THE EFFECT OF POTASSIUM AND WATER QUALITY ON THE YIELD AND OIL QUALITY OF ROSE GERANIUM (*Pelargonium graveolens* L.)

by

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DECLARATION

I, **Zenzile Peter Khetsha** declare that this dissertation: **The effect of potassium and water quality on the yield and oil quality of rose geranium (*Pelargonium graveolens* L.)** submitted to the Central University of Technology, Free State in fulfilment of the requirements for the degree Magister Technologiae: Agriculture is my own independent work and I have not previously submitted this dissertation to obtain a qualification at another university. I further disclaim the copyright of this dissertation in favour of the Central University of Technology, Free State.

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ABSTRACT

The main objective of the study was to determine the effect of different potassium concentrations and water quality (salt) compared with the current scientifically accepted potassium threshold level and standardised water quality on the yield, oil composition and leaf morphology of rose geranium (*Pelargonium graveolens* L.) when grown in different potting-bag sizes and root media under temperature controlled condition.

To achieve this objective, two trials were conducted. The first experiment evaluated potassium concentrations at 1.3, 3.3, 5.3 and 7.3 mmol L^{-1} and potting-bag size of 5 and 10 L. Treatments were arranged in a randomised complete block design assigned in a split plot layout. The main plots consisted of potassium concentration and the potting-bag sizes were allocated to sub-plots. Plant height, potassium content, linalool, geraniol, geranyl formate and the citronellol to geraniol ratio (C:G) were affected by potassium. Plant height, number of branches, the branch to height ratio (B:H), foliar fresh mass (FFM) and oil yield were significantly increased when 5 L potting bags were used. Plant foliar mass was significantly increased by the interaction between 5.3 mmol K L^{-1} and 5 L potting bags. In the second experiment salt levels applied at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} and root media (sand and sawdust) were evaluated. A split plot experimental layout was also used in this trial, with the salt levels allocated to the main plots. The sub-plots were allocated to the root medium. High salt level of 4.0 mS cm^{-1} reduced the number of leaves, plant height, number of branches, B:H ratio, leaf area, chlorophyll content and foliar fresh mass significantly. The number of leaves, leaf area and FFM were significantly increased where sawdust was used. Time of the day significantly affected stomatal conductance, and the opening of most stomata occurred at 10:00. Geranyl formate and the C:G ratio were significantly affected by salt at 4.0 mS cm^{-1}.

Salt induced the development of capitates trichomes. The abaxial leaf surface had a higher number of trichomes than the adaxial leaf surface. A strong polynomial (r^2=0.97) relationship was found between capitates trichomes and salt levels. High densities of capitates trichomes were found at high salt level of 4.0 mS cm^{-1}. Although the development of asciiform trichomes was induced, it was at an insignificant level. Trichome densities are therefore not affected by salt. It was therefore concluded that the

application of 5.3 mmol K L^{-1} concentration and the use of 5 L potting bags improves the yield and oil quality of rose geranium. It was evident from this study that rose geranium might have some degree of tolerance to salt. It was therefore concluded that rose geranium is a moderately salt-sensitive crop.

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GENERAL INTRODUCTION

General Introduction

1.1 Motivation and background to the study

Rose geranium is indigenous to South Africa and is produced in the Mpumalanga Lowveld, KwaZulu-Natal, Western Cape, Limpopo, Gauteng, to North West and Eastern Cape Province and alsowhere growing conditions are suitable (DAFF 2012). New production techniques of rose geranium are being considered to increase its yield and oil quality (Sedibe and Allemann 2012). Production in South Africa is low owing to nutrition, which seem to have an impact on oil quality. Rose geranium oil is used globally in the aromatherapy, pharmaceutical, food and perfume industries (DAFF 2009; SEDA 2009).

Sedibe and Allemann (2012) and Eiasu (2009) reported that the yield and oil composition of rose geranium is affected by fertilizer application and environmental factors. Therefore, an integrated nutrient solution management regime is necessary to ensure proper growth and normal development of rose geranium to improve the yield and oil quality.

Potassium is the third most important macro-element after nitrogen and phosphorus (Dibb 1998). Potassium is involved in many activities of plant growth and the quality of crops and it also helps to induce stress tolerance in plants (Wen Xu *et al*. 2011). Potassium acts as a catalyst and is involved in the activation of more than 60 enzymatic reactions. It is also involved in metabolic activities such as osmoregulation, water transport in the xylem, water assimilation, protein biosynthesis, osmotic adjustment, electrical neutralisation of anionic groups and control of cell membrane polarisation (Wen-Xu *et al*. 2011; Nguyen *et al*. 2010). Hence Singh and Ganesha-Rao (2009) and Singh (2008) observed that correct application of potassium increased the yield and total herbage of patchouli (*Pogostemon cablin* [Blanco] Benth.) and palmarosa (*Cymbopogon martini* [roxb.] Wats. Var. Motia burk) plants.

