. batch, hydrodiffusion, maceration distillation, mobile stills and continuous distillation process. After distillation, the geranium oil is packed in 180 - 200 kg steel drums, or occasionally in 40-90 kg aluminium drums (ADC, 1998).

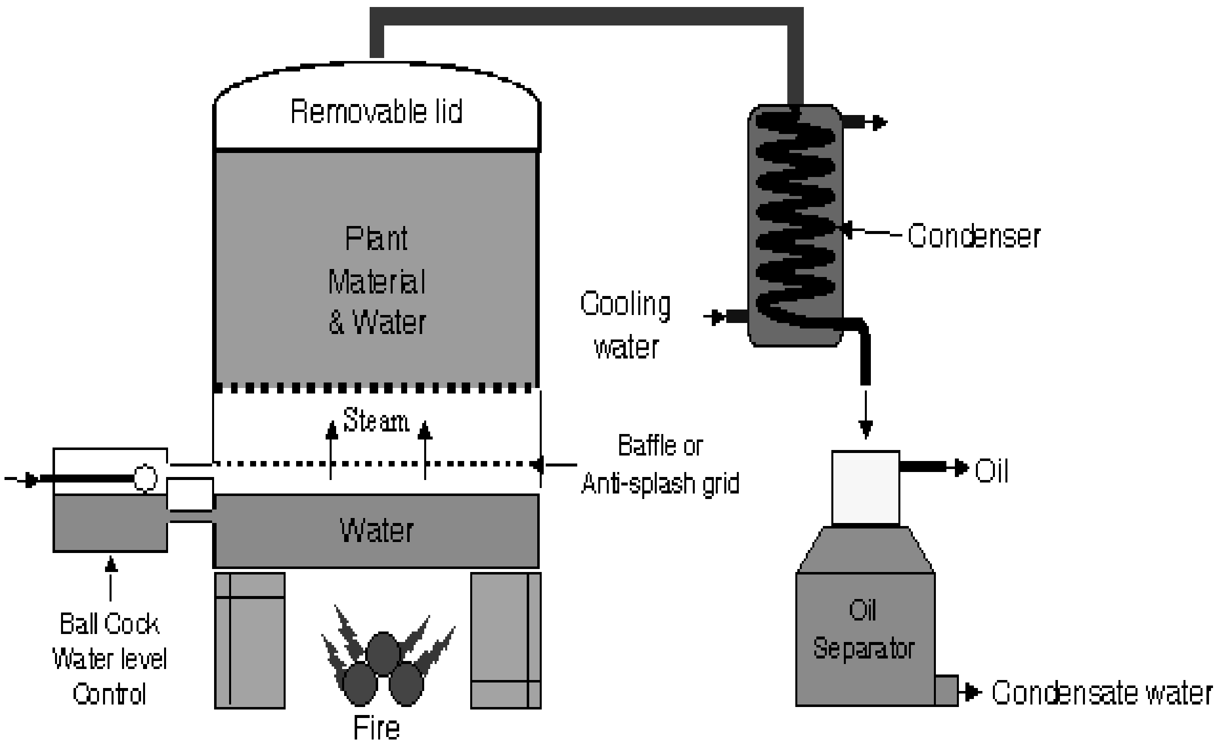


**Fig. 2.3** Schematic representation of a steam and water distillation unit (FAO, 2005; Masango, 2005).

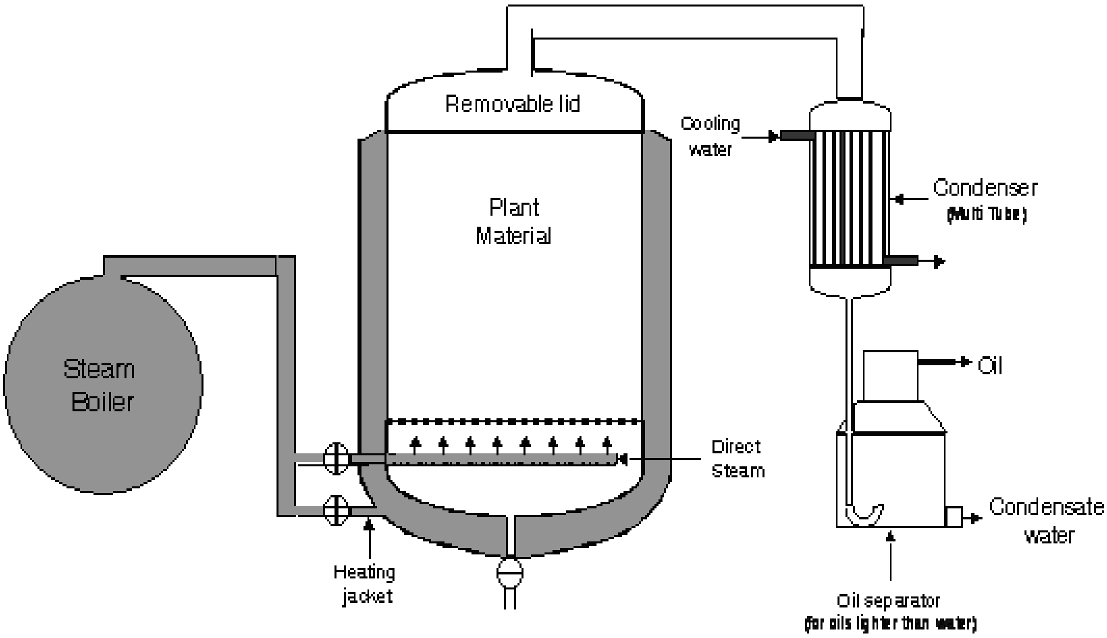


**Fig. 2.4** Schematic representation of steam distillation unit (FAO, 2005; Masango, 2005).

## 2.4 Factors affecting production and yield

### 2.4.1 Soil and climate

In South Africa rose geranium is planted during spring in areas where frost free conditions prevail. It can be grown on a wide variety of soils. However, soils that are rich in organic matter with pH values between 5.5 and 8.5 and clay contents of less than 40% are preferred. Rose geraniums grow well on the sandy soil of the coastal belt. Plants should preferably be planted on raised seedbeds. Thus, soil with a good drainage is recommended to prevent water logging (DAFF, 2009; Ayara *et al.,* 2006; ADC, 1998).

In the semiarid tropical climate of India, Rao *et al.* (1996) found that biomass, oil yield and terpenoid composition of rose geranium were influenced by seasonal changes. Significantly greater biomass and oil yields were recorded during the autumn and monsoon (rainy) seasons compared to the lower yields during the dry summer season. The biomass of rose geranium, obtained during the autumn and monsoon seasons, ranged from 13 to 17 t $ha^{-1}$ compared to the 10 t $ha^{-1}$ obtained during summer. Rose geranium oil yield obtained during the monsoon and autumn season reached 26 L $ha^{-1}$, compared to 12 L $ha^{-1}$ obtained during summer. The reduction of biomass and oil yield during the summer was attributed to the climate, characterized by high temperatures with low humidity levels. The terpenoid composition was strongly influenced by these seasonal climatic changes, and winter months favoured the accumulation of geraniol and its esters (Rao *et al.,* 1996).

Local studies showed that some chemicals contained in rose geranium oil are influenced by changes in night temperature. Geraniol decreased with decreasing night temperatures and citronellol increased while the ratio between citronellol and geraniol (C:G ratio) increased (Motsa, 2006; Motsa *et al.,* 2006).

### 2.4.2 Plant age

The age of rose geranium plants is reported to have an effect on yield and oil quality. Ram and Kumar (1996) reported higher oil yields with high citronellol and geraniol contents in fully expanded leaves that were harvested at four weeks than those that were harvested later. Only isomenthone was relatively low at the four week harvesting stage. However, biomass increased with shoot age (six to eight weeks) and older shoots had high levels of isomenthone and decreased citronellol and geraniol contents. Similar results were reported by Motsa *et al.* (2006) who also discovered that the C:G ratio increases with shoot age. For South African conditions, rose geranium plants should thus be harvested within eight to twelve weeks after transplanting (Motsa *et al.,* 2006).

### 2.4.3 Light intensity

Photosynthetic activities are reduced at low light intensities and in water-stressed crops. Dry matter formation and the accumulation of essential oils in thyme leaves are closely related to photosynthesis, affecting shoot growth and the production of essential oil (Letchamo & Xu, 1996). Duriyaprapan and Britten (1982) reported that Japanese mint (*Mentha arvensis*) exposed to solar radiation levels of 100, 64, 49 and 28% for 10 weeks, responded to increased shading intensity, and these conditions resulted in stem length elongation and reduced leaf area index. Little response to shading was found in the relative growth rate (RGR) or net assimilation rate (NAR) but the mean leaf area ratio (LAR) increased with no significant differences in oil yield.

Kaul *et al*. (1997) reported that foliage and oil yield of rose geranium cultivars (Bourbon, Kelkar and Algerian) decreased significantly when grown under trees with 50 to 60% shade. Partial shading resulted in slight changes in linalool, citronellol and geraniol contents. Partial shade had no effect on the odour quality and other organoleptic properties (colour, specific gravity, refractive index and optical rotation) of the oils of all three cultivars tested and all these characteristics were within the limits of BIS (Bureau of Indian Standards) standards.

### 2.4.4 Plant population

The recommended in-row spacing for rose geranium is 30 cm with an inter-row spacing of 70 to 80 cm (ADC, 1998). Rao (2002) reported that a narrow inter-row spacing (60-70 cm) at an in-row spacing of 30 cm, significantly increased rose geranium plant height. Plants that were cultivated at a narrow inter-row spacing were 17.7% taller than those that were cultivated at a wider spacing (90-120 cm). Total biomass increased with narrower inter-row spacing. The total biomass was as high as 57.4 t $ha^{-1}$ compared to 24.7 t $ha^{-1}$ obtained at wider inter-rows. The oil yield of rose geranium was also improved with narrow inter-rows. Oil yields reached 52.7 kg $ha^{-1}$ compared to 26.5 kg $ha^{-1}$ observed at wider spacing. For South African conditions, an in-row spacing of 40 cm, with an inter-row width of 50 cm (50 000 plants $ha^{-1}$) is recommended in high rainfall areas or for plants grown under irrigation. In low rainfall areas this plant density can be reduced to 30 000 plants $ha^{-1}$ (DAFF, 2009).

### 2.4.5 Weed management and intercropping

The rooted cuttings of field grown rose geranium require a minimum of 30-35 days for establishment and a further 45-50 days for the canopy to close and thus enable the crop to compete successfully with weeds. Rose geranium is susceptible to weed competition during the first 90 days after planting and the field must be kept weed free during this period to minimize yield losses. This long critical period should include four manual weeding operations (Kothari *et al*., 2002; Rajeswara & Bhatacharya, 1997).

Unrestricted weed competition causes a reduction in oil yield of up to 70% with losses being directly attributed to the reduction in plant spread, reduced number of branches $plant^{-1}$ and reduced leaf area (Kothari *et al*., 2002; Rajeswara & Bhatacharya, 1997). Kothari *et al*. (2002) reported that the application of the pre-emergence herbicides pendimethalin or oxyfluorfen is a more effective method of controlling weeds in rose geraniums than three hands weeding, hoeing and mulching done 45 days after transplanting. Oil quality (citronellol, geraniol, linalool, isomenthone, 10-epi-γ-eudesmol, geranylformate, citronellyl formate, *cis*-Roseoxide and *trans*-Roseoxide contents) was not affected by weeds (Kothari *et al*., 2002).

Rao (2002) reported that intercropping of rose geranium with cornmint (*Mentha arvensis)* had no impact on rose geranium plant height, total biomass and rose geranium oil yield. However, intercropping rose geranium with cornmint improved the chemical composition of the rose geranium oil.

### 2.4.6 Mulching and fertilization

Application of 160 kg N $ha^{-1}$ in association with a rice straw mulch optimized geranium oil yield in India (Ram *et al.,* 2003). Similar beneficial effects of organic mulches were reported in numerous studies on various crops by Milkha *et al*. (2001); Palanda *et al*. (1999); Ram and Kumar (1997) as well as Petra *et al*. (1993). Simultaneous application of paddy mulch straw and 160 kg N $ha^{-1}$ fertilizer increased the citronellol concentration while geraniol decreased with this application combination (Ram *et al.,* 2003).

Plants make use of nitrogen in the form of nitrate or ammonium. Some plant species have shown a strong preference for one form over another. In most summer crops, soil nitrate is preferentially assimilated by plant roots. In soilless production systems, ammonium is used at low concentrations since too much $NH_4$ may acidify the rhizosphere to toxic levels (Pidwirny, 2006; Combrink, 2005). In South Africa soilless growers use some ammonium in their nutrient solution mostly to regulate the pH in the root zone (Combrink, 2005). Nitrate is the only form used in abundance by most growers in the nutrient solutions and it is applied at levels as high as 13 meq $L^{-1}$ for crops with very high N needs (Combrink, 2005).

Singh (1999) reported that the application of 200 kg N $ha^{-1}$ gave optimum herb and oil yields. Dicyandiamide (DCDU) coated urea gave a higher yield than prilled urea (PU). The foliage yield was 44 t $ha^{-1}$ and 36 t $ha^{-1}$ for DCDU and PU, respectively. Oil yield obtained was 122 and 102 kg $ha^{-1}$ for DCDU and PU, respectively. Mosdell *et al.* (1986) described dicyandiamide (DCD) as a nitrification inhibitor that have 67% N compound of low volatility that is incorporated into fertilizer granules. DCDU is when urea is amended with DCD so that 10% of the N is derived from DCD to form DCDU. Prilled urea is formed by dropping liquid urea from a prilling tower into droplets that dry into roughly spherical shapes of 1 mm to 4 mm in diameter.

Ayara *et al.* (2006) found that the application of organic N at a rate of 100 kg $ha^{-1}$ increased fresh foliage and oil yield by 57.5%. When organic N application was increased to 300 kg $ha^{-1}$, the oil yield increased by 180.7%, compared to the zero N application. Generally citronellol percentages tend to increase with increasing N levels but in this study, the higher organic N levels reduced the guaia-6,9-diene content. However, it can be concluded that the application of N will in most cases increase both foliage and oil yields of geraniums.

Work done by Dethier *et al.* (1997) and Tiwari and Banafar (1995) showed that fertilizer applications have varying effects on essential oil yield and oil composition. Essential oils are terpenoids based on an integral $C_5$ unit (isoprenoid). Biologically active isoprenoid requires acetyl-CoA, ATP and NADPH for synthesis. Consequently, biosynthesis of essential oil is dependent on the plant's inorganic phosphorus content (Loomis & Corteau, 1972). Several reports on increased oil yields due to application of P were reported by Ichimura *et al.* (2008) on sweet basil, Sigh *et al.* (2005) on lemon grass, Ali *et al.* (2002) on linseed and Lickfelt *et al.* (1999) on oil seed rape.

Phosphate requirements for most hydroponically grown crops ranges between 0.7 to 1.5 meq $L^{-1}$ (Combrink, 2005). De Villiers (2007) reported that the number of branches on *Agathosma betulina* (buchu) increased at increased P concentration, as was also found by Ramezani *et al.* (2009) on basil (*Ocimum basilicum*). De Villiers (2007) also found that buchu dry mass increased when the levels of phosphate in the nutrient solution was increased from 0 to 0.7 meq $L^{-1}$. A dry mass of field grown feverfew plants increased following an application of 150 kg $ha^{-1}$ of triple super phosphate (Saharkhiz & Omidbaigi, 2008). A deficiency in phosphate often inhibit the uptake of other anions ($NO_3$ & $SO_4$), as was reported by Shen *et al.* (2005), Hinsinger *et al.* (2003) and Neumann *et al.* (1999).

Sulphur (S) is a major plant macro-nutrient. Soil contains inorganic and organic forms of sulphur. Under aerobic conditions, inorganic S is present as sulphate ($SO_4$), the only form in which plants absorb sulphur (Maathius, 2009). Nitrogen and S requirements are said to be closely related as both nutrients form part of amino acids such as cysteine, cystine and methionine, required for the synthesis of protein. Sulphur is involved in the formation of chlorophyll, activation of enzymes and is part of co-enzyme A, pyrophosphate and vitamins such as biotin and thiamine (B1). It is also involved in the formation of glucoside (mustard oil), SH-sulphydryl linkages and oil in oilseeds (FAO, 2009; Droux, 2004; Marschner, 1986). The sulphur content varies among plant species and ranges between 0.1 to 6% of the plant's dry mass (Droux, 2004). Sulphate is responsible for the pungency taste of onions (Randle *et al.,* 1993) and for the production of sesquiterpene lactones in lettuce.

## 2.5 General effect of water quality on plants

The success of crops that are grown on soilless substrates depends on the water quality used for the preparation of nutrient solution. Although water quality is said to be a complex concept, it is not only the concentration of specific ions and phytotoxic substances that are important for plant nutrition, the presence of organisms that can clog the irrigation systems is also crucial (Tognoni *et al.,* 1998).

Literature shows that information on salt tolerance exists in more than 130 crop species; but information is still lacking for some crops (Shannon & Grieve, 1999). Gratten and Grieve (1999) reported that the composition of salts in water varies widely across the globe, with Na, Ca and Mg being the dominant cations found in most saline water resources, while the dominant anions are Cl, $SO_4$ and $HCO_3$.

*Salinity measurements*

Electrical conductivity is measured on the vacuum-extracted and filtered water extracts in units of deciSiemens per meter (dS $m^{-1}$), milliSiemens per centimetre (mS $cm^{-1}$) and millimhos per centimetre (mMho $cm^{-1}$) (Stanghellini *et al*., 1998). Siemens (S) is the international unit used for conductivity. A unit 1 000 times smaller (mS) is used to measure the conductivity of water (1 000 mS=1S) and Mho is equivalent to siemens. To measure EC in nutrient solutions, mMho is occasionally used. The unit for resistance (Ohm) is well-known and widely used to express salt content of soils. The problem with Ohm as a unit for salt content in nutrient solutions is that a high resistance indicates a low salt content (Combrink, 2005).

*Effect of salinity on plants*

High salinity levels reduce plant growth rate and may lead to fewer or smaller leaves. The initial and primary effect of salinity is due to its osmotic effect (Jacoby, 1994; Munns & Termaat, 1986). Roots are also reduced in length and mass and may become thinner and plant maturity rate may be delayed or advanced depending on species. The severity of the response of plants to salinity is mediated by environmental interactions such as relative humidity, temperature, radiation and air pollution (Shannon & Grieve, 1999). Ion toxicities or nutrient deficiencies may arise because of a predominance of a specific ion or competition effects among cations or anions.

The osmotic effect of salinity reduces plant growth, changes leaf colour and developmental characteristics such as root/shoot ratio and maturity rate. The effects are manifested in the leaf and meristem damage or nutrient disorders. High concentrations of Na or Cl may accumulate in the leaves, resulting in leaf scorching. A Ca deficiency is common when the soil water has a high Na:Ca ratio (Shannon & Grieve, 1999).

Furthermore, Al-Khafaf *et al.* (1989) reported that plant roots are affected by salinity. These results showed that root and shoot dry-mass accumulation varied significantly at different salinity levels and water distribution. No root growth was observed in layers with electrical conductivity more than 12 dS $m^{-1}$. Root growth of maize seems to be detrimentally affected by salinity levels exceeding 12 dS $m^{-1}$.

A moderate salinity may have favourable effects on yield, quality and disease resistance (Shannon & Grieve, 1999). In spinach yield may increase with a mild increase in the salinity level. Sugar content increases in carrots and starch content decreases in potatoes as salinity increases (Shannon & Grieve, 1999). Cabbage heads are firm at low salinity levels, and become less compact as salinity levels increase (Shannon & Grieve, 1999). Celery has been reported to be resistant to high salinity (Leonardi, 1998; Aloni & Pressman, 1987). Yields of *Amaranthus* spp. were not affected when the salinity level of a nutrient solution was increased to 3 mS cm with application of NaCl (Sedibe *et al.,* 2005). The leaf oil yield of coriander (*Coriandrum sativum* L.) was increased significantly by 18 and 43% with 25 and 50 mM NaCl respectively but decreased significantly under a high salinity level (75 mM). Most of the oil quality parameters of coriander were also affected at a high salinity levels. Total fatty acid content of the adaxial and abaxial part of the leaf was also affected by salinity (Neffati & Marzouk, 2008).