Water quality is associated with salinity and other chemical aspects, such as the pH, electrical conductivity and micronutrients (Combrink and Kempen 2011). Poor water

quality affects the balance between potassium, sodium, magnesium and calcium and this also hinders the uptake and distribution of potassium and nitrogen (Combrink and Kempen 2011; El-Baz *et al*. 2003). Enzymatic activation processes such as osmoregulation, water assimilation, water transport and photosynthesis are affected by the reduced osmotic potential resulting from increased salinity levels and deficiency of potassium, magnesium and calcium (Wen Xu *et al*. 2011). Reduced osmotic potential also influences the ratio between roots to shoots, leaf growth, photosynthesis, root volume and oil yield and it delays maturity (El-Baz *et al*. 2003).

Plant growth and biomass yield of sage (*Salvia officinalis* L.), peppermint (*Mentha piperita* var. officinalis), rosemary (*Rosmarinus officinalis* L.) and coriander (*Coriandrum sativu* L.) decreased with increased salinity levels (Neffati *et al*. 2011; Hendawy and Khalid 2005; Solinas and Deiana 1996; El-Fadl *et al*. 1990). On the contrary, increased salt level favoured high oil quality in basil (*Ocimum basilicum* L.), and sage (Attia *et al*. 2010; Taarit *et al.* 2010).

1.2 Justification for the study

The current local oil production of rose geranium less than five tons ha^{-1} does not meet the global demand due to erratic climate; therefore production under climate controlled environment should be an alternative for mass production to increase oil yield. Moreover, nutrition, water quality and other environmental factors affect the production of rose geranium. Rose geranium is a herbaceous crop and yield and biosynthesis of oil is affected by the mass of plant foliage, therefore the main focus in its production should be to increase the number of leaves, branches and the amount of herbage mass per unit area, as this is where most of the trichomes are located. Moreover, oil-producing trichomes are located on the green parts of the plant, especially on the leaves, stems and flowers. Most studies on fertilizer focused on nitrogen because of its effect on total biomass (Sedibe 2012; Araya *et al*. 2006). There is a high correlation between biomass and oil yield. The application of potassium, water quality, potting-bag size and type of root medium need to be studied further.

1.3 Problem statement of the study

Poor oil yield and quality of rose geranium is affected by fertilizer application and environmental factors. Little is known about the effect of potassium, water quality, potting-bag size and root-media on yield and oil quality of rose geranium.

1.4. Hypothesis

Since potassium is involved in the activation of metabolic reaction and its uptake is affected by the salinity content, using soil less root media and potting bag; it is therefore hypothesised that by adding potassium, selecting root media and potting bags of different sizes and managing salt levels of the nutrient solution may improve yield and oil composition of rose geranium.

1.5 Objectives

1.5.1 Main objective

The main objective of the study was to determine the effect of different potassium concentrations and water quality compared with the current scientifically accepted potassium threshold level recommended for other ornamentals and standardised water quality on the yield, oil composition and leaf morphology of rose geranium (*Pelargonium graveolens* L.) when grown in different potting-bag sizes and root media under temperature controlled condition.

1.5.2 Specific objectives

The specific objective of this study was;

1.5.2.1 To determine the effect of potassium concentrations (1.30, 3.30, 5.30 and 7.30 mmol L^{-1}) on yield and oil composition of rose geranium (*Pelargonium graveolens* L.) grown in 5 L and 10 L potting bags.

1.5.2.2 To determine the effect of water quality (NaCl) concentrations (1.68, 2.40, 3.20 and 4.0 mS cm^{-1}) on oil yield and oil composition of rose geranium (*Pelargonium graveolens* L.) grown in sand and sawdust growth media.

1.5.2.3 To determine the effect of salinity (NaCl) (1.68, 2.40, 3.20 and 4.0 mS cm^{-1}) on leaf morphology of rose geranium (*Pelargonium graveolens* L.).

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CHAPTER 2

LITERATURE REVIEW

Literature review

2.1 Introduction

Rose geranium is native to South Africa and was developed from a cross between *Pelargonium radens* (L.) and *Pelargonium capitatum* (L.). The Bourbon cultivar of rose geranium is popular in South Africa and across the world because of high-quality oil traits (Motsa *et al*. 2006). Majority of *Pelargonium* species originated in South Africa (RSA) and were introduced to Britain and Netherlands through trade for medicinal, cosmetic and aromatherapy use (Sedibe and Allemann 2012).

Leading countries in the production of rose geranium are Algeria, China, France, Morocco, Madagascar, Spain and Russia (DAFF 2012). Rose geranium oil is highly exported to China, United Kingdom (UK), Japan, India, Germany, United States of America (USA) and European Union (EU) (DAFF 2009; SEDA 2009). South Africa's current main competitors are Egypt, India and China (DAFF 2012).

2.2 Descriptions of rose geranium

Rose geranium belongs to the Geraniaceae family (Weiss 1997). It is characterised by large, bushy, upright and branching shrubs that grow up to 1.3 m (Motsa *et al*. 2006). Its leaves are alternate, opposite, and compound; they have a deep lobed fan shape with five to seven divided lobes and usually with palmate venation and stupules (Motsa *et al*. 2006). The stem has a multiple soft green to grey colour and is covered with various types of bristles (long and fine); some plants may have short bristles. The roots grow to a depth of 30 cm and spread extensively in the soil. In its natural habitat, rose geranium starts flowering in spring and continue flowering until the end of summer (Gupta *et al*. 2001). It has a complete flower with a stamen, single pistil and style. The plant produces greenish oil with a pleasant fragrance from the leaves, stem and flower (Demarne 2002).

Rose geranium population of 30 000 to 60 000 plants ha^{-1} can yield a turnover of 15 to 50 tons of fresh foliage. This can yield between 5 and 22.5 kg ha^{-1} of essential oil (DAFF 2009).

2.3 Marketing and uses of rose geranium oil

The annual global demand for rose geranium oil is approximately 600 tons annually and it is expected to increase between 20 to 25 tons globally in future (SEDA 2009; Demarne 2002). Egypt is the only African country that produces more than 55 tons of oil per year (Motsa *et al*. 2006). The supply of rose geranium oil in the global market is relatively small compared to demand. South Africa produces less than five tons to the market because of erratic climatic conditions and most South African oil is absorbed by the local markets (Sedibe and Allemann 2012; Eiasu 2009).

Rose geranium oil is used in the perfumery industry for production of expensive perfumes such as Polo Blue by Ralph Lauren, Kouros by Yves Saint Laurent and Paul Smith Women by Paul Smith (Gomes *et al*. 2006). The oil is also used as a flavouring agent in alcohol, food and soft drinks. It helps to reduce blood circulation related problems (Lawless 1995). In the respiratory system, the oil treats sore throats and tonsillitis (Lawless 1995). In the genito-urinary and endocrine systems, it reduces the secretions of the adrenocortical glands and premenstrual syndrome fluids. The aroma of rose geranium reduces nervous tension, stress and neuralgia (Lawless 1995).

2.4 General effect of minerals on plants

Rose geranium yield depends on correct use of fertilizer application rates and environmental factors (Sedibe and Allemann 2012). The most important plant nutrients for optimum growth are nitrogen, phosphorus, potassium, calcium, sulphur and magnesium, as well as the micro-nutrients (Nguyen *et al*. 2010). There are no cost-effective fertilizer substitutes for potassium, nitrogen and phosphorus. Sedibe (2012) and Araya *et al*. (2006) studied the effect of nitrogen on rose geranium; however potassium and other cations such as calcium and magnesium were not studied. Moreover, potassium is the third most important macronutrient and is commonly

available in the form of soluble potash for the use in agriculture. Potassium is applied with other minerals such as nitrogen and soluble phosphorus (Ober 2006). Potassium is involved in various metabolic activities involving enzyme activities. It activates more than 60 enzyme activities, which activate water assimilation and transportation (Wen-Xu *et al*. 2011). Potassium is also involved in protein synthesis; photosynthesis, osmotic adjustment and ionic balance (Tounekti *et al*. 2010). Potassium controls the osmoregulation and electrical neutralisation of anion groups and controls the polarisation of cell membrane (Very and Sentenac 2003). Furthermore, it alters the plant water content, chlorophyll and photosynthetic rate (Wen-Xu *et al*. 2011).

Yield of some essential oil plants was increased by potassium; this was reported by Puttanna *et al*. (2010) for rosemary whereby application of potassium at 100 kg ha^{-1} with nitrogen at 150 kg ha^{-1} yielded 350 L ha^{-1} $year^{-1}$ compared to the control. The oil yield and total herbage of patchouli (*Pogostemon cablin* [Blanco] Benth.) was increased by 29.9% and 27.0% potassium compared to the control (Singh and Ganesha-Rao 2009). The application of 41.5 kg potassium ha^{-1} in the form of muriate potash increased the herbage and oil yield of patchouli to 23.88 t ha^{-1} and 166.04 kg ha^{-1}, respectively (Singh and Ganesha-Rao 2009). In addition, Singh (2008) reported that the herbage and oil yield of palmarosa (*Cymbopogon martini* [roxb.] wats. Var. motia burk) increased by 23.7% when muriate of potash was applied at a rate of 123.0 kg ha^{-1}. The foliar mass, shoot height and root length of *Houtuynia cordata* (Thunb.) were increased by increasing nutrient solution potassium to 1.28 mM (Wen-Xu *et al*. 2011).

Apart from potassium Sedibe (2012) and Araya *et al*. (2006) reported that nitrogen, sulphate and phosphate affect yield and oil composition of rose geranium. Nitrogen is a major nutrient; it accounts for about 80% of the total mineral nutrients absorbed by plants (Maathuis 2009). Phosphorus in the soil is available to plants as phosphate (PO_4) and about 90% of phosphorus is in fixed form ($CaPO_4$). Plants use phosphorus for cellular metabolism, embryonic development of seeds, germination and seedling growth. Calcium is an important intermediate macronutrient required by plants. It plays a vital role in processes that preserve the structural and functional integrity of plant membranes by stabilising the cell wall. Soils contain inorganic and organic forms of

sulphur. Sulphate (SO_4) is the only form in which sulphur is available to plants. Toxicity of sulphate to plants was reported under saline conditions by Maathuis (2009). Sulphur increases nitrogen uptake and this was observed by Eman and El-Ashry (2009). Magnesium in the soil usually varies from 0.05 to 0.5% and plants need only 0.5 mM. While the total magnesium application level varies between 0.3 and 1.0%. Magnesium is active in plants as the central element of the chlorophyll molecule; it is a carrier of phosphorus within the plant, acts as an enzyme activator and is a constituent of many enzymes (Eman and El-Ashry 2009).