In another study on coriander, Neffati *et al.* (2011) reported that salinity had an impact on the yield, oil composition and antioxidant activities of coriander fruits. The oil yield was increased by 77 and 84% at 50 and 75 mM NaCl respectively in comparison to the control. Linalool and camphor contents increased with increasing NaCl concentrations. The highest antioxidant compound in methanol extract was exhibited on the control. In the control plants, the total phenolic concentration was 1.04 mg GAE $g^{-1}$ DM (dry mass) and decreased by 43% and 66% at 50 and 75 mM NaCl, respectively.

*Salt tolerance parameters*

Researchers have identified several indices that can be used to measure salt tolerance. The following parameters were suggested by Shannon and Grieve (1999);

- Tolerance during germination;
- Conservation of shoot dry mass;
- Root mass;
- Shoot number;
- Resistance to leaf damage;
- Maintenance of flowering;
- Seed and fruit set;
- Leaf size;
- Plant survival under stress;
- Accumulation of specific ions in shoots or leaves and
- The production of metabolites.

## 2.6 Substrates used for soilless production

There are several substrates used for soilless crop production. The Belgians and the Dutch use rockwool as a substrate. Most South African growers use sawdust as is used in Canada and countries with forest industries (Combrink, 2005). On small scale light expanded clay aggregates, sand, gravel and pumice are used. Certain factors must be considered when choosing a substrate: Roots require $O_2$ for respiration and should not be exposed to the accumulation of $CO_2$; roots need a moist environment to protect the root hairs from drying out; the ratio between nutrient elements in the root zone solution should be optimized; the concentrations of ions, as indicated by the EC, should not be so high as to restrict the absorption of water and not be too low as to cause nutritional deficiencies; an optimum pH in the root medium should be maintained to avoid precipitation of insoluble salts such as commonly found with Fe deficiencies under alkaline conditions; roots should be protected against temperature extremes (optimum root temperatures for summer crops vary between 17 and 25$^{o}$C); allellopathic substances may be released by organic material under wet, anaerobic conditions and roots should also be protected from nematodes and soil borne pathogens (Combrink, 2005).

Some growers use washed river sand as substrate. Sand is useful for perennial crops such as roses and rose geranium since organic substrates may decompose over time with associated aeration and pH problems. Sand has a high density; therefore strong containers should be used (woven plastic bags etc). Sand is also a good choice for growers who would like to recycle their nutrient solutions (Combrink, 2005).

*Sawdust*

Sawdust is easy to handle due to its low density. It is produced in sawmills, or in furniture factories. It may be mixed with wood shavings to improve drainage when used as a substrate. Sawdust from chemically treated wood is not suitable for soilless production due to the possibility that toxic substances may be present (Combrink, 2005; Bradley & Marulanda, 2000).

Sawdust is used for only one growing season, because its water retention may increase to the detriment of aeration due to its decomposition by micro organisms. Hence, sawdust is relatively not durable (Combrink, 2005; Bradley & Marulanda, 2000).

*Vermiculite*

Vermiculite is a commonly used inorganic medium and is mined in the US and Africa. The raw material contains Al, Fe and Mg silicate. Processing of this material includes heating the vermiculite (biotite mica) to a temperature of up to 1 000°C, which converts water trapped between the layers of rock-like material into steam. The production of steam creates pressure that expands the material, increasing the volume of the particles of the material between 15 to 20 times. Vermiculite is sterile because of the high temperature used during processing. Vermiculite is unique and characterized by a high water-holding capacity due to its large surface area per volume. It has a low bulk density, high pH, and relatively high cation exchange capacity, attributed to its structure (Bradley & Marulanda, 2000).

Vermiculite is mostly used as a propagating substrate. Vermiculite will progressively release plant nutrients and on average it has 5-8% K and 9-12% Mg. Vermiculite can absorb and store P with some of the P remaining in an available form to the plant roots (Bradley & Marulanda, 2000).

Vermiculite is manufactured in four different grades, differentiated by particle size. Insulation grade vermiculite and that which is marketed for poultry litter (which has not been treated with water repellents) has been used with some success. Vermiculite which has been treated with water repellent, such as block fill should not be used as substrates. Because vermiculite tends to compact over time, it should be incorporated with other materials such as peat or perlite to maintain sufficient porosity. It cannot be used in conjunction with sand or as a sole medium component, because as its internal structure deteriorates, air porosity and drainage decreases (Bradley & Marulanda, 2000).

*Light expanded clay aggregates*

Light expanded clay is produced in an oven, when moist clay is heated up to 1100$^{o}$C and steam is released causing expansion. When the granules cool down, a rigid porous granular material is formed. The material is then sieved into different fractions. Different substrate products can be made from clay. It is of great importance that only special clay that has a low content of soluble salts is used (Bradley & Marulanda, 2000; Art Spomer, 1998).

Light expanded clay has a moderate density and a porous structure. Expanded clay substrates are well aerated, containing relatively small amounts of water. Therefore, it is advisable to irrigate frequently and to keep a constant water layer in the substrate. It has been quantified that expanded clay can be used and reused for five years and still remain in good condition and can be steam sterilized (Bradley & Marulanda, 2000; Art Spomer, 1998).

*Pumice*

Pumice is produced by the cooling down of volcanic lava. The escaping steam and gas contribute to its porous nature. This aluminosilicate material contains K, Na, Mg, Ca, and traces of Fe. Pumice absorbs K, P, Mg, and Ca from the nutrient solution making it available for plant use at a later stage. Crushed pumice is used together with compost to enrich the soil (Bradley & Marulanda, 2000; Özçelik *et al.,* 1997; Noland *et al.,* 1992).

*Sand*

Sand is a name designated for a group of granular material consisting mainly of quartz. It is inert, with particle sizes that may range from 0.05 to 2 mm. Sand has a high density and coarse sand have a small water retention potential. The main problem with fine textured sand is its low air content. The particle size distribution varies from one batch to another leading to a variation in air content. A coarse type of sand is preferred for soilless crop production (Abou-Hadid *et al.,* 1987; Combrink, 2005; Bradley & Marulanda, 2000).

Sand from coastal areas may contain sea shell particles. These and other limestone particles may raise the pH when used as a substrate. A rise in pH may lead to nutrient deficiencies, particularly of trace elements such as Fe. Coarse sand maintains good drainage with a low water holding capacity, and a high bulk density (Bradley & Marulanda, 2000; Abou-Hadid *et al.,* 1987). Sand is not influenced by chemical and biological factors when used as substrate and it can be sterilized by steam (Bradley & Marulanda, 2000).

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# CHAPTER 3

## GENERAL MATERIAL AND METHODS

# GENERAL MATERIAL AND METHODS

## 3.1 Experimental site

The majority of the trials (Chapter 4, 5 and 6) were conducted in a greenhouse on the West campus facility of the University of the Free State in Bloemfontein. Bloemfontein is situated in the Free State province of South Africa and is at an altitude of 1351 m above sea level (29$^o$06'S and 26$^o$18'E). The temperature in the greenhouse was controlled by a wet wall (pad) and two fan systems. Weather data during the experimental period was collected by an automated weather station located at West campus (Table 3.1).

Table 3.1 Weather data for the 2009 growing season

| | Temperature ($^o$C) | | Rainfall | *RH |
|---|---|---|---|---|
| **Months** | **Avg. max.** | **Avg. min.** | **(mm)** | **(%)** |
| Jan | 26.4 | 15.4 | 67.0 | 88.2 |
| Feb | 27.4 | 16.2 | 67.9 | 86.5 |
| Mar | 28.3 | 13.4 | 20.2 | 80.4 |
| Apr | 25.7 | 10.3 | 21.3 | 76.0 |
| May | 20.4 | 6.0 | 29.2 | 82.7 |
| Jun | 16.7 | 4.9 | 19.6 | 84.3 |
| Jul | 16.1 | 1.7 | 10.0 | 71.2 |
| Aug | 21.0 | 4.8 | 8.7 | 72.1 |
| Sep | 25.9 | 7.0 | 0.0 | 55.8 |
| Oct | 26.5 | 12.2 | 97.4 | 79.9 |
| Nov | 29.6 | 13.6 | 77.5 | 78.7 |
| Dec | 32.7 | 15.0 | 57.6 | 77.5 |

*Relative humidity

## 3.2 Planting and irrigation

Rose geranium cuttings (±10 cm in length) were rooted for eight weeks by a commercial grower (Pico-gro, RSA). The plants were planted in 5 L pots filled with sterile silica sand (2 mm). Each treatment was replicated five times in a randomized complete block design. A plant population of 133 000 plants $ha^{-1}$ was used at an in-row spacing of 15 cm and an inter-row spacing of 50 cm. Each experimental unit consisted of eight pots containing one plant each.

A 'drain-to-waste' drip system was used to fertigate all the pots, using four irrigation cycles per day, scheduled at 08:00, 11:30, 14:00 and 18:00 for 30 min for each irrigation cycle. The emitter flow rate was set at 4 L $hour^{-1}$. As plants developed and the demand for more water and nutrients increased, the applied volumes were increased to ensure that 10 to 25% solution drained to waste to prevent salt accumulation in the potting bags as recommended by Combrink (2005).

## 3.3 General plant management

Fresh nutrient solution was mixed once every two week. During the experimentation periods no diseases occurred, but aphids had to be controlled. With no registered insecticide available for rose geranium, chlorpyrifos (organophosphate) was used to control these insects, using levels prescribed for ornamentals (0.5 ml $L^{-1}$). A full cover spray was applied and this application was repeated after three and six days.

Each nutrient solution was made up in 1000 L tanks, which served as reservoirs for the various treatments. The feeding water (Bloemfontein municipality), had an EC of 0.2 mS $cm^{-1}$ and pH of 6.92. Expressed as meq $L^{-1}$, it contained 0.48 Na, 0.05 K, 1.00 Ca, 0.45 Mg, 0.01 nitrate-N, 0.15 $SO_4$, 0.21 Cl and a total alkalinity of 1.18 ($HCO_3$). The alkalinity was lowered to 0.04 meq $L^{-1}$ by applying nitric acid at 0.78 meq $L^{-1}$. These ions were taken into account when nutrient solutions were prepared (Combrink, 2005). Micronutrients used in all the experiments are detailed in Table 3.2 (Combrink, 2005).

**Table 3.2** Micro nutrient concentrations used in all the nutrient solution treatments

| Micronutrient | Fertilizer | Application (mg $L^{-1}$) |
|---|---|---|
| Iron (Fe) | Libfer; 13% Iron-EDTA | 1.12 |
| Boron (B) | Boric acid | 0.21 |
| Molybdenum (Mo) | Ammonium molybdate | 0.05 |
| Manganese (Mn) | Manganese sulphate | 0.55 |
| Cupper (Cu) | Copper sulphate | 0.03 |
| Zinc (Zn) | Zinc sulphate | 0.20 |

## 3.4 Parameters

Harvesting took place four months after planting, the number of branches per plant, plant height, foliar fresh mass (FM) and foliar dry mass (DM) were determined. By dividing the number of branches by plant height (m), the branch:height (B:H) was used as an indication of bushiness. Foliar dry mass was determined by drying of the harvested material at 68$^{o}$C for 72 hours. The dried leaves were milled to 0.25 mm diameter using a micro hammer mill (Culatti, Zurich) (Jones *et al.,* 1991). These leaves were subsequently used for the analyses of Ca, Mg, P, K, S, and N.

Plant tissue Ca, Mg, P, and K contents were determined using the Inductively Coupled Plasma Optical Emission Spectrometric (ICP-OES) (Optima 4300 DV, ICP-OES, PerkinElmer Inc. USA). The ICP-OES is a multi-element instrument that analyses all elements, with only a few seconds between each element. Each element was measured at an appropriate emission wavelength, chosen for high sensitivity and lack of spectral interference. The wavelengths used for Mg, Ca, P, and K were 383.826 nm; 422.673 nm; 213.618 nm and 769.896 nm, respectively (Zasoski & Burau, 1977). Sulphur content was also determined by ICP-OES using an extract solution, but a different instrument was used (JY Horiba Ultima, USA) and was set at a wavelength of 181.978 nm, after the system was purged with nitrogen necessary for wavelengths below 188 or 189 nm to prevent interference from atmospheric oxygen using the procedure of Zasoski and Burau (1977).

Nitrogen was determined following the Dumas combustion method in a Leco FP-528 combustion nitrogen analyzer (LecoCorp. St. Joseph, MI, USA) (Etheridge *et al.,* 1998; Matejovic, 1995). Samples were weighed into a porcelain sample holder and thereafter introduced in the combustion chamber (1300°C) using an automated sample loader (CNS 2000, Operation Manual, Leco, St. Joseph, MI, USA). The combustion process converts covalently bound nitrogen into nitrogen gas ($N_2$). The $N_2$ content was measured by a thermal conductivity cell. The leaves used to determine chlorophyll was selected from the top third of different branches of a single plant, subsequently non destructive hand held chlorophyll content meter was used (CCM-200, Opti-Science, USA).

Approximately 2 - 5 kg of fresh biomass was collected at harvesting for the extraction of essential oil. The oil was extracted from the leaves using water and steam distillation for one hour (Motsa *et al.,* 2006) in a customised 5 kg test distiller manufactured by PA Pretorius (Fig. 3.1). Oil quality was determined by gas chromatography (GC). An Aligent GC (FID) model 6890N fitted with 30 m x 0.25 mm DB-5 fused silica capillary column and film thickness of 0.25 µm was used for oil analysis. Samples of 1.0 µl of a 10% solution of the respective oil in hexane were introduced by split injection with a split ratio of 100. Both the injection port and oven temperatures were set at 250 and 70$^{o}$C, respectively. Helium was used as a gas carrier at linear velocity of 37 cm sec$^{-1}$. The temperature programs started at 70$^{o}$C isothermally for 3 minutes, increasing gradually by 3$^{o}$C min$^{-1}$ to 280$^{o}$C and were held for 10 min (Adams, 2004; Novák *et al.,* 2001).

**Fig. 3.1** The customized 5 kg test distiller.

## 3.5 Data analysis

Analyses of variance were conducted using the General Linear Model (GLM) of the Statistical Analysis System (SAS) version 9.3 (SAS, 2004). Regression analysis was also run on the SAS program. Significant results were analysed using Tukey's least significant difference test (LSD), described by Steel and Tourie (1980). Statistically significant difference between treatment means was determined at the 5% level of significance.

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# CHAPTER 4

## OIL YIELD AND QUALITY RESPONSE OF ROSE GERANIUM (*Pelargonium graveolens* L.) TO PHOSPHATE

**OIL YIELD AND QUALITY RESPONSE OF ROSE GERANIUM (*Pelargonium graveolens* L.) TO PHOSPHATE**

**Abstract**

Phosphorus is an essential plant macro nutrient, forming part of the structure of nucleic acids and phospholipids. It also plays a role in energy metabolism, where hydrolysis of pyrophosphate and various organic phosphate bonds are utilized to drive this chemical reaction. A study was conducted to evaluate the effects of phosphate levels on plant height, number of branches, foliar dry mass, oil yield, oil quality and foliar minerals of hydroponically grown rose geranium. The study was carried out in a greenhouse at the University of the Free State using a randomized complete block experimental design. Plants were grown for four months and the levels of phosphorus evaluated were 0.1, 0.8, 1.50 and 2.20 meq $L^{-1}$ and each treatment was replicated five times. Cations were applied at 1.0 meq $L^{-1}$ $NH_4$, 5.5 meq $L^{-1}$ K, 6.5 meq $L^{-1}$ Ca and 2.5 meq $L^{-1}$ Mg. Plant height and the number of branches were not affected significantly by phosphate. However, foliar mineral P, Mg and K were affected. Oil yield of rose geranium increased linearly with increased phosphate concentrations. Both guaia-6,9-diene content and citronellol:geraniol ratio were better at the highest phosphate level indicating that oil quality can be enhanced by using relatively high phosphate levels in nutrient solutions.

## 4.1 Introduction

Phosphorus (P) is the second most limiting macro nutrient after nitrogen. On average dried plant material contains about 0.2% phosphorus. This element is an important component of molecules such as nucleic acids, phospholipids, and ATP. Hydrolysis of pyrophosphate and various organic phosphate bonds are used for the metabolism of energy. High energy phosphate is held as part of the chemical structures of adenosine diphosphate (ADP) and ATP. Phosphorus controls the reaction of key enzyme reactions and it also regulates metabolic pathways. It is a vital component of the substances that are building blocks of genes and chromosomes. Supply of P is essential for the development of new cells and in the transfer of genetic code from old to new cells (Theodorou & Plaxton, 1993).

Low P availability triggers a series of morphological and physiological responses in the plant that maximizes phosphorus acquisition (Ticconi & Abel, 2004; Raghothama, 1999). As P becomes deficient, a decline in plant and leaf growth occurs as reported by Kavanova *et al.* (2006); Assuero *et al.* (2004); Chiera *et al.* (2002); Radin and Eidenbock (1984).

There are over 250 species of *Pelargonium* and hundreds of hybrids accounting to thousands of cultivars. Most of the known species are commonly found in the Western Cape region. Some of these species have scented leaves that produce essential oils, such as *P. capitatum, P. graveolens, P. odoratissium* and *P. radens* (Leistner, 2000). Rose geranium is one of the most important essential oil crops in South Africa's agricultural industry. Geranium oil is derived from the scented Bourbon type (Reunion type) originating from a cross between *P. capitatum* and *P. radens* (Lis-Balchin, 1996). Rose geranium is indigenous to South Africa, but is widely cultivated in Egypt, India and China. To a lesser extent, it is also grown in Central Africa, Madagascar, Japan, Central America and Europe. The soft drink industry is the major market for locally produced rose geranium oil (Lis-Balchin, 1996).

Research efforts are currently being undertaken in South Africa to cultivate crops with an oil quality matching the Bourbon standard so that a better price can be negotiated on the global market. In order to increase production of geranium oil in South Africa, every effort has to be made to introduce the crop to potential producers and to develop the necessary agrotechnologies for commercial cultivation. Although there is still some conflicting information on the factors that affect oil yield of rose geranium, research has shown that oil yield and quality are highly dependent on the correct management of fertilization and climate (DAFF, 2009; Prakasa Rao *et al.,* 1995). Application of P has been reported to increase yield of lemon grass (Sigh *et al.,* 2005), while similar results have been reported for sweet basil (Ichimura *et al.*, 2008) and in oil seed rape (Lickfelt *et al.,* 1999).

The objectives of this study was to evaluate the effect of phosphate on growth, oil yield and quality of rose geranium, as well as an attempt to set standards for levels to be used in the nutrient solution of hydroponically grown plants.

## 4. 2 Material and methods

The basic experimental procedure followed is laid out in chapter 3 and only specific procedures followed for this experiment are detailed here.

The effect of four P concentrations (0.1, 0.8, 1.5 and 2.2 meq $L^{-1}$) was evaluated for its effect on growth, herbage yield as well as oil yield and quality of rose geranium. In order to maintain a constant EC with increasing P levels in the nutrient solutions, the levels of nitrate and sulphate were proportionally reduced (Table 4.1) as described by Combrink (2005). The cation composition of all nutrient solutions was kept constant (Table 4.1). The pH and the EC of the nutrient solution were maintained at 6.98 (±0.02) and 1.60 (±0.1) mS $cm^{-1}$, respectively.

**Table 4.1** Macro nutrient compositions of the nutrient solutions containing different phosphate concentrations used to irrigate rose geranium plants

| **Phosphate** | **Ions (meq $L^{-1}$)** | | | | | | |
|---|---|---|---|---|---|---|---|
| **(meq $L^{-1}$)** | **$NH_4^+$** | **$K^+$** | **$Ca^{2+}$** | **$Mg^{2+}$** | **$NO_3^-$** | **$H_2PO_4^-$** | **$SO_4^{2-}$** |
| **0.1** | 1 | 5.5 | 6.5 | 2.5 | 11.04 | 0.1 | 3.80 |
| **0.8** | 1 | 5.5 | 6.5 | 2.5 | 10.52 | 0.8 | 3.62 |
| **1.5** | 1 | 5.5 | 6.5 | 2.5 | 10.00 | 1.5 | 3.44 |
| **2.2** | 1 | 5.5 | 6.5 | 2.5 | 9.48 | 2.2 | 3.26 |

## 4.3 Results and discussion

### *Plant parameters*

The number of branches per plant was not significantly affected by phosphate (Table 4.2). Contrary to the results obtained in this study, significant increases in the number of branches per plant have been reported with increased P-levels on various other essential oil crops. De Villiers (2007) reported an increase in the number of branches per plant of *Agathosma betulina* (buchu) and an increase in the number of branches per plant were also reported for *Ocimum basilicum (*sweet basil*)* by Ramezani *et al.* (2009).

**Table 4.2** Effect of phosphate concentrations on the number of branches, height and branch:height ratio of rose geranium

| **Phosphate concentration (meq $L^{-1}$)** | **Branches per plant (B)** | **Height (m) (H)** | **B:H ratio (branches $m^{-1}$)** |
|---|---|---|---|
| 0.1 | 20.8 | 0.49 | 42 |
| 0.8 | 21.0 | 0.48 | 43 |
| 1.5 | 24.0 | 0.41 | 59 |
| 2.2 | 23.2 | 0.46 | 50 |
| **$LSD_{F(0.05)}$** | ns | ns | 14 |

$LSD_{F(0.05)}$=least significant difference at 5% level of significance
ns not significant at $P$<0.05

Plant height and the number of branches of rose geranium were not affected significantly by the P concentrations when a strict Tukey test was used. Significant differences were found when a less strict Fishers LSD test was used. The effect of P on the B:H ratio was significant ($P$=0.04). The B:H ratio was higher at 1.5 and 2.2 meq $L^{-1}$ P, when compared to the values obtained at 0.1 and 0.8 meq $L^{-1}$ P. Results obtained on *Agathosma betulina, Tanacetum parthenium* and *Ocimum basilicum* by Ramezani *et al.* (2009); Saharkhiz and Omidbaigi (2008); and De Villiers (2007), respectively, showed that P increased the number of branches and also increased plant height. The ratio of branches per meter is an indication of branching or bushiness, and this peaked at a relatively high P level of 1.5 meq $L^{-1}$ (Table 4.2).

Dry mass of the harvested foliar material increased significantly ($P$=0.05) with P concentrations at the relatively high P concentration of 2.2 meq $L^{-1}$ compared to all other P concentrations (Fig. 4.1). The biomass of hydroponically grown buchu (*Agathosma betulina*) increased when the concentration of P was increased from 0 to 0.7 meq $L^{-1}$, but further increases showed no added increases in shrub yield (De Villiers, 2007). A similar increase was noted on feverfew (*Tanacetum parthenium* L.) grown conventionally on a soil with a pH of 6.7 (100 kg P $ha^{-1}$) as reported by Saharkhiz and Omidbaigi (2008). The increase in dry mass can be ascribed to the role of P in cell division, root growth, reproductive growth and vegetative growth of plants (FSSA, 2007; Ram *et al.,* 2003; Bidwell, 1979). It is evident that rose geranium requires more P than other essential oil crops grown hydroponically.

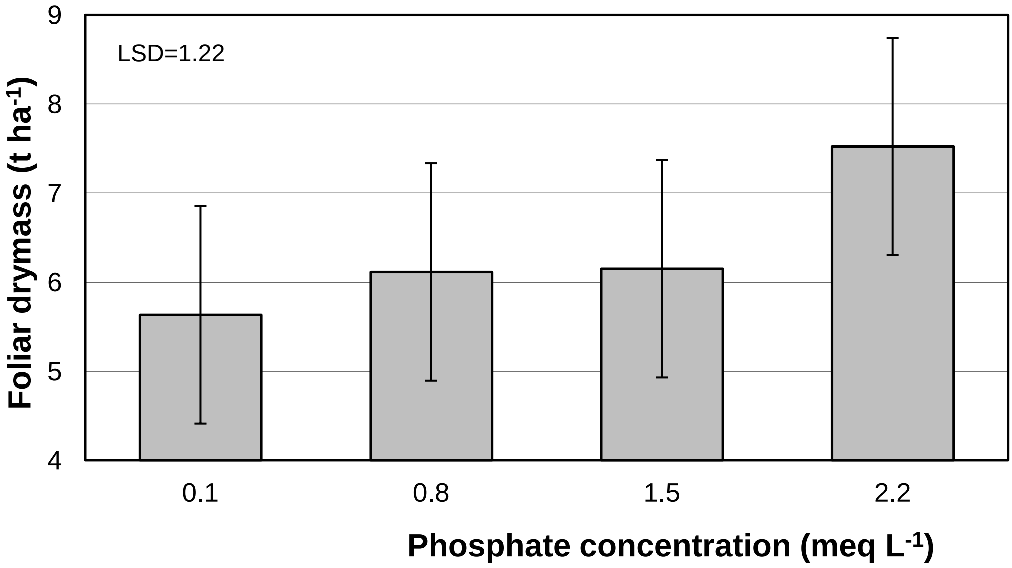


**Fig. 4.1** Effect of increasing phosphate concentrations in nutrient solutions on foliar dry mass of hydroponically grown rose geranium.

***Mineral content***

The results of this study show that different P-levels in the nutrient solutions had significant effects on the plant tissue P (*P*=0.0004), Mg (*P*=0.0039) and K (*P*=0.04) contents (Figs 4.2 and 4.3). From Fig. 4.2 it can be seen that both P and Mg contents increased significantly as the P concentration in the nutrient solution was increased from 0.1 to 0.8 meq $L^{-1}$. Although foliage P content increased with increasing P concentrations in the nutrient solutions there was no increase by lifting the P-level from 0.8 to 2.2 meq $L^{-1}$. Although no significant differences in foliage-Mg were found by lifting the P-levels higher than 0.8 meq $L^{-1}$, it is clear that foliage Mg peaked at 1.5 meq $L^{-1}$.

A number of studies have indicated that the uptake of anions by the roots may be inhibited by a phosphorus deficiency (Shen *et al.*, 2005; Hinsinger *et al.*, 2003; Neumann *et al.*, 1999). This was not found in this study with foliar N and S contents unaffected.

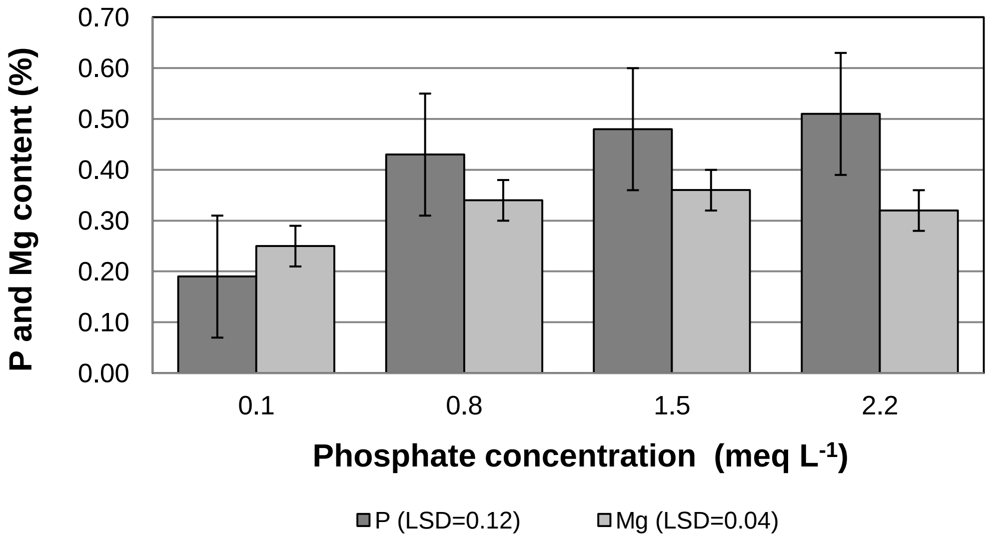


**Fig. 4.2** Phosphate concentrations in the nutrient solution affect the P, and Mg content of rose geraniums foliage grown hydroponically.

The potassium content in the plant tissue showed a slight increase (non-significant) as the P concentration increased from 0.1 to 1.5 meq $L^{-1}$ and as the Mg content also peaked at 1.5 meq $L^{-1}$ (Fig 4.2; Fig. 4.3). Low leaf K concentrations at a low P levels may be attributed to exudation of carboxylates which is accompanied by K efflux through co-transport (Sas *et al.*, 2001).

Calcium content of the foliage was not significantly influenced by P-levels as was found by Shen *et al.* (2005) on lupines (*Lupinus albus* L.).

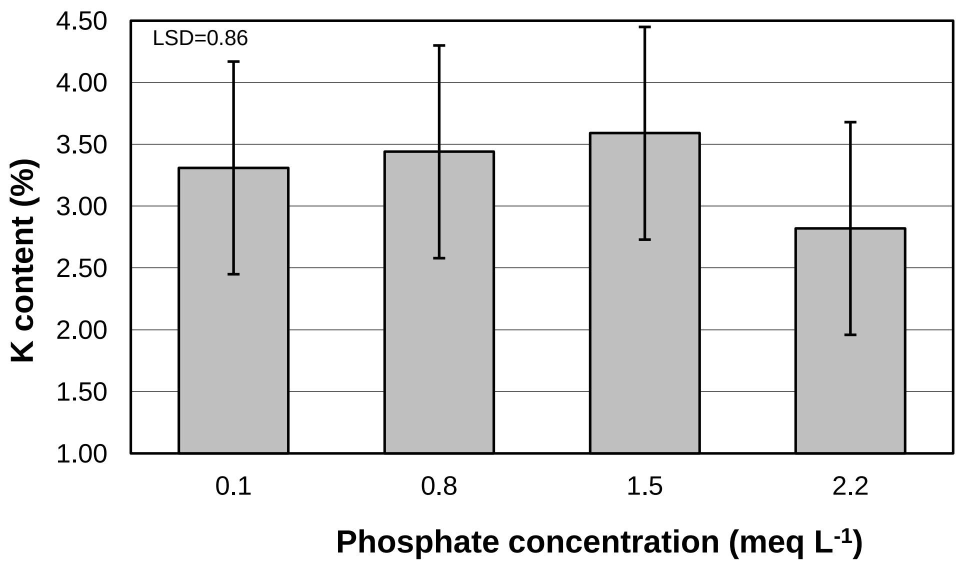


**Fig. 4.3** Phosphate concentrations in the nutrient solution affect the K content of rose geraniums grown hydroponically.

### *Oil yield and oil quality*

Oil yield did not increase significantly when the P concentration was increased from 0.1 to 1.5 meq $L^{-1}$. However, the oil yield increased abruptly (Fig. 4.4) when the P concentration was increased to 2.2 meq P $L^{-1}$. This significant ($P$=0.01) increase in oil yield was brought about by an increased leaf oil concentration as well as the increased leaf yield, shown in Fig. 4.1.

In most nutrient solutions, phosphate is applied at relatively low concentrations (0.8 to 1.5 meq $L^{-1}$) and can thus be lost when exposed to high pH environments. In a standard nutrient solution soluble mono calcium phosphate [$Ca(H_2PO_4)_2.H_2O$] will occur at a pH below 6. Should the pH of the solution rise to 7 or higher, less soluble di-calcium phosphate [$CaHPO_4.2H_2O$] and almost insoluble tri-calcium phosphate [$Ca_3(PO_4)_2$] will start precipitating (Combrink, 2005). These results indicate that an exceptionally high level of P is needed. However, it should be noted that the pH of the nutrient solution was close to 7. Research findings of Ntwana (2007) showed that plant height and shrubs of buchu were reduced by 64% at high pH values, probably due to a lowering of soluble P.

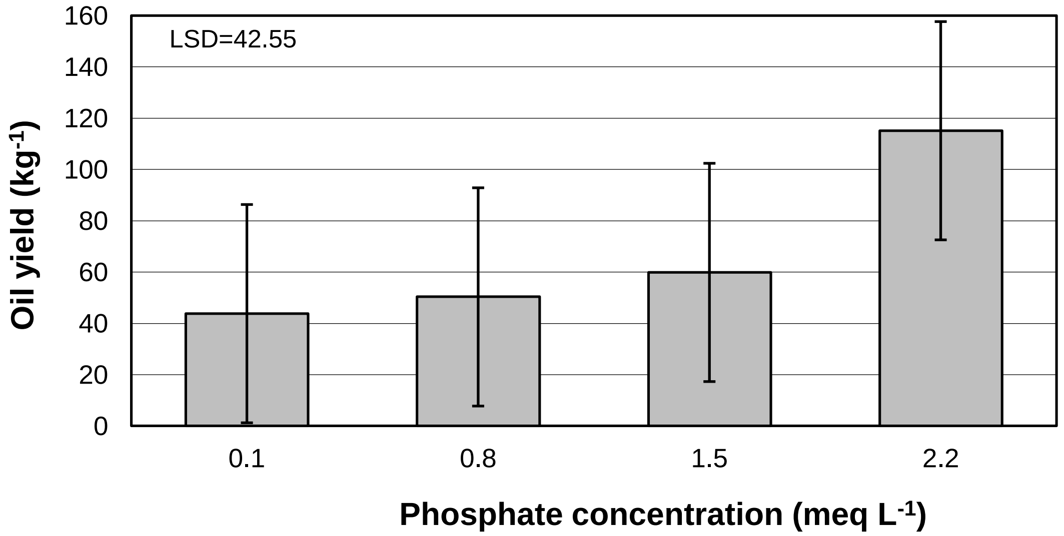


**Fig. 4.4** Phosphate concentrations in the nutrient solution affect oil yield of hydroponically grown rose geranium.

The results of this study showed highly significant effects of P levels on linalool (*P*=0.04), citronellol (*P*=0.01), geraniol (*P*=0.0007), Guaia-6,9-diene (*P*=0.003), citronellylformate (*P*=0.04) and the citronellol:geraniol (C:G) ratio. However, P levels had no significant effect on the synthesis of geranylformate, citronellol and isomenthone (Table 4.3). The citronellol:geraniol ratio was consistently higher than 3.0 at P concentrations of 0.1 to 1.5. However, at 2.2 meq $L^{-1}$ (the highest level tested) this ratio abruptly decreased to 2.85 (Table 4.3).

Unlike other essential oil industries (aromatherapy; alcoholic beverages and soft drinks etc.), the perfume industry has well detailed oil quality standards laid out for rose geranium. These requirements are based on the ratio between citronellol and geraniol. The ratio required for a good quality assessment must range between 1 and 3. Oil with a C:G ratio of more than three is considered to be of poor quality for the perfume industry, but it can still be used by other industries for the manufacturing of creams, soaps, toiletries and aromatherapy (Goto *et al.,* 2005; Weiss, 1997; Ram & Kumar, 1996). This shows that hydroponically grown rose geranium may need P levels of at least 2.2 meq $L^{-1}$ to produce perfume quality oil. Due to variations in temperature and humidity in the greenhouse, a gradient might have occurred and some of the oil components could have been affected (Ramezani *et al.,* 2009; Prakasa Rao *et al.,* 1995). However, all the secondary metabolites evaluated in the rose geranium oil were within the ranges recorded for open field produced rose geranium in South Africa (Ayara *et al.,* 2006; Motsa *et al.,* 2006).

**Table 4.3** The effect of phosphate concentrations in the nutrient solution on oil quality parameters of hydroponically grown rose geranium

| Phosphate concentration (meq $L^{-1}$) | Isomen-thone | Linalool | Citronellol (C) | Geraniol (G) | Citronellyl-formate | Geranyl-formate | Guaia-6,9-diene | C:G ratio |
|---|---|---|---|---|---|---|---|---|
| **0.1** | 1.53±0.38 | 0.79±0.26 | 27.25±0.82 | 8.86±0.80 | 19.43±0.64 | 11.79±0.30 | 9.00±0.52 | 3.09±0.23 |
| **0.8** | 1.73±0.25 | 1.04±0.38 | 26.96±0.45 | 8.46±0.25 | 19.36±0.69 | 11.60±0.72 | 9.48±0.32 | 3.19±0.94 |
| **1.5** | 1.27±0.79 | 0.43±0.02 | 24.72±1.01 | 7.00±0.65 | 20.25±0.52 | 11.65±0.78 | 10.01±0.44 | 3.54±0.23 |
| **2.2** | 2.17±0.93 | 0.81±0.26 | 25.85±1.01 | 9.19±1.14 | 18.96±0.86 | 11.75±1.05 | 8.68±0.37 | 2.85±0.40 |
| **$LSD_{T(0.05)}$** | ns | 0.41 | 1.51 | 0.87 | 0.84 | ns | 0.48 | 0.31 |

ns = not significant at $P<0.05$
$LSD_{T(0.05)}$ = least significant difference at 5% level of significance
± standard deviation

## 4.4 Conclusion

It is evident that rose geranium needs to be grown at a relatively high phosphate concentration in order to produce the highest yields of both herbage and oil, as well as the best quality oil. Good management of P in the nutrient solution will not only increase foliar mass production and oil yield of the material, but mineral uptake and oil quality will also improve. Future studies should focus on the effect of pH levels when testing phosphate requirement in plants.

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# CHAPTER 5

## EFFECT OF NITRATE AND AMMONIUM ON OIL YIELD AND QUALITY OF HYDROPONICALLY GROWN ROSE GERANIUM (*Pelargonium graveolens* L.)

# EFFECT OF NITRATE AND AMMONIUM ON OIL YIELD AND QUALITY OF HYDROPONICALLY GROWN ROSE GERANIUM (*Pelargonium graveolens* L.)

## Abstract

Nitrogen plays an important role in the growth, photosynthesis, reproduction and maintenance of genetic identity of plants. The potential to produce good quality rose geranium oil using different concentrations of nitrogen was investigated at the University of the Free State. Four levels of nitrate (8, 10, 12 and 14 meq $L^{-1}$) and four levels of ammonium (0.0, 0.5, 1.0 and 1.5 meq $L^{-1}$) were evaluated in two separate trials, using a randomized complete block design with five replications. The results showed a significant effect of nitrate on plant height, number of branches, foliar dry mass, chlorophyll content and oil yield. The height of rose geranium grown at 8 and 10 meq $L^{-1}$ nitrate concentrations were 46 cm and 57 cm, respectively. The number of branches was increased significantly by nitrate application and 57 branches were produced at 10 meq $L^{-1}$ compared to the 47 branches produced at 8 meq $L^{-1}$. The best foliar dry mass of 10 t $ha^{-1}$ was also produced at the nitrate concentration of 10 meq $L^{-1}$ compared to the 4 t $ha^{-1}$ produced at 8 meq $L^{-1}$. Chlorophyll content at 12 meq $L^{-1}$ nitrate was significantly higher than that obtained at 8 meq $L^{-1}$. The greatest oil yield (145 kg $ha^{-1}$) was also found at 10 meq $L^{-1}$. The citronellol:geraniol ratio was slightly higher than the upper limit required for good oil quality. However, the geraniol and citronellylformate contents were within range for top oil quality. Plant height, foliar dry mass, oil yield or chlorophyll content were not affected significantly by ammonium concentrations, but the content of sulphate and nitrogen increased at higher concentrations of applied ammonium with no detrimental effects on oil quality.