The availability of micronutrient elements depends on the optimum pH of the soil, as it is a natural binding site of organic and inorganic particles. The most important trace elements are iron, boron, zinc, molybdenum, copper and manganese. Their functions vary depending on the plant species (Combrink and Kempen 2011). Plants require trace elements in minute quantities and deficiencies do not significantly reduce yield and oil quality (Grattan and Grieve 1999).

2.5 General effect of water quality (salt) on plants

Most water resources in South Africa have high levels of sodium. Salt in the soil and water is caused by high concentrations of sodium and other elements such as calcium, magnesium, chlorine, sulphate and bicarbonates (Sedibe 2012). Salt leads to poor water and nutrient uptake by most plants. The suppression of these nutrients leads to imbalances in the ratios between sodium and potassium, sodium and calcium, calcium and magnesium, as well as the ratio between chlorine and nitrate (El-Baz *et al*. 2003).

Electrical conductivity (EC) is used to measure salinity; the international units used for EC are Siemens [milliSiemens (mS m^{-1}) and deciSiemens (dS m^{-1})]. Crops grown in greenhouse and those grown in the field differ according to the impact of salt (Sonneveld 2000). This is caused by high salinity causing ions in the soil and the substrates used (Sonneveld 2000). Salts affect the osmotic potential in the root zone and rhizosphere. The reduced osmotic potential results affect plant physiology and metabolic activities. It reduces root mass and length, root length and shoot ratio and it delays plant maturity and photosynthesis.

Salt tolerance differs according to plants species. Shannon and Grieve (1999) described 130 species that are tolerant to salt concentrations; however, information is still lacking on other crops. The responses of plants to salinity depend on the environmental interactions between the relative humidity, temperature, radiation and air pollution (Sedibe 2012). Moreover, high salt levels reduce growth, the number of leaves and root length (Sedibe 2012).

Salinity has favourable effects on some oil compound and it improves disease resistance of plants (Stutte 2006; Shannon and Grieve 1999). High salinity increased the oil yield of coriander (*Coriandrum sativum* L.) when NaCl was applied at at 50 to 75 mM. It resulted in a 53% and 55% oil yield increase compared to the 30% yield obtained in the control (Neffati *et al*. 2011). Linalool content of basil and coriander was increased by 57% and 45%, respectively at high salinity condition (Attia *et al*. 2010). Furthermore, 25 and 50 mM salinity levels increased yield by 18 and 43%, respectively, with a decrease in herbage yield at increased application (Neffati and Marzouk 2008). Improved oil quality was reported in sage (*Salvia officinalis* L.), where salinity increased oxygenated monotepernes by 48% (Taarit *et al*. 2010).

2.6 Cultivation requirements of rose geranium

2.6.1 Root media

Different root media are used in soil-less production of rose geranium. Substrates are selected based on cost, use and availability (Sedibe and Allemann 2012). Some root media are mixed to meet certain requirements. The most common advantage of using mixed root media is to enhance drainage and aeration. Root media must be able to hold moisture, enhance root-oxygen exchange and should provide an optimised ratio between nutrient elements in the root zone (Combrink 2005).

Sawdust, wood shavings and wood chips are used by most South African vegetable (Sedibe and Allemann 2012). These root media constitute a rather broad category of wood formed from sawmills and other wood industries (Ingram *et al*. 1993). This type of root media is only used once in a season to avoid accumulation of micro-organisms due

to decomposition (Sedibe and Allemann 2012). Sand and gravel are characterised by coarse-texture with particle size that range from 0.05 to 2 mm. Different types of texture are found in sand. Both types have different densities, aeration and water holding capacity. The most commonly used type is coarse sand; it has good drainage and is not influenced by chemical and biological factors (Sedibe and Allemann 2012). Various studies have been conducted on root media. Boyle and Craker (1991) conducted a study on ratios between mixture of sphagnum peat, perlite and field soil root media. Results showed that plants grown in mixture of sphagnum peat, perlite and field soil were shorter than those grown in a soil-less mixture of sphagnum peat and perlite. Geply *et al.* (2011) conducted a trial on different growth media including top soil, river sand and sawdust. Results showed that growing *Jatropha curcas* (L.) in river sand was best compared to other media.

Other substrates used for production are peat medium, which is commonly used in pot cultivation culture and characterised by high fibre content to provide adequate increased water-holding capacity. Substrates such as vermiculite and perlite media used for propagation of seedlings in the nurseries. Vermiculite releases plant nutrients and can also absorb and store some nutrients (Combrink 2005).

2.6.2 Potting bags

Potting bags of various sizes are used to grow greenhouse plants. This could be during the seedling preparation and transplanting stage of plants. Most pots that are used are made of plastic and fibre-glass, cast concrete, clay, polyurethane, foam and wood and also vary in size and shape. Plastic pots have also been used, as they are relatively cheaper (Combrink 2005). The choice of potting bag also depends on the root media, space in the growing tunnels, size and drainage (Geply *et al*. 2011). South African growers prefer planting a single plant in a container; however Europeans plant more than one plant per pot, especially in rockwool substrate.