## 5.1 Introduction

*Pelargonium* species are known to have originated in South Africa, but some species are also found in neighbouring countries such as Zimbabwe and Mozambique (Van Der Walt & Vorster, 1988). A number of these species, such as *P. capitatum, P. graveolens, P. odoratissium* and *P. radens* have scented leaves and produce essential oils (Leistner, 2000). Of these *P. graveolens* was developed from a cross between *P.capitatum* and *P. radens* to produce the commercially important rose geranium cultivar. Rose geranium is widely cultivated in Russia, Egypt, Congo, Japan, Central America and Europe.

The oil of rose geranium is used as a flavouring agent in many major food categories, alcoholic beverages and soft drinks. The oil is also used as an important ingredient in the perfume and cosmetic industries as well as being used as a skin care product to treat acne, bruises, burns, dermatitis, and oily complexions. It is also used medicinally to treat sore throats and tonsillitis. Rose geranium oil is used in the treatment of adrenocortical glands and menopausal problems (Lawless, 1995). Moreover rose geranium has an important role in improving the quality of life and is being used in aromatherapy as a relaxant bath to reduce nervous tension (Lawless, 1995; Lis-Balchin, 1996). The current international demand for the oil of this species is more than 600 tons, and this demand is mostly met by countries such as South Africa, China, Morocco, Reunion and Egypt (Swanepoel, 2009; Lis-Balchin, 1996).

There is a steady growth in international markets for South African produced rose geranium oil. International markets need about 2.5 t $year^{-1}$ of locally produced rose geranium oil (NEDLAC, 2011). It is a mammoth task for South African producers to meet the growing market demand since bad weather conditions contribute to reductions in oil yield and quality. In order to reduce the effect of climate on oil yield and quality, production under protection is encouraged. For these conditions, however, nutrient management becomes crucial.

Nitrogen plays an important role in growth, photosynthesis, reproduction and the maintenance of the genetic identity of plants. After nitrogen has been taken up by plants, most of it is reduced to the amine form ($-NH_2$). Nitrogen is involved in the formation of organic compounds such as amino acids, proteins, adenosine triphosphate, adenosine diphosphate, deoxyribonucleic acid, phospholipids and chlorophyll (FSSA, 2007).

Nitrogen is taken up from the soil in either the ammonium or nitrate form. Some plant species have a strong preference for one form over the other. In most cases, summer crops prefer nitrate over ammonium. Ammonium is a small ion that is easily absorbed by plant roots but may be toxic at high concentrations (FSSA, 2007; Pidwirny, 2006). Ammonium may be phytotoxic at high root zone temperatures and at high concentrations for most greenhouse crops (Kafkaffi, 2000). Steiner (1984) did not include ammonium in his nutrient solution and used nitrate as the sole source of nitrogen. Modern soilless growers use small quantities of ammonium in their nutrient solutions for its acidifying effect, preventing rises in pH and the precipitation of Fe and Zn (Combrink, 2005). The standard recommendation used by the first generation of soilless plant growers in South Africa, was not to have ammonium in nutrient solutions at levels higher than 10% of the total N application. This recommendation was changed when Combrink (2005) prescribed very low ammonium levels for pepper, higher levels for tomatoes and cucumbers and relatively high levels for orchids and disas.

The N requirement of rose geranium has been studied extensively under open field conditions. For optimum yield, rose geranium needs N at about 100 to 200 kg N $ha^{-1}$, depending on the use of organic mulch as well as the form and the source of nitrogen used (Ayara *et al.*, 2006; Ram *et al.,* 2003; Singh, 1999; Singh *et al.*, 1996). The yield and quality response of hydroponically grown rose geranium to the application of nitrate and ammonium in nutrient solutions have not yet received attention.

The objectives of this study were to evaluate nitrate and ammonium as sources of nitrogen and to set standards for soilless production of rose geranium.

## 5.2 Material and methods

The basic experimental procedure followed in this chapter is laid out in chapter 3 and only specific details for this experiment are explained.

*Experimental design*

The pH and the EC of the nutrient solution in both studies were maintained at 6.98 (±0.02) and 1.60 (±0.1) mS $cm^{-1}$, respectively. The EC level of the solution was maintained by proportionally lowering the concentrations of phosphate and sulphate at an increased nitrate concentration. Potassium, calcium and magnesium were also lowered proportionally at an increased ammonium concentration (Tables 5.1 and 5.2).

The effect of four concentrations of nitrate (8, 10, 12 and 14 meq $L^{-1}$) and ammonium (0.0, 0.5, 1.0 and 1.5 meq $L^{-1}$) were evaluated for their effects on growth and herbage yield, as well as oil yield and oil quality of rose geranium. Nitrate and ammonium were evaluated separately, in two trials, using randomized complete block designs with five replicates used in each of the experiments. Concentrations of nitrate and ammonium in the nutrient solutions are given in Tables 5.1 and 5.2.

**Table 5.1** Macro nutrient compositions of nutrient solutions containing different nitrate concentrations used to irrigate rose geranium plants grown hydroponically

| **Nitrate (meq L$^{-1}$)** | **Ions (meq L$^{-1}$)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **$NH_4^+$** | **$K^+$** | **$Ca^{2+}$** | **$Mg^{2+}$** | **$NO_3^-$** | **$H_2PO_4^-$** | **$SO_4^{2-}$** |
| **8.00** | 1.00 | 5.5 | 6.5 | 2.50 | 8.00 | 2.11 | 4.83 |
| **10.00** | 1.00 | 5.5 | 6.5 | 2.50 | 10.00 | 1.50 | 3.44 |
| **12.00** | 1.00 | 5.5 | 6.5 | 2.50 | 12.00 | 0.89 | 2.05 |
| **14.00** | 1.00 | 5.5 | 6.5 | 2.50 | 14.00 | 0.28 | 0.66 |

**Table 5.2** Macro nutrient compositions of nutrient solutions containing different ammonium concentrations used to irrigate rose geranium plants grown hydroponically

| **Ammonium (meq L$^{-1}$)** | **Ions (meq L$^{-1}$)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **$NH_4^+$** | **$K^+$** | **$Ca^{2+}$** | **$Mg^{2+}$** | **$NO_3^-$** | **$H_2PO_4^-$** | **$SO_4^{2-}$** |
| **0.00** | 0.00 | 5.88 | 6.95 | 2.67 | 10.00 | 1.50 | 3.44 |
| **0.50** | 0.50 | 5.69 | 6.72 | 2.59 | 10.00 | 1.50 | 3.44 |
| **1.00** | 1.00 | 5.50 | 6.50 | 2.50 | 10.00 | 1.50 | 3.44 |
| **1.50** | 1.50 | 5.31 | 6.28 | 2.41 | 10.00 | 1.50 | 3.44 |

## 5.3 Results

### 5.3.1 Nitrate trial

The tallest plants were found at 10 meq L$^{-1}$ nitrate concentrations, significantly (*P*=0.02) taller than plants produced with 8 meq L$^{-1}$ or 14 meq L$^{-1}$ nitrate, but not significantly taller than plants at a nitrate concentration of 12 meq L$^{-1}$ (Fig. 5.1). The trend indicates that the tallest plants can be grown with a nitrate level between 10 and 12 meq L$^{-1}$.

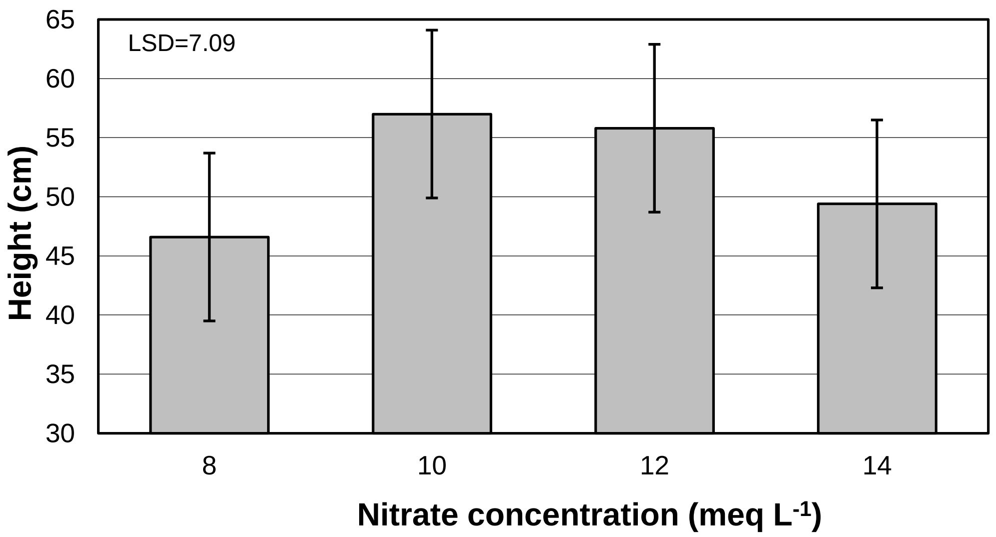


**Fig. 5.1** Effect of nutrient solution nitrate concentrations on plant height of hydroponically grown rose geranium.

The number of branches was used as an indication of bushiness. Better branched plants were produced at 10 meq $L^{-1}$ nitrate, significantly (*P*=0.007) bushier than plants produced with 8 meq $L^{-1}$ nitrate. Plants tended to produce less branches at 12 and 14 meq $L^{-1}$ nitrate, although not significantly less between the two treatments (Fig. 5.2).

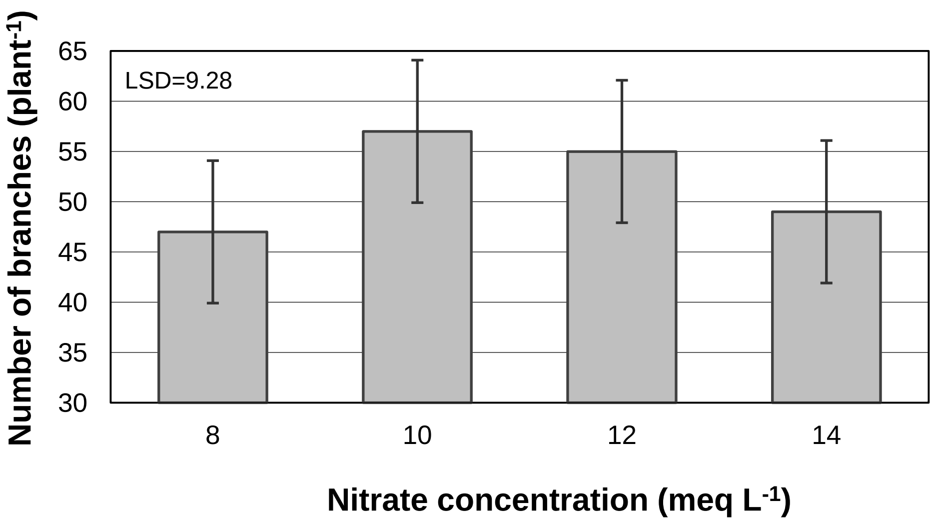


**Fig. 5.2** Effect of nutrient solution nitrate concentrations on the number of branches produced by hydroponically grown rose geranium plants.

Nitrate concentrations of 10 or 12 meq $L^{-1}$ resulted in foliar dry mass yields that were significantly higher than the dry mass produced at 8 or 14 meq $L^{-1}$ (Fig. 5.3). This definite peak between 10 and 12 was also found with height and branching as parameters. It is therefore probable that the increase in both plant height and the number of branches caused the increase in dry mass. The effects of leaf number and size can not be ruled out as these parameters were not measured.

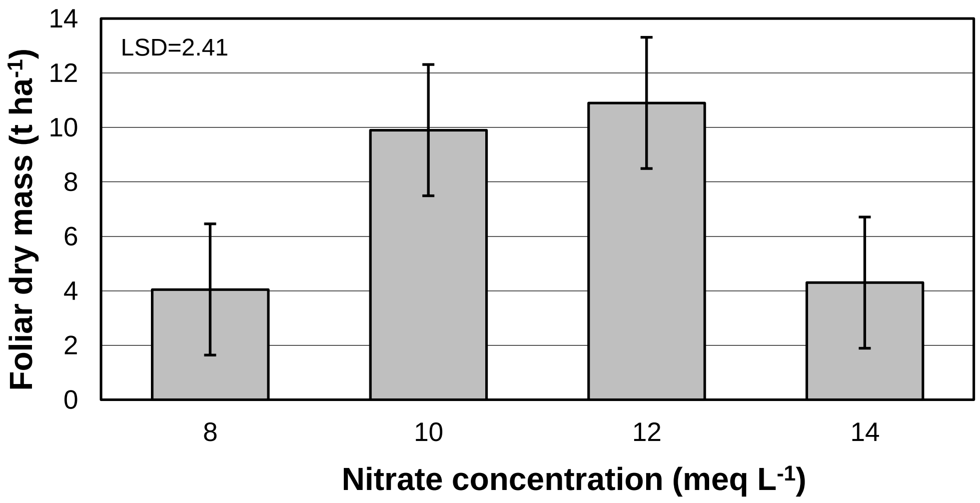


**Fig. 5.3** Effect of nutrient solution nitrate concentrations on the foliar dry mass of hydroponically grown rose geranium.

The highest chlorophyll content of leaves was observed on plants grown at 12 or 14 meq $L^{-1}$ nitrate concentrations. The chlorophyll content was significantly ($P$=0.003) greater at 12 and 14 meq $L^{-1}$ than that found in leaves from plants produced at 8 and 10 meq $L^{-1}$ nitrate (Fig. 5.4).

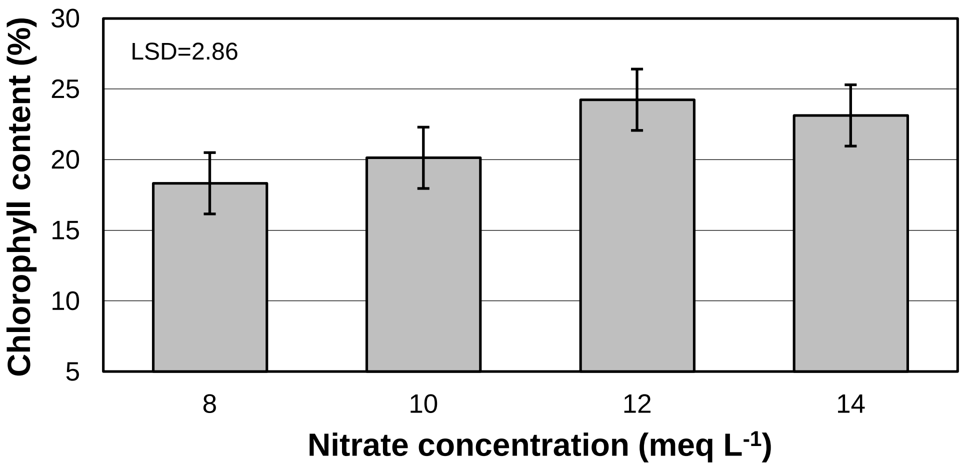


**Fig. 5.4** Effect of increasing nutrient solution nitrate concentrations on leaf chlorophyll content of hydroponically grown rose geranium.

Magnesium, P, S and K content of the leaves were not affected by nitrate concentrations. Foliage Ca was significantly (*P*=0.02) affected, but the results were inconsistent, and consequently not presented.

Although the oil content in the leaves increased almost linearly (*P*=0.04) as nitrate concentrations increased (Fig. 5.5). The oil concentration at the highest nitrate level (14 meq $L^{-1}$) was only significantly greater than that found at the lowest nitrate level (Fig. 5.5).

Different nitrate concentrations in the nutrient solutions significantly (*P*=0.003) affected the oil yield, as shown in Fig. 5.6. Oil yield peaked at 12 meq $L^{-1}$ nitrate, where yields were significantly higher than at the low (8 meq $L^{-1}$) and high (14 meq $L^{-1}$) nitrate concentrations, although not significantly better than at 10 meq $L^{-1}$ nitrate. This decrease was probably due to the decreased foliage mass produced by plants at 14 meq $L^{-1}$ nitrate (Fig 5.3).

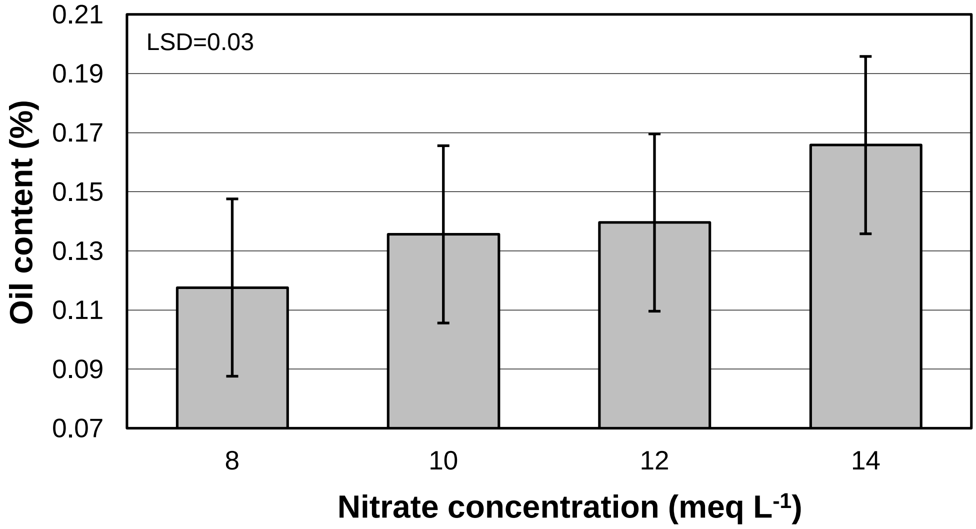


**Fig. 5.5** Effects of nutrient solutions nitrate concentration on oil content of rose geranium.

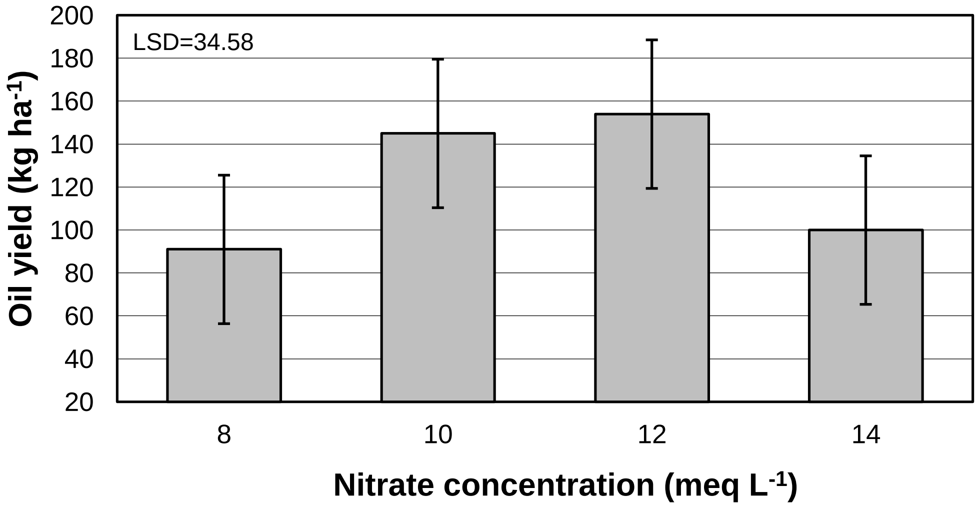


**Fig. 5.6** Effect of nutrient solution nitrate concentrations on oil yield of rose geranium foliage.

Nitrate concentration showed no significant effects on isomenthone, linalool, citronellol, geraniol, and geranylformate contents. However, the citronellol:geraniol (C:G) ratios (*P*=0.0004) and citronellylformate content (*P*=0.04) of the oil was affected by nitrate concentrations. Significantly higher C:G ratios in the oil was obtained where plants were cultivated in nutrient solutions containing nitrates at 10 and 12 meq $L^{-1}$. The C:G ratio of the oil should be less than 3 to be considered by the perfume industry. Other industries, however, do use oil with a C:G ratio higher than 3, as such oil is used in the manufacturing of creams, soaps, toiletries and for aromatherapy treatment (Weiss, 1997; Ram and Kumar, 1996).

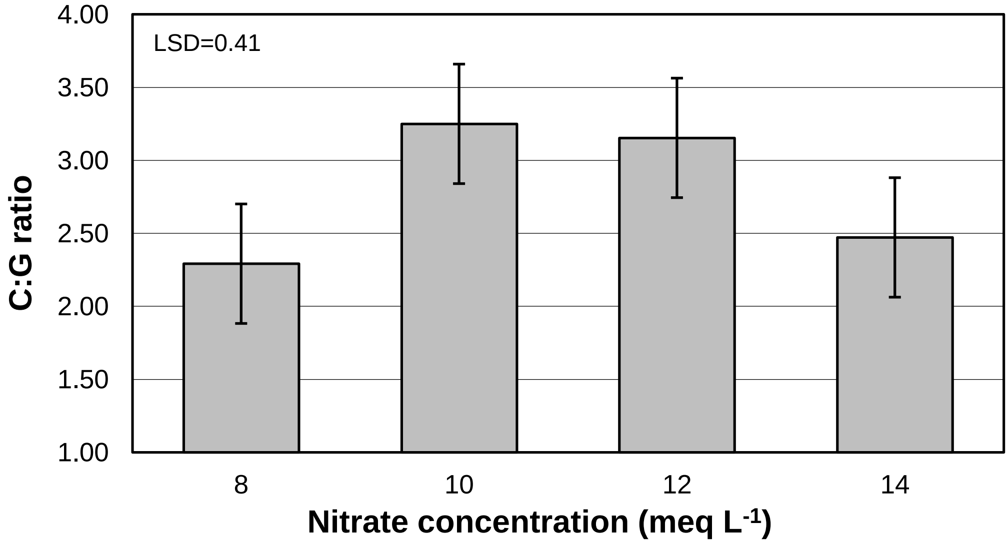


**Fig. 5.7** Effect of nitrate concentrations of the nutrient solution on the citronellol:geraniol (C:G) ratio of hydroponically grown rose geranium.

By increasing the nutrient solution's nitrate, the citronellylformate content of rose geranium oil increased and reached its upper limit at 12 meq $L^{-1}$, before decreasing at the highest concentration (14 meq $L^{-1}$), as shown in Fig. 5.8.

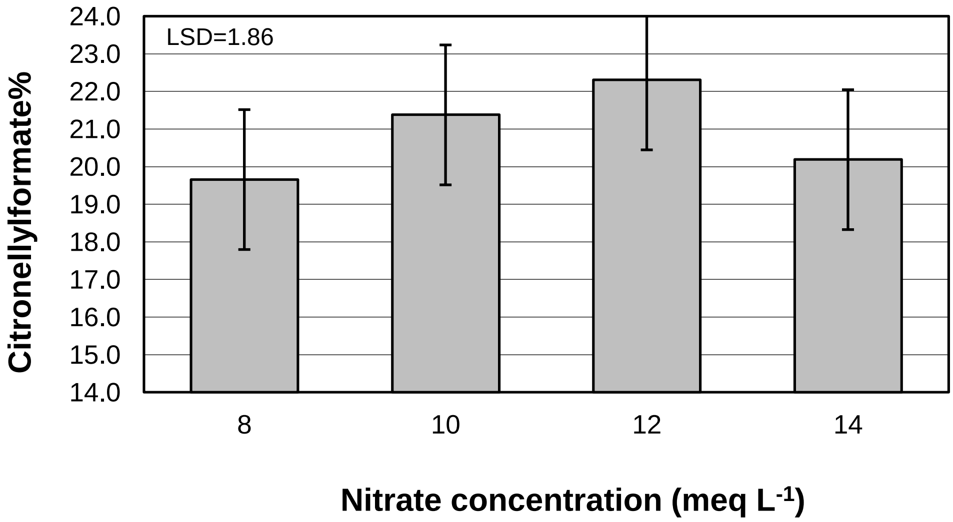


**Fig. 5.8** Effect of nitrate concentrations of the nutrient solution on citronellylformate concentration of hydroponically grown rose geranium.

### 5.3.2 Ammonium trial

Unlike nitrate, ammonium concentration in the nutrient solution did not have any significant effect on height, branching, foliar mass, oil content and oil yield of the geranium plants. However, ammonium significantly affected foliar N ($P$=0.005) and the foliar sulphur content ($P$=0.0002). These minerals accumulated in the foliage with increasing concentrations of ammonium, as shown in Figs 5.9 and 5.10, with the highest levels occurring at the highest ammonium concentrations. No significant difference in the N content of the plants was noted between 1.00 and 1.50 meq $L^{-1}$ $NH_4$, but the S content of plants grown in the 1.5 meq $L^{-1}$nutrient solutions was significantly greater than that found in plants at 1.00 meq $L^{-1}$. Where biomass as well as oil yield were significantly affected by nitrate concentrations in the nutrient solutions, it is interesting to note that nitrate concentrations did not affect foliage N concentration as was the case with ammonium.

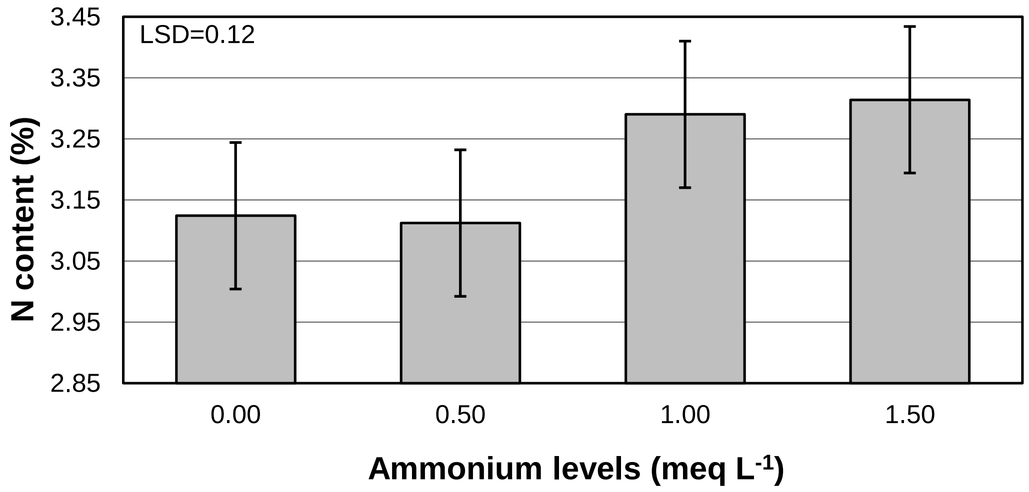


**Fig. 5.9** The effect of ammonium concentrations of the nutrient solution on foliage nitrogen content.

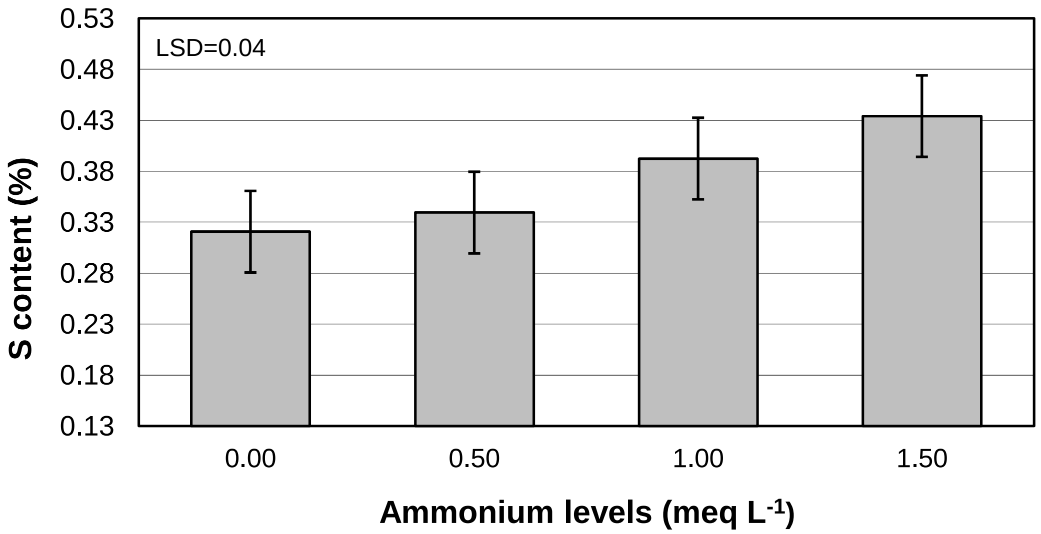


**Fig. 5.10** The effect of ammonium concentrations of the nutrient solution on sulphur concentrations in the foliage of hydroponically grown rose geranium.

Ammonium levels in the nutrient solutions had no significant effect on the isomenthone, linalool, citronellol, citronellylformate, geraniol contents as well as the C:G ratio. However, geranylformate ($P$=0.04) and guaia-6,9-diene ($P$=0.01) contents were significantly affected by ammonium (Fig. 5.11). Both compounds peaked with ammonium at 1.00 meq $L^{-1}$, where their concentrations were significantly higher than at 0, 0.5 and 1.5 meq $L^{-1}NH_4$.

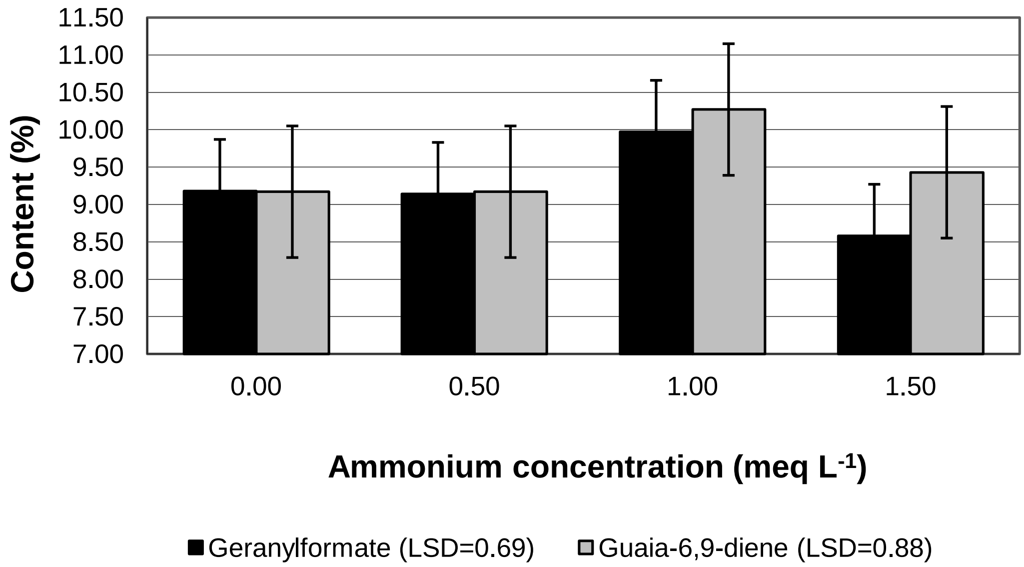


**Fig. 5.11** Guaia-6,9-diene and geranylformate contents of hydroponically grown rose geranium, grown at different ammonium concentrations.

## 5. 4 Discussion

The application of $NO_3^-$ had an effect on plant height, number of branches, foliar dry mass, chlorophyll content and oil yield. These parameters were not affected by $NH_4$ concentration. Ammonium can be used in rose geranium nutrient solutions at levels of up to 1.5 meq $L^{-1}$ without any fear of possible negative side effects. This is an important finding as ammonium is used in nutrient solutions to manage root zone pH for soilless production systems (Combrink & Kempen, 2011).

The optimum $NO_3$ concentration for most parameters measured appears to be between 10 and 12, at about 11 meq $L^{-1}$ or slightly higher. Steiner's universal nutrient solution contains 12, 1 and 7 meq $L^{-1}$ $NO_3$, $H_2PO_4$ and $SO_4$ respectively (Steiner, 1984). Thus, nitrate represents 60% of Steiners' total anion application. In this trial the optimum anion ratio can be estimated at about 11 meq $NO_3$ $L^{-1}$ (74%), associated with 1.2 meq $H_2PO_4$ $L^{-1}$ and 2.75 meq $SO_4$ $L^{-1}$.

Studies conducted under open field conditions, also indicate that a high nitrogen application of 100 to 200 kg N $ha^{-1}$ is needed to produce high rose geranium yields (Ayara *et al.,* 2006; Ram *et al.,* 2003; Singh, 1999). Application of 100 kg organic N $ha^{-1}$ increased herbage yield by 57% and oil yield by 47% (Ayara *et al.,* 2006; Ram *et al.,* 2003). Dry mass of oregano (*Origanum vulgare* L.) was increased by 26% when N fertilizer was used (Aziz *et al.,* 2009). Good moisture and N supply increase root biomass, improving the uptake of nutrients and water by roots of palmarosa (*Cymbopogon martinii*) (Singh & Sharma, 2001). This would then appear to be consistent with the results obtained for this study.

Various studies showed a positive relationship between chlorophyll and N-content of the leaves (Wang *et al.,* 2004; Scheepers *et al.,* 1992). Chlorophyll content is used as an alternative measure for nitrogen status of most plant species (Fontes & de Araujo, 2006). This relationship between chlorophyll and foliar N content was not found in this study, possible due to the increase in biomass production that occurred at increased nitrate levels. It is interesting to note that higher ammonium concentrations did increase foliar N (Fig. 5.9) without affecting either yield or leaf chlorophyll content.

It is known that high concentrations of ammonium in nutrient solutions may suppress the uptake of calcium. However, the foliage Ca content was not affected by the application of ammonium at levels of up to 1.5 meq $L^{-1}$ in this study. Environmental conditions varied in the greenhouse that could have stimulated or suppressed transpiration, so causing differences in foliar Ca concentrations. As a result, as well as the fact that foliage Ca concentrations were well within limits for healthy plant growth (1.72%-2.06%), the inconsistent effects of nitrate concentrations on foliage Ca are not shown. Calcium accumulation in plant tissues is affected by Ca uptake by the roots as well as the transpiration rate of the involved plant species (Combrink, 2005; Adams & Holder, 1992).

Oil quality is an important factor as it affects the market price of rose geranium oil. The perfume industry has well detailed quality requirements for rose geranium oil. These are based on the ratio between citronellol and geraniol. The ratio required for this must range between 1 and 3. Oil that have C:G ratios of more than three is considered to be of poor quality, therefore such oil is used in the manufacturing of creams, soaps, toiletries and aromatherapy treatment (Ram & Kumar, 1996; Weiss, 1997). The rose geranium oil obtained in this study at low or high nitrate levels had good C:G ratios associated with lower biomass and oil yields.

The different $NO_3$ levels affected citronellylformate and the C:G ratio, while $NH_4$ affected the geranylformate and guaia-6,9-diene contents. Citronellylformate is an ester obtained from citronellol and is used for imparting floral notes in fragrances and flavour compounds. The application of $NO_3$ at 10 meq $L^{-1}$ seems to optimize the synthesis of secondary metabolites such as citronellylformate. The application of $NH_4$ had positive effects on the metabolism of guaia-6,9-diene and geranylformate (Fig. 5.11). Other than nitrogen application, weather parameters such as temperature and rainfall also affect oil content and the concentration of chemical compounds such as geraniol, citronellol and citronellylformate in rose geranium oil (Prakasa Rao *et al.,* 1995).

One of the effects of ammonium on plant growth is blockage of root metabolism that may increase the permeability of root cell membranes. A decrease in foliar sulphate is used as an indicator of toxic effects of ammonium on the root metabolism (Ragab, 1980). In the ammonium study, foliar S increased, indicating that the ammonium concentrations used were not toxic (Fig. 5.9). The reason why N and S accumulated in leaf tissue is unclear.

The contribution of nitrate and ammonium to the uptake of N differ between species. Ammonium is mediated by families of active membrane transporters ($K_M^{ammonia}$<0.5-40 μM) (Hell & Hillebrand, 2001). Both $NH_4$ and $NO_3$ are transported through the same pathway. If $NH_4$ and $NO_3$ are present in the same nutrient solution, ammonium will influence the uptake and metabolism of $NO_3$. Hence, optimum plant growth is achieved with well balanced ratios of ammonium and nitrate (Ragab, 1980).

## 5. 5 Conclusion

Rose geranium needs to be grown at a relatively high nitrate concentration of 10 to 12 meq $L^{-1}$ to ensure high herbage and oil yields. Ammonium concentrations evaluated had no effect on herbage and oil yield but affected foliar N and S contents. Given that some oil quality parameters were also affected by ammonium, this provide useful information to growers that 1.00 meq $L^{-1}$ ammonium might be sufficient for hydroponically grown rose geranium. It is evident that further investigation of the ratio of ammonium to nitrate is required for hydroponically grown rose geranium.

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# CHAPTER 6

## RESPONSE OF HYDROPONICALLY GROWN *Pelargonium graveolens* (L.) TO SULPHATE

# RESPONSE OF HYDROPONICALLY GROWN *Pelargonium graveolens* TO SULPHATE


## Abstract

*Pelargonium graveolens,* extensively known as rose geranium, is the most important plant in the South African essential oil industry. The oil is used as an ingredient in the perfume and cosmetic manufacturing industries. The objective of this study was to determine the effects of varying sulphate concentrations in nutrient solutions on growth, yield, oil yield and quality of hydroponically grown *Pelargonium graveolens* and to set standards to be used in the production of rose geranium. Four concentrations of sulphate were investigated 0.36, 1.90, 3.44 and 4.98 meq $L^{-1}$ and these treatments were assigned in a completely randomized block design using five replications. The results of the study showed a significant effect of sulphate on the number of branches, plant height and branch:height (B:H) ratio and foliar dry mass. Plant growth and oil yield peaked at the 3.44 meq $L^{-1}$ level. Oil quality was unaffected based on the ratio between citronellol and geraniol.


## 6.1 Introduction

The global market for essential oils is estimated to be worth more than R6 billion. The United States of America, Western Europe and Japan are the major importers, consuming 40%, 30% and 7%, respectively (United Nations, 2004). The major producers are Algeria, Congo, France, Madagascar, Morocco, Reunion, Russia, Spain and South Africa (Lis-Balchin, 1996).

It has been estimated that the market size for South African produced rose geranium oil will increase from the current 2.5 t to 50 t $year^{-1}$ (NEDLAC, 2011). Due to inconsistent, erratic and extreme climatic conditions, an increase in production should be met by increasing yields through the use of better production systems, rather than by expanding the area under production. Plants that are cultivated under protection grow rapidly on soilless substrates that are fertigated with a well balanced nutrient solution (Bone & Waldron, 1999). Controlled environmental conditions enhance plant development and yield subsequently by optimizing mineral utilization by plants.

Sulphur (S) is a major plant macro-nutrient contained by soil in organic and inorganic forms. Under aerobic conditions, inorganic S is present in the sulphate ($SO_4$) form. This is the form in which plant roots absorb sulphur (Maathius, 2009). Sulphur form part of the amino acids cysteine, cystine and methionine, which are required for the synthesis of proteins. Sulphur is also involved in the formation of chlorophyll, activation of enzymes and is part of co-enzyme A. Excessive or deficient S may interfere with the synthesis of proteins (Sexton *et al.,* 1998). SH-sulphydryl linkages are involved in the formation of glucoside of mustard oil (FAO, 2009; Droux, 2004; Marschner, 1986). In onions, sulphate is responsible for the pungent taste (Randle *et al.,* 1993) and in lettuce it is responsible for the production of sesquiterpene lactones. The application of sulphate also improve the oil yield and quality (glucosinolate) of oilseed rape (*Brassica napus*) (Čeh *et al.,* 2008; Fismes *et al.,* 2000; Asare & Scarisbrick, 1995). No information on the effects of S on yield and quality of rose geranium could be found. The effects of sulphate concentrations on the oil quality of rose geranium were therefore investigated. The objectives of this study were to determine the effect of varying sulphate concentrations in nutrient solutions on the growth, yield and quality of hydroponically grown *Pelargonium graveolens,* as well as an attempt to set standards for sulphate to be used in nutrient solutions for this species.