Pot size is an important aspect when selecting containers for cultivation. Bigger pots hold more root media than small pots, which allows better penetration and distribution of roots. A bigger pot has better moisture holding with less irrigation intervals, while

smaller pots are prone to drying out quickly at high temperatures and may require frequent irrigation. Selection of a pot is affected by the rooting system of a plant and size (NeSmith and Duval 1998).

The pot bag has an effect on plant leaf area, shootand root biomass and morphological characteristics of plants (NeSmith and Duval 1998). Good balance between the roots and aerial shoots can be restricted by smaller pots. Yang *et al*. (2010) reported a significant effect of pot size where shoot mass of maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) was decreased on plant grown in smaller pots. Pooter *et al*. (2012) reported that lilies (*Nympaea* spp.) yield was increased when grown on bigger pots (Al-Menaie *et al*. 2012).

2.6.3 Climatic requirements

2.6.3.1 Temperature requirements

Rose geranium is moderately sensitive to cold conditions and does not grow well in winter. It is normally grown in summer in an open field conditions where there is full sunlight. The night and day temperature required by rose geranium is between 10-16°C and 18-24°C, respectively. The growth of the plant ceases at a temperature below 6°C and fatality occurs at temperatures below 3°C and above 33°C. Indoor production is advised during cold seasons, mainly in greenhouses or any climate-controlled facilities (Weiss 1997).

2.6.3.2 Soil water requirements

Although rose geranium is drought-tolerant, it can respond well to irrigation. A herbage yield of 28.2 t ha^{-1} with a corresponding oil yield of 0.73 kg ha^{-1} was produced after subjecting rose geranium to three months of continued irrigation (Miller 1996). The effect of drought varies in plants according to the duration and severity of drought, the tolerance level of the plant and plant material to be harvested. Essential oil plants are directly affected by moisture stress (Singh *et al*. 1996). Water application to rose geranium varies according to cultivation practices, irrespective of field production or tunnel production. Using drip irrigation and sub irrigation, water should be applied in

such a way that the upper crop part is not wetted to prevent fungal infections. Rose geranium should be allowed to dry out completely between watering intervals (Meyers 2006).

2.6.4 Harvesting

Hand and mechanical harvesting methods are used to harvest rose geranium (Ram *et al*. 2001). The foliage should be harvested at least 15 cm above the ground to promote the regeneration of the shoots (Singh and Ganseha-Rao 2009). Younger leaves have the best quality oil that emits a strong rose odour (Rajeswara-Rao *et al*. 1993).The right time for harvesting is from four to six months after planting, depending on climatic condition and management practices (Rajeswara-Rao *et al*. 2000). Kothari *et al*. (2004) and Rajeswara-Rao *et al*. (2000) indicated that the perfect time for harvesting is during the flowering period, since flowers contain some essential oil along with other green parts and as well when rose geranium leaves turn yellow.

2.7 Distillation and identification of oil compounds

Various methods are used to extract essential oil; enfleurage, expression, maceration, steam distillation and solvent distillation are the most widely used methods (Hauser 2008Rose geranium growers use the steam distillation method to extract the oil from the plant material. This system is designed to protect the essential oil from heat and water, volatility and water solubility and acts as a barrier to prevent the oil from overheating (Gogoi 2005).

The identification of essential oil is primed by the comparison of retention times of the chromatogram peaks with those of authentic compounds run under identical conditions. The retention process index is computed from gas chromatograms by logarithmic interpolation between *n*-alkanes. The homologous series of *n*-alkanes is used as standard with literature data indications. The oil concentration data is then obtained by electronic integration of peak areas (Swamy and Rao 2009).

2.8 Trichomes

Some essential oil plants have various types of trichomes that cover the plant surface. Trichome is a small hair or other outgrowth from the epidermis of the plant, typically unicellular and glandular. Trichomes secrete secondary metabolite that repels herbivores and arthropods. Trichomes vary in shape and the compound they secrete. Compound synthesis trichomes are referred to as glandular trichomes, while non-secreting trichomes are referred to as non-glandular trichomes. Glandular trichomes have an important metabolic use and contain oil.

Trichomes are observed under scanning electron microscope is used to view the picture of a bewildering diversity of shapes and structures. Variations between trichome vary between species; differences occur on number of lobes and shape (Payne 1978). In the glossary as stipulated by Payne (1978), the three trichomes occurring on rose geranium are classified.

The non-glandular trichomes (Figure 2.1 a. and b.) and asciiform, brevicollate (Figure 2.2) are found on the leaves of rose geranium. Trichomes are characterised by size and morphology; smaller trichomes are regarded as asciiform and characterised by a short segmented apitate with a columnar hatchet-shaped tip that has a slightly bent apical cell pointing at the leaf apex (Payne 1978) whereas the bigger trichomes are characterised by a short neck with a bigger round tip and are known as brevicollate trichomes (Payne 1978). The non-glandular or attenuate trichomes are characterised by the long and gradual taper (Figure 2.1).