## 6.2 Material and methods

This section only describes deviations from the general material and methods, described in chapter 3.

The trial was carried out during autumn and winter, using a randomized complete block design with five replications. Sulphate concentrations of 0.36, 1.90, 3.44 and 4.98 meq $L^{-1}$ were selected based on the concentrations used for other crops in soilless production systems as no norms for sulphate fertilization exist for rose geranium. Nitrate and phosphate concentrations were reduced proportionally as the sulphate concentration was increased in order to maintain a consistent EC of 1.57 mS $cm^{-1}$ between the different treatments (Combrink, 2005). The anion and cation concentrations for the various treatments are shown in Table 6.1. The concentrations of the trace elements that were used are described in chapter 3.

**Table 6.1** Macro nutrient composition of nutrient solutions containing different sulphate concentration used to irrigate rose geranium plants

| **Sulphate (meq L$^{-1}$)** | **Ions (meq L$^{-1}$)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **$NH_4^+$** | **$K^+$** | **$Ca^{2+}$** | **$Mg^{2+}$** | **$NO_3^-$** | **$H_2PO_4^-$** | **$SO_4^{2-}$** |
| **0.36** | 1.00 | 5.5 | 6.5 | 2.50 | 12.68 | 1.90 | 0.36 |
| **1.90** | 1.00 | 5.5 | 6.5 | 2.50 | 11.34 | 1.70 | 1.90 |
| **3.44** | 1.00 | 5.5 | 6.5 | 2.50 | 10.00 | 1.50 | 3.44 |
| **4.98** | 1.00 | 5.5 | 6.5 | 2.50 | 8.66 | 1.30 | 4.98 |

## 6.3 Results and discussion

### Plant parameters

**Table 6.2** Effects of sulphate concentrations on the number of branches, plant height (cm), branch:height ratio, foliar dry mass, oil yield and oil content of hydroponically grown rose geranium

| **Sulphate concentration (meq L$^{-1}$)** | **Parameter** | | | | | |
|---|---|---|---|---|---|---|
| | **Number of branches (B)** | **Height (H)** | ***B:H ratio** | **Foliar dry mass (t ha$^{-1}$)** | **Oil yield (kg ha$^{-1}$)** | **Foliar oil content (%)** |
| 0.36 | 17.20±2.94 | 51±6.66 | 33±2.00 | 5.36±1.12 | 46.70±9.92 | 0.97±0.33 |
| 1.90 | 27.60±4.77 | 42±4.68 | 63±2.46 | 6.88±1.43 | 55.76±9.47 | 0.87±0.27 |
| 3.44 | 20.40±1.94 | 51±5.57 | 40±2.81 | 8.74±0.86 | 59.92±23.07 | 0.70±0.30 |
| 4.98 | 21.80±5.06 | 42±4.33 | 51±6.90 | 5.17±0.77 | 32.97±10.44 | 0.63±0.19 |
| **$LSD_{T(0.05)}$** | **4.89** | **8.07** | **11.78** | **1.43** | **ns** | **ns** |

$LSD_{T(0.05)}$=least significant difference at 5% level of significance

ns not significant at $P$<0.05

± standard deviation

*B:H ratios were calculated by dividing the number of branches per plant by plant height (m) to express B:H as branches m$^{-1}$

Both the number of branches per plant (*P*=0.004) and plant height (*P*=0.04) were significantly affected by sulphate concentrations. Plants grown at a sulphate concentration of 1.90 meq $L^{-1}$ produced the most branches, but plant height varied inconsistently between sulphates levels tested. Shorter plants appeared to develop more branches than taller plants, thus increasing the number of branches per metre. At 1.90 meq $L^{-1}$ level, 27.6 braches developed, significantly (*P*=0.004) more than at other sulphate concentrations. However, at the higher sulphate level of 3.44 meq $L^{-1}$, the foliar dry mass production was significantly (*P*=0.05) better than at the other sulphate concentrations. The sulphate levels did not have any effect on the oil yield and content of rose geranium (Table 6.2). Most studies on S do not show any significant effect of S on vegetative growth (Kowalska, 2005; Bellert *et al.,* 1998).

**Foliar mineral**

There was no significant effect of $SO_4$ on the uptake of K, Ca and Mg. Sulphate did, however, have a highly significant (*P*=0.0001) effect on the S content of the plant tissue (Table 6.3). The S content of the foliage increased linearly with increasing sulphate concentrations in the nutrient solution. Sulphate accumulation in vacuoles is an indication of a higher S intake than the plants' need. In other plant species, $SO_4$ will accumulate in the form of secondary organic metabolites such as glucosinolates and γ-glutamylpeptides which are found in cabbage and onions, respectively (Kowalska, 2005; Randle *et al.,* 1999). Foliar S was well within the range required for normal growth of plants (0.1 - 6%) for all the levels tested (Droux, 2004). The results obtained were similar to the results obtained in previous studies on *Brassica napus* and *Allium cepa* reported by Malhi and Gill (2002) and Randle *et al.* (1999), respectively.

**Table 6.3** Sulphate concentrations in nutrient solutions affecting the mineral composition of rose geranium foliage

| **Sulphate concentration (meq $L^{-1}$)** | **Plant tissue minerals (%)** | | | |
|---|---|---|---|---|
| | **S** | **K** | **Ca** | **Mg** |
| 0.36 | 0.28±0.03 | 3.67±0.39 | 1.75±0.22 | 0.37±0.04 |
| 1.90 | 0.35±0.02 | 3.31±0.43 | 1.70±0.27 | 0.36±0.04 |
| 3.44 | 0.43±0.02 | 3.58±0.35 | 1.54±0.26 | 0.35±0.03 |
| 4.98 | 0.54±0.08 | 3.69±0.40 | 1.57±0.32 | 0.33±0.02 |
| **$LSD_{T(0.05)}$** | **0.07** | **ns** | **ns** | **ns** |

$LSD_{T(0.05)}$=least significant difference at 5% level of significance

ns not significant at $P<0.05$

± standard deviation

**Oil quality**

Dethier *et al.* (1997) reported that fertilizer applications have varying effects on essential oil yield and oil composition. Good quality oil is in high demand particularly in the perfume industry. Lower quality and less expensive oil are used in the manufacturing of creams, soaps, toiletries and for aromatherapy treatment (Goto *et al.,* 2005; Weiss, 1997; Ram & Kumar, 1996). In this study, most of the oil quality parameters were unaffected by the $SO_4$ concentrations. The chemical compounds that were significantly affected were citronellylformate ($P$=0.04), geranylformate ($P$=0.01) and the C:G ratio ($P$=0.01). However, these results were inconsistent (Table 6.4). This might have been partly caused by gradients in greenhouse temperature and humidity rather than as a result of the $SO_4$ concentrations (Prakasa Rao *et al.,* 1995). Christensen *et al.* (2003) showed that sulphate affects peppermint oil quality, and also had problems with external factors affecting the results. The fact that the trial was conducted in autumn could have contributed to the high C:G ratios found.

**Table 6.4** Effect of sulphate concentration in nutrient solutions on oil quality parameters of hydroponically grown rose geranium

| Sulphate concentration (meq $L^{-1}$) | Parameter | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Isomethone | Linalool | Citronellol | Geraniol | Citronelly-formate | Geranyl-formate | Guaia-6,9-diene | C:G ratio |
| 0.36 | 1.61±0.52 | 0.80±0.29 | 26.50±1.17 | 8.50±0.52 | 20.63±1.70 | 12.26±1.03 | 9.31±0.55 | 3.13±0.30 |
| 1.90 | 1.38±0.22 | 0.69±0.15 | 28.39±1.92 | 7.41±0.57 | 21.84±1.17 | 11.25±0.36 | 8.91±0.46 | 3.85±0.41 |
| 3.44 | 1.61±0.74 | 0.83±0.21 | 27.11±1.83 | 8.32±1.17 | 19.71±0.98 | 11.71±0.74 | 9.10±0.74 | 3.29±0.35 |
| 4.98 | 2.08±1.16 | 0.39±0.05 | 26.03±2.29 | 7.43±0.88 | 21.43±0.72 | 12.44±0.46 | 9.47±0.59 | 3.51±0.25 |
| **$LSD_{T(0.05)}$** | ns | ns | ns | ns | 1.5 | 0.7 | ns | 0.4 |

C:G ratio=citronellol:geraniol ratio

$LSD_{T(0.05)}$=least significant difference at 5% level of significance

ns not significant at $P<0.05$

± standard deviation

## 6.4 Conclusion

The results of this study show that rose geranium can be grown with sulphate at concentrations up to 4.98 meq $L^{-1}$. No detrimental trend could be noted in any parameters with increasing sulphate concentration in the nutrient solution. It can be concluded that, although oil quality was not of good for the perfume industry, although, was not detrimentally affected by sulphate for use by other industries. The relatively high C:G ratio was probably caused by the sulphate concentration of the nutrient solution. It can also be concluded that rose geranium can be produced with sulphate concentrations in nutrient solutions between 0.36 and 3.4 meq $L^{-1}$, although much more work in this regard is needed to optimize the $SO_4$ concentration.

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# CHAPTER 7

## EFFECT OF SHADING AND MOISTURE STRESS ON YIELDS AND OIL QUALITY OF HYDROPONICALLY GROWN ROSE GERANIUM (*Pelargonium graveolens* L.)

# EFFECT OF SHADING AND MOISTURE STRESS ON YIELDS AND OIL QUALITY OF HYDROPONICALLY GROWN ROSE GERANIUM (*Pelargonium graveolens* L.)

## Abstract

Rose geranium oil is used as a flavouring agent in many major food categories, alcoholic and soft drinks. Relatively high radiation levels occur in South Africa during summer months, and some hydroponically grown crops may need shading for optimum production. This study was conducted during spring and summer to evaluate the effects of shading and moisture stress on growth, oil yield and oil quality of rose geranium. A split plot layout was assigned in a randomized complete block experimental design. Shading treatments of 0%, 20%, 40%, 60% and 80% were allocated to the main plots, while subplots were exposed to two moisture stress levels (0 and -0.15 MPa of osmotic pressure). The results show that rose geranium performs well when grown under 40% shade, where the number of branches, foliar fresh and dry mass, branch:height ratio as well as the oil yield peaked. Proline content was significantly higher at a low level of shading, where high levels of radiation were found, and where moisture stress was applied. Moisture stress did not, however, affect any of the quality parameters measured for rose geranium oil. Most of the chemical substances produced at the 40% shading level were at acceptable levels for good oil quality. The number of oil glands $cm^{-2}$ of leaf area was not affected by shading although the oil gland density tended to be lower with increased levels of shading. For conditions similar to those in Bloemfontein, it is suggested that 40% shading be used for the production of rose geranium in summer.

## 7.1 Introduction

Oil of rose geranium is used as a flavouring agent in many major food categories, as well as in alcoholic and soft drinks. Fresh rose geranium leaves infuse a rosy flavour to desserts and jellies (Gough, 2002). The oil is also used as an important ingredient in the perfume and cosmetic industries (Kulkarni *et al.,* 1997). It has a floral rosy, green herbal fresh mint scent and is used in the production of high quality perfumes. It is used more frequently in masculine perfumes, than in women's fragrances (Gomes *et al.,* 2006). The Bourbon cultivar of rose geranium produces the best quality oil and is used mostly as a component for fine perfumes, but its annual production is relatively small (Gomes *et al.,* 2006).

The production of rose geranium requires an evenly distributed annual rainfall of 1 000 to 1 500 mm and yield losses may occur during dry seasons (Weiss, 1997). Although Swamy *et al.* (1960) reported that rose geranium can tolerate drought, plant growth and oil yield may be impeded by severe drought conditions. Singh (1999) reported that herb and oil yields increased when the moisture regime was raised from 0.3 to 0.6 ratio of irrigation water to cumulative pan evaporation (IW:CPE) on red sandy loam soils (alfisols). Irrigation water can improve herb- and oil yield of rose geranium while the chemical compounds such as geraniol and citronellol are not affected (Singh *et al.,* 1996).

The total radiance at the upper boundary of the atmosphere is 1360 $Jm^{-2}s^{-1}$. Most of this radiation is lost by absorption and scattering caused by dust, water vapour, $CO_2$ and $O_3$ in the atmosphere. Most greenhouse growers in South Africa use lime or removable paints to reduce radiation in greenhouses and in plastic tunnels due to high natural radiation levels. More expensive structures with light reflecting screens (nets) are also used to reduce light intensity for high-income crops (Combrink, 2005).

Although, rose geranium plants grow well under full sunlight in summer, lower light intensities may improve plant growth and yield. Herbage, oil yield and quality decreased when rose geranium was grown in the shade of trees (Kaul *et al*., 1997). Dry matter yield and the accumulation of essential oils are closely related to photosynthesis, affecting shoot growth and the production of volatile oils of thyme (Letchamo & Xu, 1996). Duriyaprapan and Britten (1982) reported an increase in stem length and leaf area when Japanese mint (*Mentha arvensis)* was exposed to increased shading.

The average daily radiation in Bloemfontein during the summer months varies from 500 to 800 $MJm^{-2}$. The aim of this study was to evaluate the effects of shading and moisture stress on biomass yield, oil yield and oil quality of rose geranium.

## 7.2 Material and methods

### *7.2.1 Experimental site*

The trial was conducted at the West campus facility of the University of the Free State in Bloemfontein, South Africa, during spring and summer. The facility is located at latitude 29$^{o}$06'S and longitude 26$^{o}$18'E, and an altitude of 1351 m above sea level. An automated weather station, located at the facility was used to collect weather data throughout the trial period (Table 7.1).

**Table 7.1** Weather data for the 2009-2010 growing season

| **Month** | **Temperature** | | **Rain** | **RH** | **Daily radiation** |
|---|---|---|---|---|---|
| | **Max** | **Min** | **mm** | **%** | **($MJm^{-2}$)** |
| Sept | 25.9 | 7.0 | 0 | 32.5 | 832.5 |
| Oct | 26.5 | 12.2 | 97 | 55.9 | 679.3 |
| Nov | 28.7 | 13.6 | 9 | 53.1 | 761.8 |
| Dec | 32.8 | 15.0 | 58 | 46.5 | 794.5 |
| Jan | 28.4 | 16.8 | 133 | 68.9 | 677.5 |
| Feb | 29.9 | 16.8 | 112 | 42.7 | 727.1 |
| March | 28.8 | 15.1 | 86 | 66.0 | 679.2 |
| April | 25.9 | 13.6 | 21 | 71.3 | 603.0 |
| May | 21.6 | 6.7 | 17 | 62.9 | 669.7 |

### *7.2.2 Experimental procedure*

The basic experimental procedure followed in this study is described in chapter 3, only specific details for this experiment are detailed here.

A completely randomized block experimental design with a split-plot experiment layout was used. Shading treatments (0, 20, 40, 60 and 80%) were allocated to main plots, while moisture stress treatments (0 and -0.15 MPa of osmotic pressure) were applied to the subplots. All treatments were replicated three times.

Black shade netting (Knittex®) of an appropriate shade rating was used to provide the required shade treatments. The -0.15 MPa was induced by adding 100 g $L^{-1}$ of polyethylene glycol (PEG 6000) to the nutrient solution following the procedure described by Michel and Kaufmann (1973).

Mini tunnels (3 m x 0.9 m x 0.5 m) were constructed by covering metal frames with appropriate shade netting. These frames were placed 4 m apart in order to avoid shading other treatments. Each main plot consisted of six polyethylene window boxes (Versila®) 40 cm x 15 cm x 15 cm, each containing three geranium plants (10-14 cm tall) planted 13 cm apart in sterile coco-peat.

The containers were kept in a greenhouse for two months to allow the cuttings to establish prior being transferred to the mini tunnels. After four months of growth using a standard nutrient solution, the two moisture stress treatments were initiated on the sub-plots. Each sub-plot consisted of three containers and received either standard nutrient solution or the osmotically adjusted solution. Plants were harvested two months after being exposed to the moisture stress treatments.

The concentration of cations and anions in the solution, together with that provided by the water used in making up the solutions are presented in Table 7.2. Fresh solution was mixed every two weeks.

Two irrigation cycles of one hour each were scheduled during each day, at 08:00 and 16:00, during which sufficient nutrient solution was applied to allow 20% drainage in order to prevent the buildup of salts in the medium.

**Table 7.2** Macro nutrient compositions used in the nutrient solution

| Ions | Concentration (meq $L^{-1}$) |
|---|---|
| Nutrient solution | |
| *Cations* | |
| $NH_4^+$ | 1.00 |
| $K^+$ | 5.50 |
| $Ca^{2+}$ | 6.50 |
| $Mg^{2+}$ | 2.50 |
| *Anions* | |
| $NO_3^-$ | 12.00 |
| $H_2PO_4^-$ | 0.89 |
| $SO_4^{2-}$ | 2.05 |
| Water | |
| $Na^+$ | 0.44 |
| $Cl^-$ | 0.21 |
| $HCO_3^-$ | 0.40 |

**Data collection**

The number of branches per plant, plant height, branches: height ratio, foliar fresh mass (FM) and foliar dry mass (DM) were determined after harvesting. Foliar dry mass was determined by drying of the harvested material at 68°C for 72 hours.

Relative water content (RWC) was measured a week before the termination of the experiment using five leaves from each of five randomly selected plants in each sub-plot. Relative water content was determined using the procedure described by Roger (2001); Barr and Weatherley (1962).

$$RWC\% = \frac{\text{Leaf FM} - \text{Leaf DM}}{\text{Sat M} - \text{Leaf DM}} \times 100$$

Where: RWC% = relative water content, leaf FM = fresh mass of leaves, leaf DM= dry mass of leaves, Sat M = saturation mass of leaves.

A leaf sample collected from the 4th leaf below the shoot tip of the main stem was used for proline analyses using the procedure described by Bates *et al.* (1973). The concentration of proline was determined from a standard curve and was calculated on a fresh mass basis [µmol proline (g FM)$^{-1}$]

Rose geranium oil was extracted for one hour in a customised 5 kg test distiller manufactured by PA Pretorius (Motsa *et al.,* 2006). Oil quality was determined using an Aligent GC (FID) model 6890N gas chromatography (GC) fitted with a 30 m x 0.25 mm DB-5 fused silica capillary column and film thickness of 0.25 µm (Adams, 2004).

The number of oil glands on the abaxial and adaxial leaf surface of plants exposed to shade treatments was determined using a scanning electron microscope. Pieces of leaf material (1 $cm^2$) collected from the leaf tip were used. These were immediately fixed in gluderaldehyde (3% w/v), rinsed in a phosphate buffer (0.1 M, pH 7) and post fixed for 1-2 hours with osmium tetroxide (1% w/v) after being washed several times with distilled water. The leaf samples were then dehydrated in ethanol at increasing concentrations (30%, 50%, 70%, 95% and 100% w/v). Dehydration in 30%, 50%, 70% and 95% ethanol series was carried out twice for 10 minutes each, while dehydration in the 100% ethanol was done twice for a period of one hour each. The dehydrated samples were dried in a semi automatic critical point dryer apparatus (Samdri-795, tousimis, Rockville, Maryland, USA). Samples were then mounted on a metal stub using a Pratley quick seal glue (RSA). A vacuum coating spatter unit (Polaron E5200C, Watford, England) was used to gold coat these stubs. The samples were observed under a JSM 6400 scanning electron microscope (JEOL, Tokyo, Japan) using a 100x magnification to observe the oil glands on the leaves (Werker *et al.,* 1993).

## 7.3 Results and discussion

*Plant parameters*

Plant parameters were not affected by interactions between shading and moisture stress levels. Main factors had significant effects on plant height, number of branches, B:H ratio, DM, and FM.