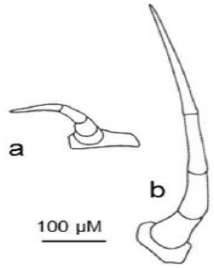

Figure 2.1 Schematic representations of non-glandular trichome (attenuate).

Source:Tissier 2012

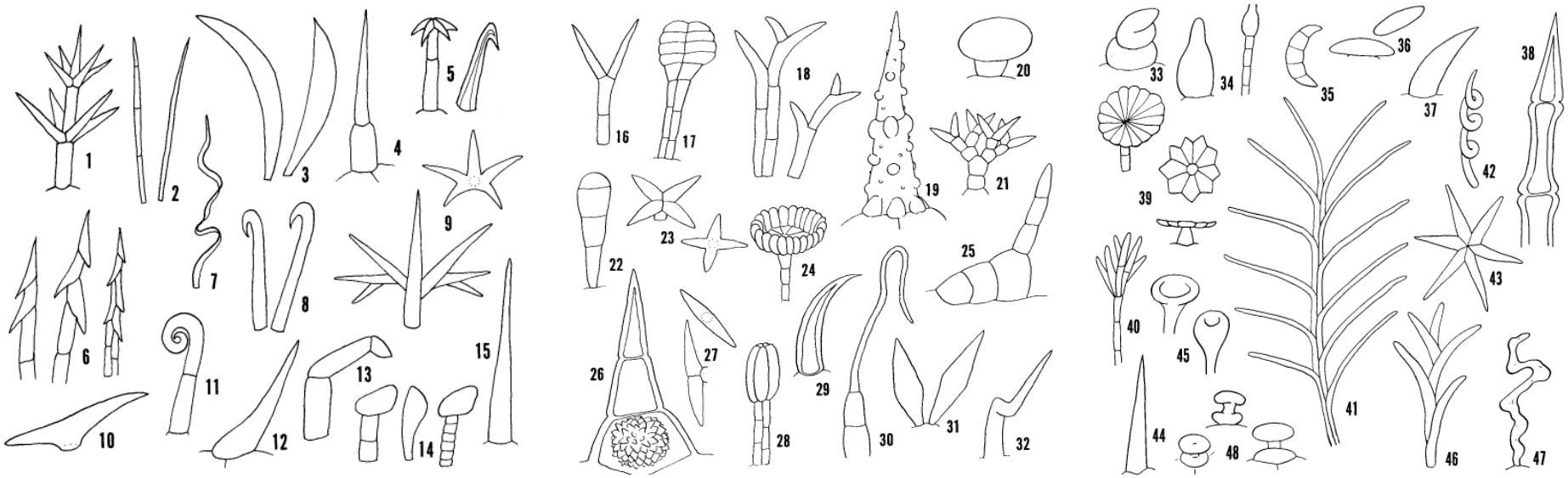

Figure 2.2 Trichome types: 1) Abietiform 2) Acerate 3) Acinaciform 4) Acuminate terminal cell 5) Anchor hairs anduncinate with terminal fluke cells 6) Ancistrous 7) Aduncate 8) Angler 9) Antler 10) Anvil 11) Apicircinatus 12) Arrect 13) Arthrodactylous 14) Asciiform 15) Attenuate 16) Bifid 17) Biseriate 18) Bootjack 19) Bosselated 20) Brevicollate 21) Brevifurcate 22) Clavate 23) Cruciate 24) Cup-shaped 25) Cushion hair 26) Cystolith hair [with cystolith in basal cell] 27) Dolabrate 28) Doliform head of colleter 29) Falcate 30) Flagelliform 31) Fusiform 32) Geniculate 33) Heliciform 34) Lageniform 35) Lunate 36) Limaciform 37) Ornithorhynchous 38) Osteolate cells of uniseriate hair 39) Peltate 40) Penicillate 41) Plumose 42) Spiral 43) Stellate 44) Subulate 45) Surculate 46) Sympodial 47) Torulose 48) Trochlear.

Source: Payne 1978

2.9 Oil quality parameters of rose geranium

Essential oils are classified as secondary metabolites and impart aromatic and medicinal properties to plants. Essential oils are a complex mixture of a large number of individual compounds with a variety of highly functionalised chemical entities. Rose geranium essential oils are grouped into the hydrocarbons; monotepernes (C_{10}) (citronellol, geraniol, linalool and their esters), sesquiterpernes (C_{15}) (guaia-6,9-diene) (Eiasu 2009) and oxygenated groups such as alcohols, phenol, oxide, aldehyde, ketone, acid, ester and ether functional groups (Demarne 2002). The key oil components measured for quality in rose geranium oil are citronellol, geraniol, iso-menthone, linalool, guaia-6,9-diene, citronellyl formate, rose oxide and geraniol formate. The most important components used for perfumery industry are geraniol, citronellol, and linalool. These three oil components are also referred to as rhodinol in the perfumery industry (Motsa *et al*. 2006).

The quality of rose geranium is classified according to its origin, the oil characteristics and citronellol and geraniol ratio (C:G). The oils that originate from China, Morocco, Algeria, Egypt and Bourbon (Reunion) differ in oil composition. The geranium oil differs according to character and physical properties (the gravity, refractive index, colour, solubility in ethanol and optical rotation), chemical properties (acid value, esters value and carbonyl value) and also its organoleptic properties (aroma). The Reunion, Algerian and Chinese cultivars have a C:G ratio of 1:1, 3:1 to 4:1, respectively (Demarne 2002).