Plants grown under 80% shading were the tallest, significantly taller ($P$=0.001) than those grown at the 0 and 20% shade treatments, but not significantly different from those at 40 and 60% shade (Fig. 7.1). Plants at both the 40 and 60% levels of shade were significantly taller than those grown in direct sunlight. The greatest number of branches was produced by plants grown under 40% shade cloth, significantly (*P*=0.002) more than at any other treatment. In Figs 7.2 and 7.3 it can be seen that the low light intensity induced by 80% shading resulted in significantly (*P*=0.0005) fewer branches than formed at higher light intensities. As the level of shading was increased from zero to 40% the B:H ratio was not significantly affected. However, by increasing the shading levels to 60% and 80%, the B:H ratio decreased significantly (Fig. 7.3).

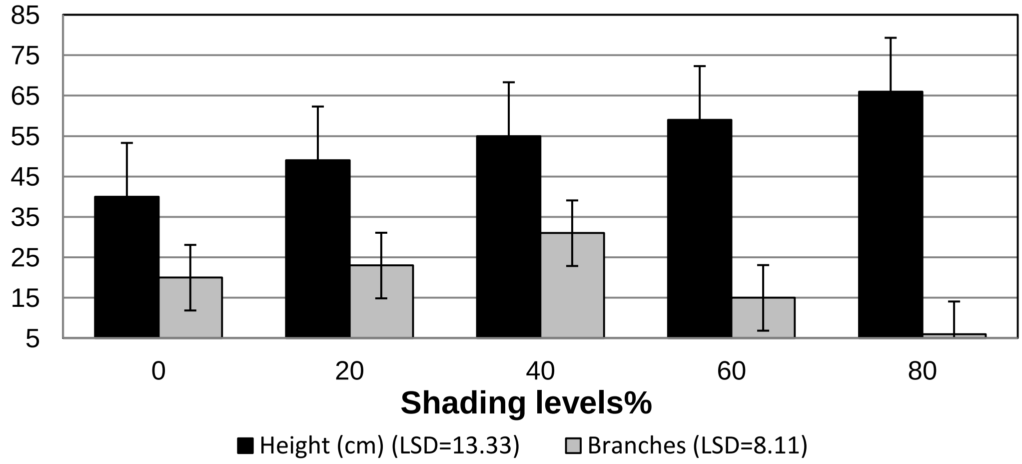



**Fig. 7.1** Shading affects the plant height and the number of branches per plant of hydroponically grown rose geranium.

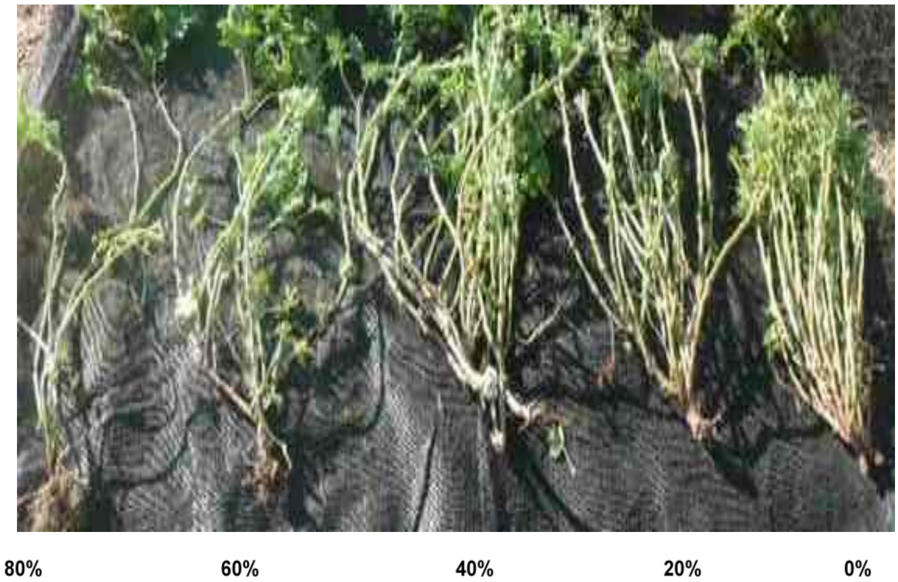



**Fig. 7.2** Effects of shading on the number of branches of hydroponically grown rose geranium.

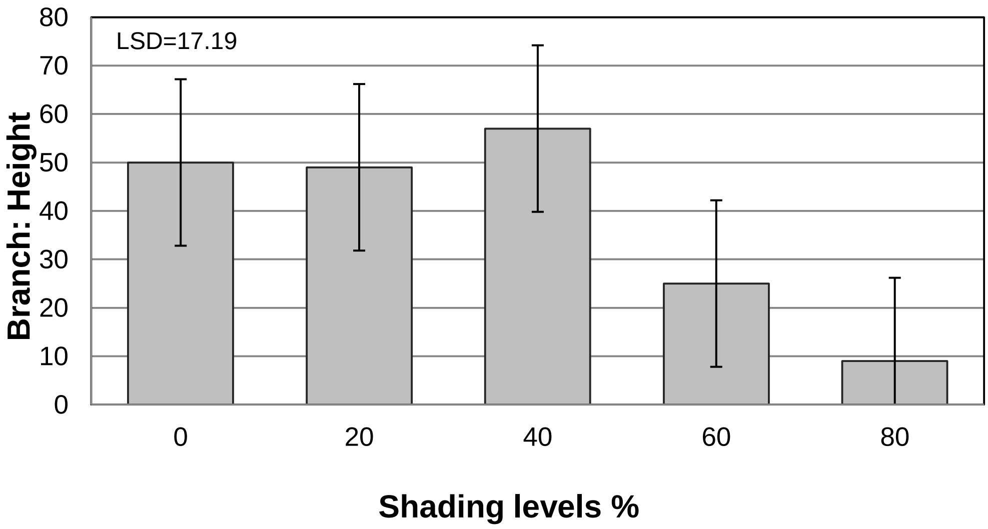



**Fig. 7.3** Effect of shading on the branch:height (B:H) ratio of hydroponically grown rose geranium.

Fresh mass of plants grown under 40% shade net was significantly greater than that produced at any other treatment (Fig. 7.4). Dry mass, on the other hand, showed no significant differences between the 0, 20 and 40% shade treatments, but a significant reduction occurred at the 60% shade treatment (Fig. 7.4).

**Table 7.3** The effects of moisture stress on foliar fresh mass (FM), relative water content (RWC) and proline content of hydroponically grown rose geranium

| Stress levels (MPa of osmotic pressure) | Foliar-FM (g plant$^{-1}$) | Relative Water Content | Proline content [μmol (g FM)$^{-1}$] |
|---|---|---|---|
| **0** | 418.67±187 | 75.06±10.50 | 0.09±0.03 |
| **-0.15** | 334.07±202 | 73.69±11.06 | 0.25±0.11 |
| **$LSD_{T(0.05)}$** | **83.94** | **ns** | **0.045** |
| **CV%** | **2.22** | **2.22** | **2.26** |

$LSD_{T(0.05)}$=least significant difference at 5% level of significance

ns not significant at $P<0.05$

± standard deviation

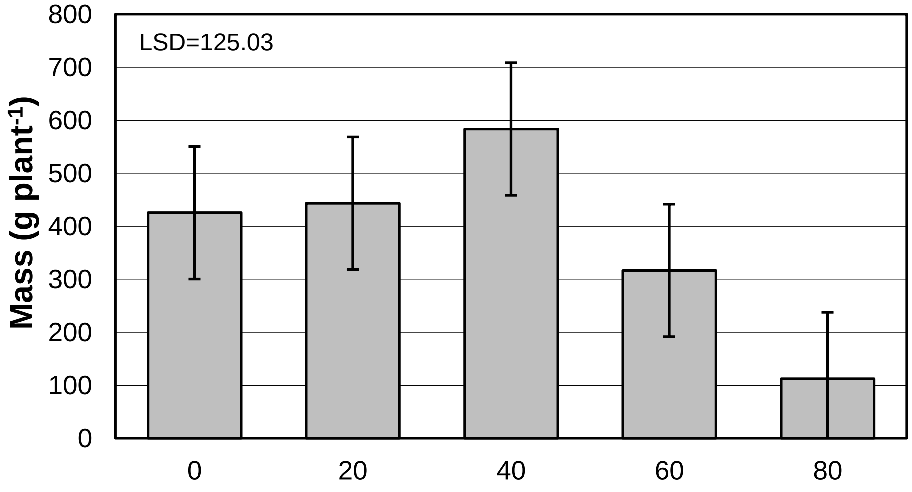
CV% coefficient of variation

Plants with weak stems and few branches were produced at the 80% shade level (Fig. 7.1). The production of branches is determined by various environmental factors such as light (quality and quantity) and temperature. Plants absorb red light and transmit most of the far-red light reducing the ratio between red and far red light. A reduction of the red to far red ratio induces shade avoidance by plants, resulting in an increase in height and a reduction in branching. The transport of phytochrome auxin from the shoot apex through polar auxin transport suppresses outgrowth of lower branches (Krishnareddy & Finlayson, 2010). As was found in this study with rose geranium, Duriyaprapan and Britten (1982) also reported that Japanese mint (*Mentha arvensis)* had longer stems when shading levels were increased.

A highly significant ($P$=0.0002) reduction in FM (Table 7.3) was found with no significant effect of moisture stress on dry matter yield. As moisture stress was only applied during the final months of the six month growing period, it is possible that the foliage of stressed plants contained less water, with a resultant decrease in FM, without a concomitant decrease in DM. Plant leaves develop thicker upper palisade, spongy parenchyma, a thicker lower epidermis and densely spaced trichomes where plants are subjected to droughts (Ennajeh *et al.*, 2010). Similar results were reported in previous studies conducted on rose geranium and thyme by Kaul *et al.* (1997) and Letchamo and Xu (1996).

In order to ensure high herbage yields, water stress should be avoided as was reported by Eiasu *et al.* (2009), Eiasu *et al.* (2008) and Rajeswara Rao *et al.* (1996). The relative water content (RWC) is a useful indicator of the water balance in plants because it expresses the absolute amount of water needed to reach full saturation (González & González-Vilar, 2003; Sinclair & Ludlow, 1985). The RWC measured in this study was not significantly affected by shading or moisture stress (Table 7.3)

a) Fresh mass




b) Dry mass

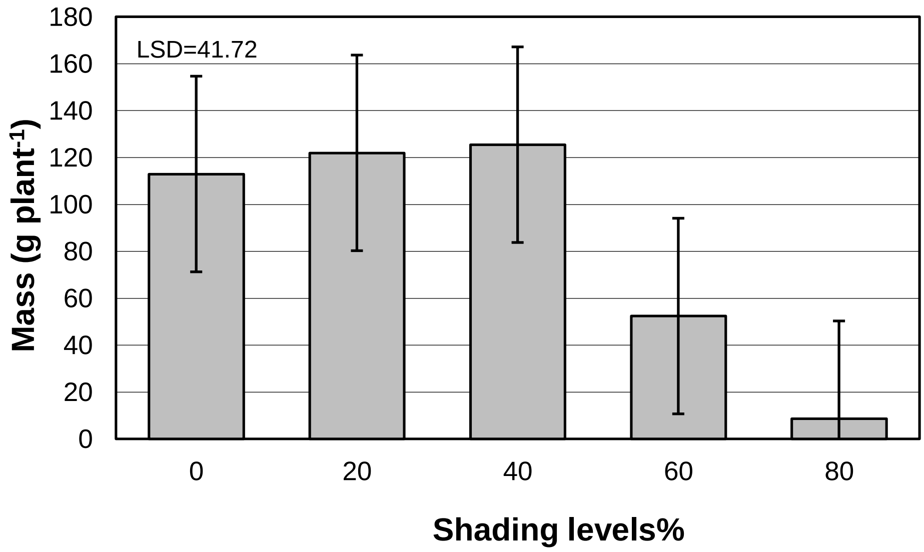



**Fig. 7.4** Effects of shading on the foliar mass of hydroponically cultivated rose geranium.

Shading had a highly significant effect ($P$=0.0001) on petiole length with a positive linear relationship ($r^2$=0.75) between petiole length and increasing shade percentages. Petioles from plants at all levels of shading were significantly longer than those grown in full sunlight (Fig. 7.5). Similar results were reported by González and Gioneli (2004) on *Convolvulus* (mallow bindweeds). Longer petioles increase mechanical strength to carry the leaves. A large number of smaller cells have a high density that provides rigidity and strength to the plant, making the plant more resistant to breaking (Weijsched *et al.,* 2008; Anten *et al.,* 2005; Givnish, 2002).

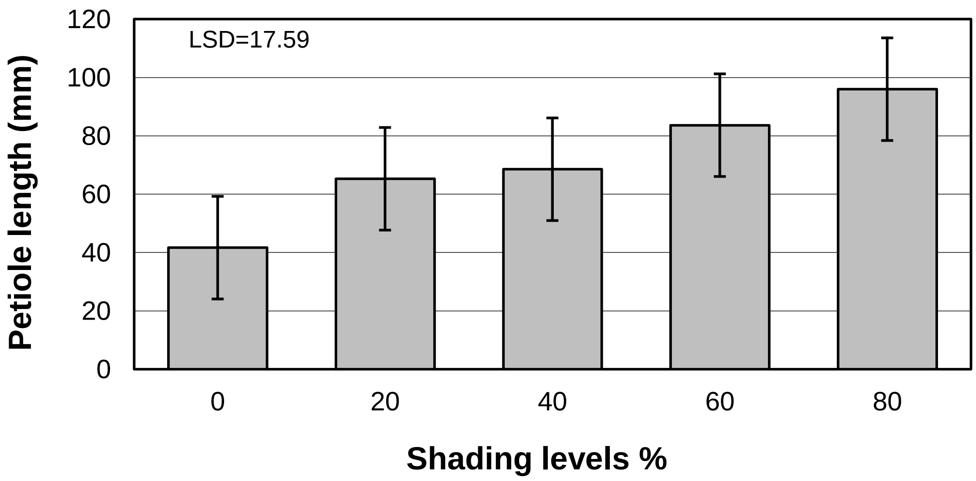



**Fig. 7.5** Effect of shading on the leaf petiole length of rose geranium.

Proline was significantly affected by shading (*P*=0.0001) and by the applied moisture stress (*P*=0.02). These effects are illustrated in Table 7.3 and Fig. 7.6. According to Ábraham *et al.* (2010) physiological status of a plant is disturbed as the accumulation of proline is triggered by biotic or abiotic stressors. In this study the exposure of rose geranium to full sunlight (Fig. 7.6) and to moisture stress (Table 7.3) induced the accumulation of proline. Plants grown at 40%, 60% and 80% shade had relatively low proline concentrations compared to plants that were exposed to full radiation and those receiving only 20% shading (Fig 7.6). In a study done by Lichtenthaler (1996) under similar conditions, the author ascribed this to an abiotic stress, due to the associated high light intensity or heat.

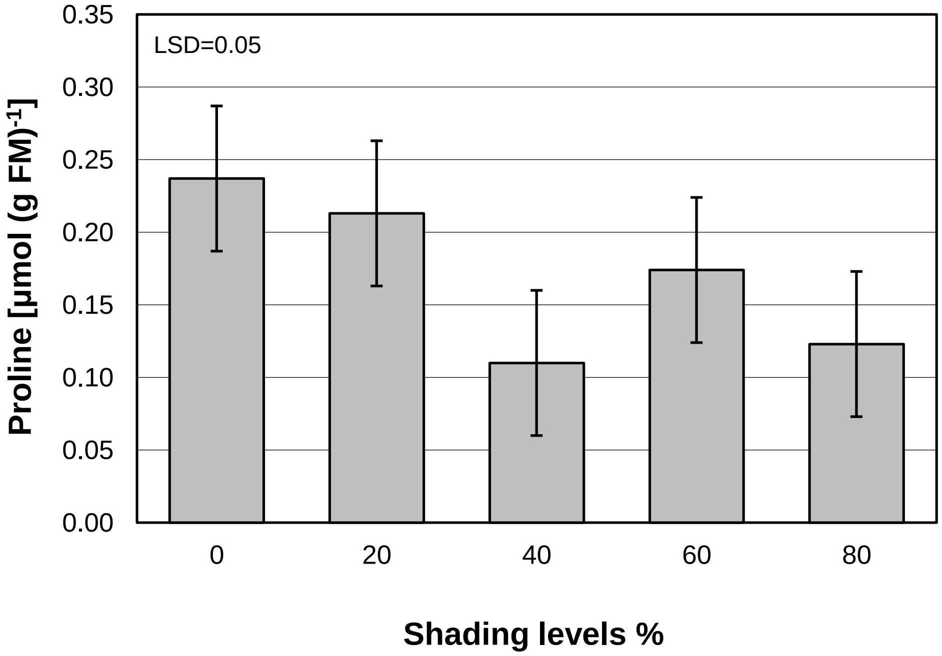



**Fig. 7.6** Effects of shading on proline content of rose geranium.

*Oil yield and oil quality*

Oil yield and oil quality are important parameters, as they directly affect the potential income that may be generated from a rose geranium crop. Motsa *et al.* (2006) ascribed higher oil yields to greater herbage yields. This was also the case in this study where a strong correlation ($r^2$=0.73) was found between FM and oil yield (Fig. 7.7). Since biomass production peaked at 40% shading (Fig. 7.4), an associated high oil yield was anticipated at this level, and was subsequently confirmed (Fig. 7.8).

The results of this study confirm results obtained by Eiasu *et al.* (2009), Motsa *et al.* (2006), Kumar *et al.* (2001) and Kaul *et al*. (1997) who found that high rose geranium herbage yield also produce high oil yield. Kaul *et al*. (1997) reported that herbage and oil yield of rose geranium cultivars (Bourbon, Kelkar and Algerian) decreased by 50-60% when grown under trees with 50 to 60% shade. They also found that partial shading resulted in slight changes in linalool, citronellol and geraniol contents (Kaul *et al*., 1997)

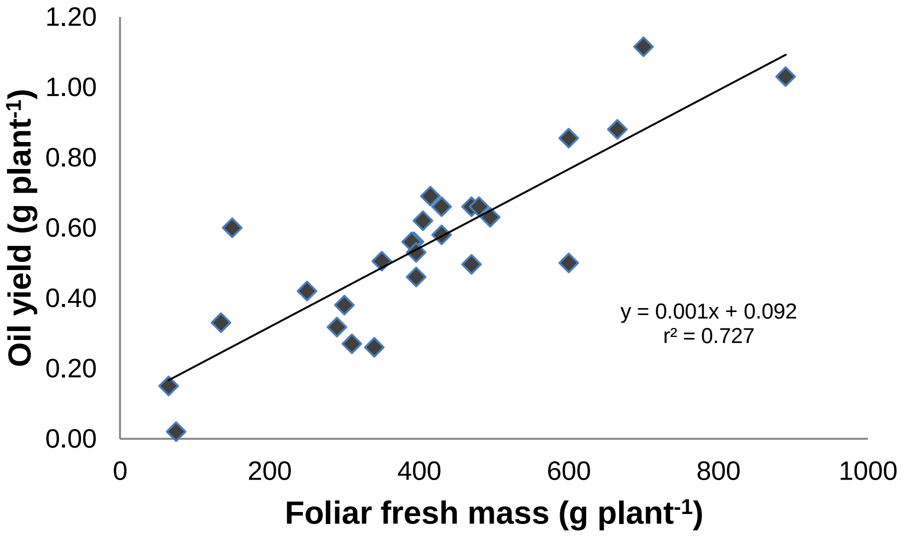



**Fig. 7.7** Relationship between foliar fresh mass and oil yield of rose geranium.

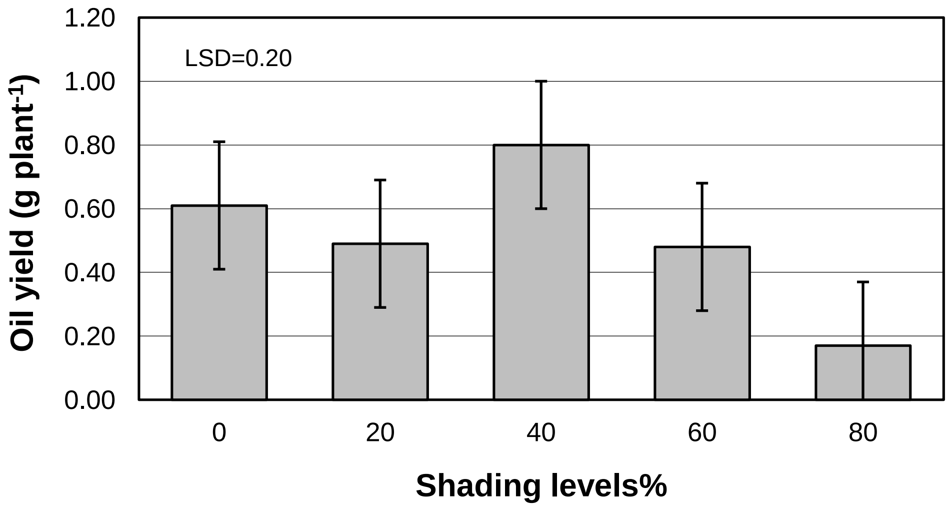



**Fig. 7.8** Effects of shading on oil yield of rose geranium.

During the microscopic study large and small glandular trichomes as well as non glandular trichomes were observed on the leaves. Small glandular trichomes had a columnar or nearly columnar shape with a slightly bent terminal (apical) cell pointing towards the leaf tip. As shading had no significant effect on the number of oil glands, this prompted a further investigation that showed a weak exponential relationship ($r^2$=0.37) between shading and the number of large glandular trichomes on the adaxial leaf surface (Figs 7.9 & 7.10).