Citronellol (3,7-dimethyl-6-octen-8-ol) is an unsaturated aldehyde (terpene) and is a constituent of rose geranium and citronella oil. It is an oily liquid compound that is slightly soluble in water and miscible with ethanol and ether, and is used as an insect repellent and it is used in the manufacturing of perfumes. It is volatile and has a very strong and sweet smell (Letizia *et al*. 2003).

Geraniol (3,7-dmethylocta-trans-2,6-dien-1-ol) is an acyclic monoterpenoid and an alcohol with a chemical formula of $C_{10}H_{18}O$ (Chen and Viljoen 2010). It is the main component of rose, palmarosa and citronella oil. Geraniol is a clear yellow oil compound and is insoluble

in water, but soluble only in organic solvents and a main component of perfumes (Chen and Viljoen 2010).

Linalool is sometimes referred to as coriandrol (3,7-dimethyl-1,6-octadien-3-ol) in literature (Letizia *et al*. 2003). In its physical form it appears as a colourless to very pale and yellow liquid. Linalool forms an important fragrance ingredient used in many fragrance compounds. It may be found in fragrances used in decorative cosmetics, fine fragrances, shampoos, toilet soaps and other toiletries, as well as in non-cosmetic products such as household cleaners and detergents (Letizia *et al*. 2003). Thus, the perfumery industries are more interested in the rhodinol composition (Kulkarni *et al*. 1997).

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CHAPTER 3

GENERAL MATERIALS AND METHODS

General materials and methods

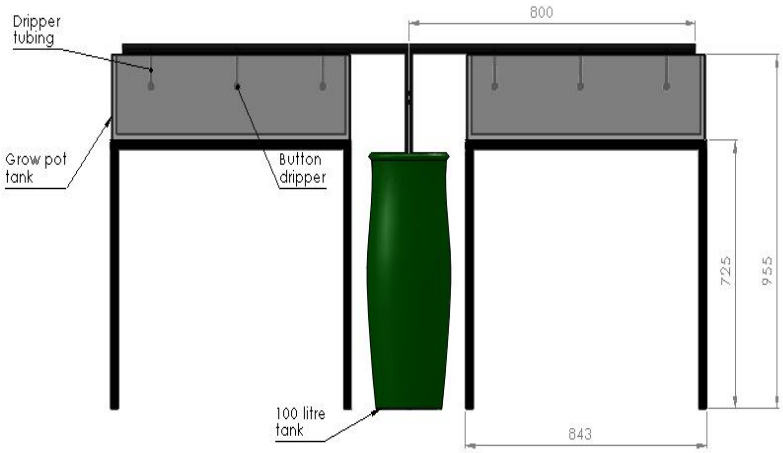
3.1 Site and climate descriptions

Experimental trials (potassium and potting-bag size; water quality and root-media) were carried out in a plant house at Bloemfontein, campus of the University of the Free State. The plant house is located in the semi-arid area with coordinates of 29º10'S and 26º17'E at an altitude of 1395 m above sea-level. The temperature was kept at a minimum and maximum temperature of 24 and 26ºC, respectively. Two axial fans and a wet-wall of the plant house were triggered by a climate adapter (Climate adapter Johnson A419 series USA) to maintain the temperature of the plant house.

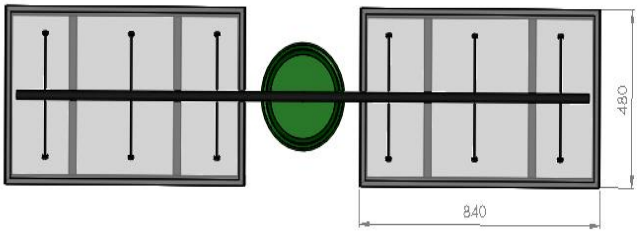
3.2 Description of irrigation systems used in all trials

A customized small-scale growing units (450x800x215 cm) adapted from the unit used by Sedibe and Allemann (2013) was used to grow rose geranium (Figure 3.1). The irrigation systems had six-dripper tubing with a flow rate of 4 L $hour^{-1}$; these drippers were allocated to six potted plants. An irrigation pump with a flow rate capacity of 700 L $hour^{-1}$ was mounted to a 20 mm tubing pipe distributed to the pot-holding tank of the growing unit. All nutrient solutions used were recirculated and replaced with fresh solution every 14 days. Three irrigation system cycles were scheduled for one hour per cycle at 7:00, 11:00 and 15:00.