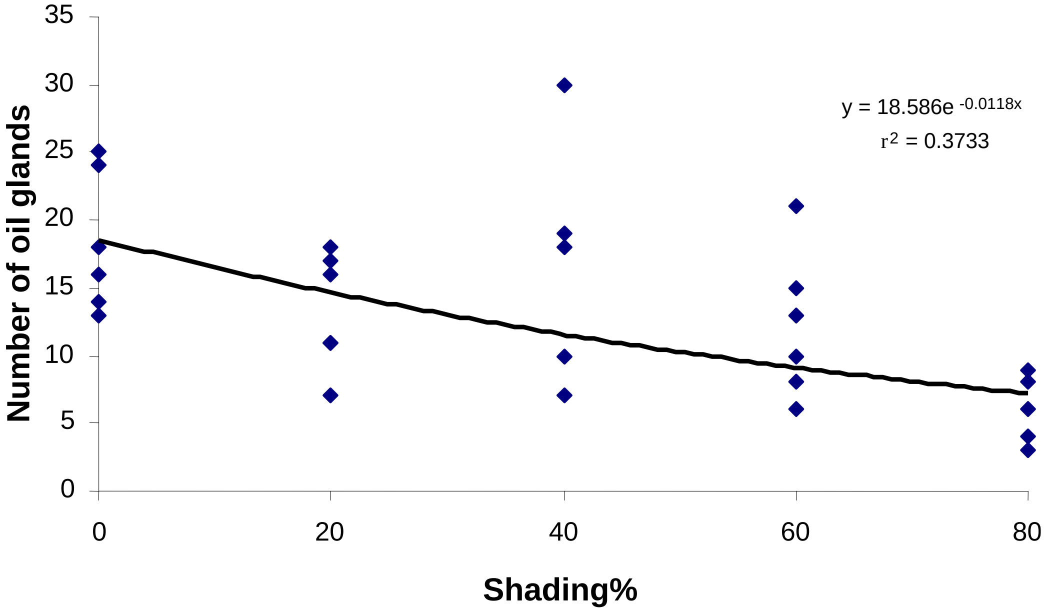



**Fig. 7.9** Exponential relationship between shading and the density of oil glands (oil glands $cm^{-2}$) on the adaxial leaf of rose geranium plants.

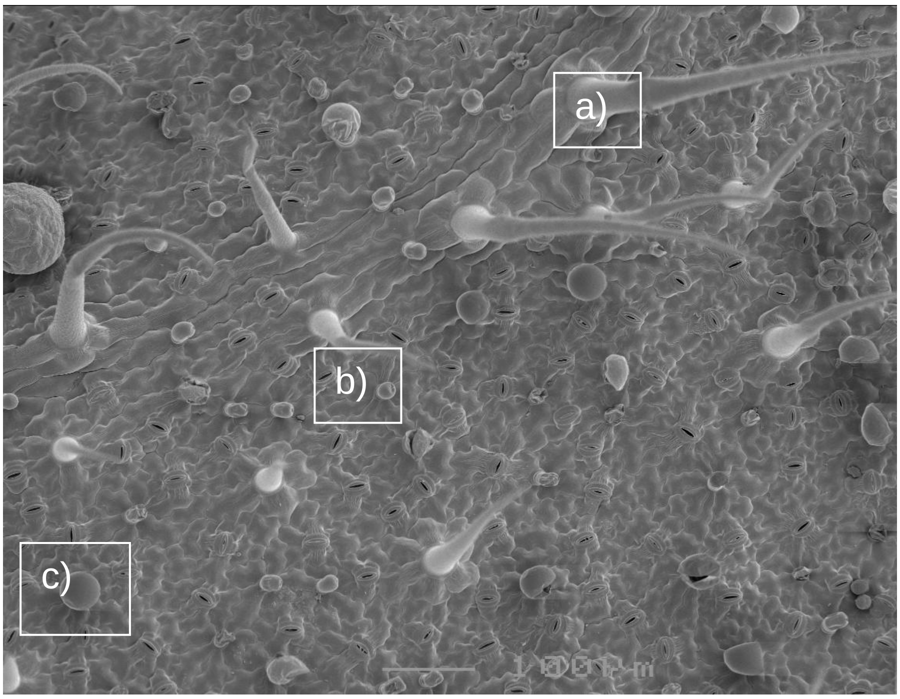



**Fig. 7.10** Trichomes on the adaxial side of a rose geranium leaf, observed with an electron microscope (100x magnifications) a) Non glandular trichomes b) Small glandular trichomes c) Large glandular trichomes.

An important oil quality parameter is the ratio between citronellol and geraniol (C:G). Where the C:G ratio exceeds 3 it signifies oil with a low odour quality (Saxena *et al.,* 2000). A good odour quality is obtained when the C:G ratio ranges between 1 and 3. The C:G ratio was not significantly affected by the levels of shading and moisture stress, but the ratio was relatively high, averaging at 3.06. Other oil quality parameters such as geraniol, *cis*-Roseoxide, geranylformate and guaia-6,9-diene were affected as shown in Figs 7.11 and 7. 12.

Geraniol is responsible for the rosy odour in rose geranium and other ornamentals. *Cis*-Roseoxide is an organic compound of the pyran and monoterpene class and is responsible for the highly volatile, floral-green top note in rose geranium oil. The results presented in Figs 7.11 and 7.12 show that both *cis*-Roseoxide ($P$=0.04) and geraniol ($P$=0.01) were significantly affected by shading. The concentration of geraniol significantly dropped at 80% shade while *cis*-Roseoxide significantly dropped at 40% shade (Figs 7.11 & 7.12).

Geranylformate ($P$=0.0081) and guaia-6,9-diene ($P$=0.004) did not differ significantly between shading levels up to the 60% shading level. Geranylformate content of geranium oil increased, while that of guaia-6,9-diene decreased at the 80% shade treatment (Fig 7.11). All of these substances were produced at acceptable concentrations for quality oil at the 40% shading level, which also gave the best oil yield. The oil obtained in this study can only be used in the manufacturing of soaps and lotions, since its C:G ratio exceeded three, so making it unsuitable for the perfume industry.

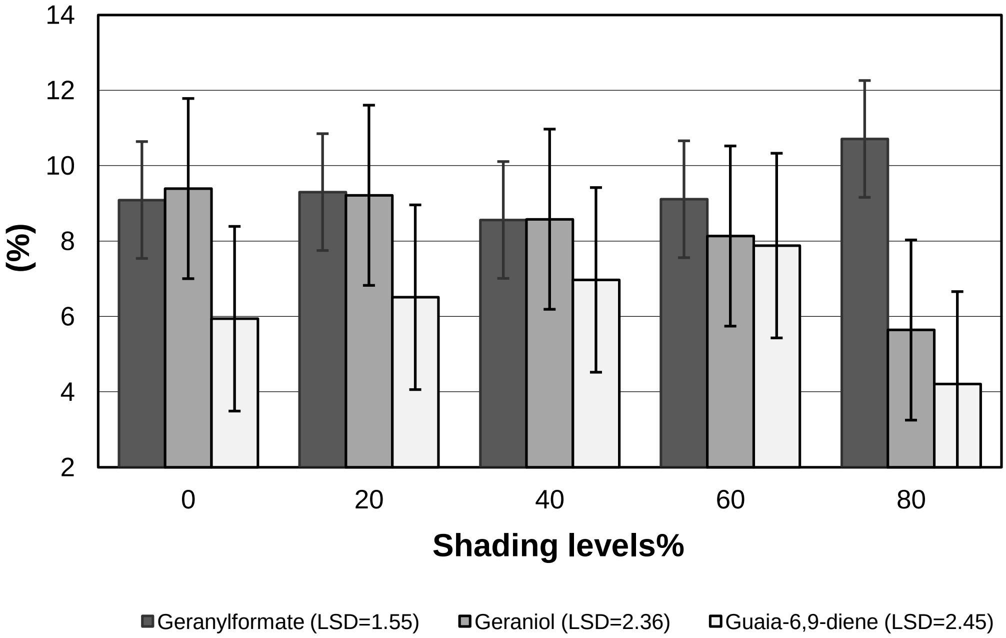



**Fig. 7.11** Effect of shading on geranylformate, geraniol and guaia-6,9-diene contents of rose geranium.

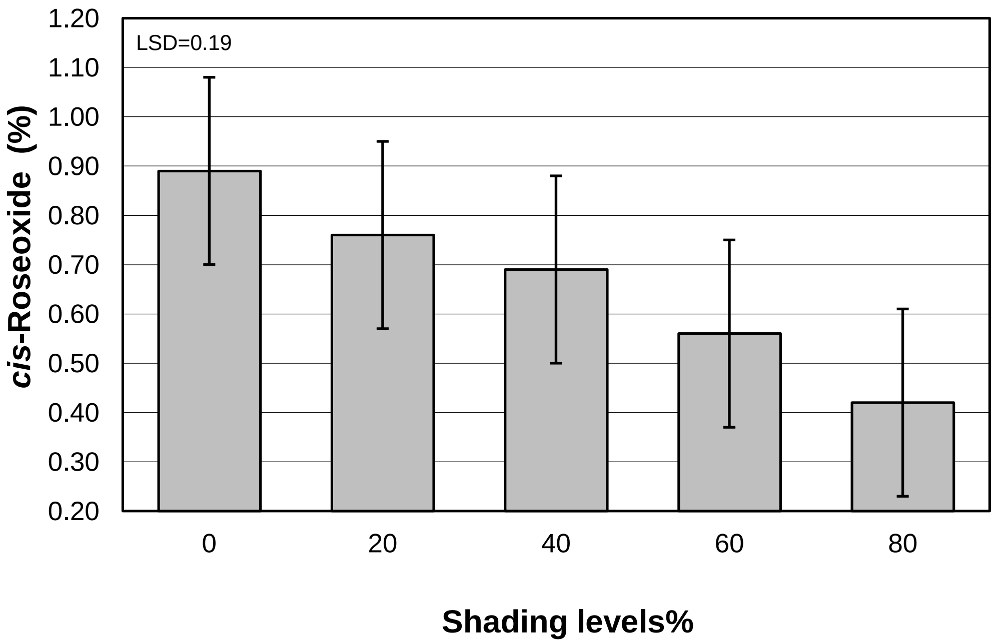



**Fig. 7.12** Effect of shading on the levels *cis*-Roseoxide in rose geranium oil.

## 7.4 Conclusion

Plant height, number of branches, foliar mass, the ratios of branches to plant height, oil yield and oil quality parameters were affected by both shading and moisture stress. Proline gave a clear indication when stress conditions occurred. Rose geranium should not be subjected to moisture stress or high light intensities. Rose geranium need to be grown with about 40% shade in summer.

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# CHAPTER 8

## CONCLUSION AND GENERAL RECOMMENDATIONS

The steadily growing international market for South African produced rose geranium oil has resulted in an increase in the area planted to 200 ha. It is a mammoth task for South African producers to meet the growing market demand, as only about 2.5 t of oil is produced annually at this stage.

The growing demand for rose geranium can be met by increasing yields through the use of better production systems, rather than by expanding the area under production. In order to increase production of rose geranium oil in South Africa, every effort has to be made to introduce the crop to potential producers and to develop the necessary agrotechnologies for commercial cultivation. Fertilizer application as well as moisture and climatic conditions all affect oil yield and quality.

Poor oil yields and quality of rose geranium in most production areas of South Africa could be ascribed to inconsistent, erratic and extreme climatic conditions, as well as insufficient knowledge on fertilizer management. Rather than by expanding the production area, it is possible to increase oil yield through the use of better production systems.

High value crops such as cucumbers and peppers are produced under controlled environmental conditions, where light intensity, temperature and humidity are controlled. Plants grown under protection are grown in soil or soil-less substrates and irrigated with balanced nutrient solutions. Two or more planting seasons can be achieved per year and more plants can be cultivated per unit area.

The main objective of this study was to determine the effects of different nutrient ratios, shading and moisture stress on oil yield and quality of hydroponically grown rose geranium. In order to do this, four trials were conducted during 2009 and 2010 growing seasons with the following specific objectives:

1) To evaluate the effect of phosphate on oil yield and quality of rose geranium, as well as an attempt to set standards for concentrations to be used in the nutrient solution of hydroponically grown plants.

2) To evaluate nitrate and ammonium as sources of nitrogen and to set standards for soilless production of rose geranium.

3) To determine the effects of sulphate levels in nutrient solutions on yield and quality of hydroponically grown, *Pelargonium graveolens* and to set standards for sulphate to be used in the nutrient solution.

4) To evaluate the effect of shading and moisture stress on yields and oil quality of hydroponically grown rose geranium.

In order to meet objectives 1, 2 and 3, trials were conducted in a greenhouse on the experimental farm of the University of the Free State in Bloemfontein. Plants were grown for four months using randomized complete block experimental designs with five replicates. The plants were planted in 5 L pots, filled with 2 mm sterile silica sand. Sand is useful for perennial crops such as rose geranium. For such crops organic substrates are avoided completely as decomposition cause's increases to the root zone alkalinity and may limit aeration.

In the first experiment, four concentrations of phosphate (0.1, 0.8, 1.50 and 2.20 meq $L^{-1}$) were evaluated. Oil yield of rose geranium increased with increased concentration of phosphate. Both guaia-6,9-diene content and the citronellol:geraniol ratio in the oil were better at the 2.20 meq $L^{-1}$ concentration indicating that oil quality could be improved by using relatively high phosphate concentrations. Foliar mass and oil yield of the material, mineral content and oil quality improved with increased concentrations of phosphorus. The phosphate requirement for hydroponically grown rose geranium seemed to be higher than the upper limit used on other hydroponically grown crops. The pH of the nutrient solution of 6.98 (±0.02) seemed to be too high for phosphate availability. Therefore, future studies should focus on the effect of pH levels when testing phosphate.

2) Four concentrations of nitrate (8, 10, 12 and 14 meq $L^{-1}$) and four concentrations of ammonium (0.0, 0.5, 1.0 and 1.5 meq $L^{-1}$) were evaluated, using two separate trials. Increased nitrate concentrations had a significant positive effect on plant height, number of branches, foliar dry mass, chlorophyll content of the leaves and

oil yield and quality. The best nitrate concentration for oil yield and quality was at 10 and 12 meq $L^{-1}$. Ammonium concentrations did not, however, have any significant effect on the measured agronomic parameters. Given that some oil quality parameters were affected by ammonium, it is evident that further investigation of the ratio of ammonium to nitrate is required for hydroponically grown rose geranium.

3) Four concentrations of sulphate (0.36, 1.90, 3.44 and 4.98 meq $L^{-1}$) were investigated. This study showed a significant effect of sulphate concentrations on the number of branches, plant height and branch:height (B:H) ratio and foliar dry mass, with most parameters peaking at 3.4 meq $L^{-1}$. Oil quality was not substantially affected by the various sulphate concentrations. It appears as though rose geranium can tolerate a wide range of sulphate concentrations with only minor effects on oil quality.

4) During spring and summer a study was conducted to evaluate the effects of shading and moisture stress on growth, oil yield and quality. A split plot experimental layout was used in a randomized complete block design, using 0%, 20%, 40%, 60% and 80% as shading treatments allocated to the main plots. The subplots were exposed to moisture stress levels of 0 and -0.15 MPa of osmotic pressure. The results of the study showed significant effects of shading on the number of branches, foliage fresh mass, foliage dry mass and the branch:height ratio due to shading. Most of these parameters peaked with 40% shade (black), where the best oil yield was also obtained. It, therefore, seems to be advisable to grow rose geranium at 40% shade.  Oil quality was not detrimentally affected at this level of shading. Moisture stress had a significant effect on foliar fresh mass (FM) and proline content of rose geranium. Stressed plants had less foliar fresh mass and the leaf proline content was higher.

After considering the effects of the different mineral levels on oil yield and quality, the following mineral composition is recommended for hydroponic production of rose geranium to provide the highest yield and oil quality:

- Nitrate concentration for oil yield is between 10 to 12 meq $L^{-1}$ and 8 and 14 meq $L^{-1}$ concentration for oil quality only.

- Phosphate concentration for oil yield and oil quality is 2.2 meq $L^{-1}$ (at pH7).
- Sulphate concentration for oil yield is between 1.9 and 3.4 meq $L^{-1}$.
- Ammonium concentration for both oil yield and quality is 1.0 meq $L^{-1}$.

The recommended macro nutrient composition for oil yield and oil quality of rose geranium is described in Table 8.1. The pH and the EC of the nutrient solution were at 6.2±0.7 and 1.6 mS $cm^{-1}$, respectively.

**Table 8.1** Recommended macro nutrient compositions to be used to fertigate rose geranium plants grown hydroponically

| **Parameter** | **Macro nutrients (meq $L^{-1}$)** | | | | | | |
|---|---|---|---|---|---|---|---|
| | **$NH_4^+$** | **$K^+$** | **$Ca^{2+}$** | **$Mg^{2+}$** | **$NO_3^-$** | **$H_2PO_4^-$** | **$SO_4^{2-}$** |
| **Oil yield** | 1 | 5.94 | 7.0 | 2.7 | 11 | 2.2 | 3.44 |
| **Oil quality** | 1 | 4.9 | 5.64 | 2.1 | 8 | 2.2 | 3.44 |

It is also recommended that rose geranium should not be subjected to moisture stress or high light intensities. Oil yield and quality can be optimized by protecting rose geranium against high light intensities during summer. Therefore, 40% shade is recommended as it provides enough protection against radiation on rose geranium during hot summer months.

The results obtained in this study present a solid foundation for future studies on fertilizer management that are aimed at developing comprehensive fertilizer programs to be used in hydroponically grown rose geranium. Future studies should focus on the following:

- The effect of pH and EC levels when testing for phosphate and sulphate requirements of rose geranium.
- The effect of the ratio of ammonium to nitrate is required for hydroponically grown rose geranium.
- The effect mycorrhizal fungi when testing for phosphate, sulphate, ammonium and nitrate.
- The effect of Ca and Mg ratios on the uptake of nitrogen and phosphorus.

- The effect of coloured shade cloth (net) on rose geranium.
- To investigate the economic viability and financial implications of growing rose geranium commercially using hydroponics as an alternative to open land farming.