a) Side view



b) Top view



c) 3D view

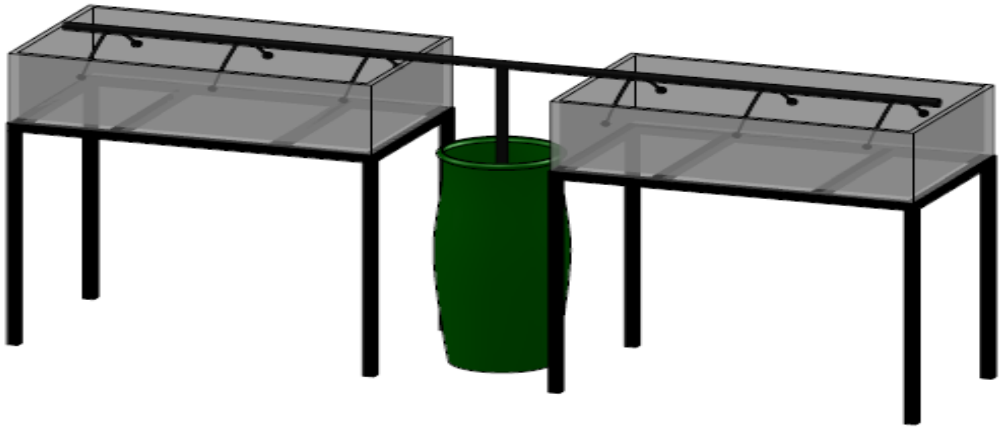


Figure 3.1 Schematic representation of small-scale growing unit and irrigation system used to grow rose geranium plants.

3.3 Crop management in all trials

Rooted cuttings (±10 cm) of rose geranium were obtained from a commercial grower (Pico-gro RSA). One rooted cutting was planted per each pot used.

Aphids and red spider mites were problematic during the experimental period. With no registered insecticide for rose geranium, aphids and red spider mites were controlled by a full cover spray of malasol at 1.75 mL L^{-1} and abamectin at 1.20 mL L^{-1}. These applications were repeated for three to six days at four-week intervals.

All nutrient solutions received the same amount of micro-nutrients (Table 3.1). Rose geranium plants were allowed to grow for four months and followed by manual hand-harvesting.

Table 3.1 Micro-nutrient composition of the nutrient solution used to fertigate rose geranium plants in all nutrient solution treatments.

Micronutrient	Fertilizer source	Application (mg L^{-1})
Fe (EDTA)	Libfer, 13% iron-EDTA	1.12
Mn	Manganese sulphate	0.54
Zn	Zinc sulphate	0.18
B	Boric acid	0.03
Cu	Copper sulphate	0.02
Mo	Ammonium molybdate	0.05

3.4 Parameters measured

The following parameters were measured in all trials. However, parameters outlined in chapter 4, 5 and 6 are specific procedures for those chapters.

3.4.1. Plant height

Plant height was determined a day before harvesting. Since rose-scented geranium is shrubby crop, measurements were carried out on the main middle stem whereby a piece of thread was tied from the bottom to the top of the plant and hanged up right. The measurement was done with a measuring tape starting from the bottom to the upright position of the plant as described by Wood and Roger (2000).

3.4.2 Number of branches

The number of branches was determined at harvest by counting the number of shoots developing from the main base stem continuing unto the last distal top node (old and new shoots were considered) following the procedure described by Damascos *et al*. (2008).

3.4.3 Chlorophyll content

Chlorophyll content was determined according to the procedure described by Chen and Black (1992) before harvest using a portable non-destructive chlorophyll meter (Opti-sciences CCM 200 USA). Readings were taken randomly from the upper six mature leaves on the crop.

3.4.4 Leaf area

Leaf area was determined using a portable leaf area meter (CI-202 USA) used by Ghasemi *et al*. (2011). Six mature leaves were harvested from the crop in the following sequence; two from the top, two from the middle and two at the bottom of the plant. Measurements were taken immediately after harvest.

3.4.5 Plant biomass

Fresh mass of the plant was determined following the procedures of Wood and Roger (2000) and Reuter and Robinson (1986) by weighing fresh plant material with PGL 2002 Adam scale (USA). Plant materials were pulled out from the medium without causing any severe trauma and blotted gently with a paper towel for the removal of any free surface moisture. Measurements were taken immediately before the likelihood of wilting occurs.

3.4.6 Essential oil extraction

Rose geranium oil was extracted from the leaves and stems using a custom-built steam distillation unit (Sedibe 2012). About 5 kg of fresh plant material was distilled for oil at a temperature of ±98°C for one hour. The mass of the oil volume (yield) was determined by weighing the oil volume using PGL 2002 Adam scale (USA) immediately after extraction as described by Swamy and Rao (2009).

3.4.7 Oil composition

Essential oil compounds were primed by comparing the retention times of the chromatogram peaks. Key oil components determined for oil composition were citronellol, geraniol, linalool, iso-menthone, citronelyll formate, geranyl formate, rose oxide (*cis* and *trans*) and guaia-6,9-diene. The retention indices were computed from a gas chromatogram that was logarithmically interpolated between the n-alkanes. A homologous series of n-alkanes (C8-C22 Polyscience USA) was used as a standard. The oil concentration data were obtained by electronic integration of peak areas as described by Heravi *et al*. (2006).

3.5 Data analysis

Experimental data in all trials was analysed using the general linear model of SAS statistical software version 9.2 (SAS 2008) to determine the analysis of variance (ANOVA). The Tukey's least significant difference (LSD_T), described by Steel and Tourie (1980) was used to determine the significant results between variants. Statistical difference between treatment means was determined at the P=0.05 probability level.

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CHAPTER 4

THE EFFECT OF POTASSIUM AND POTTING-BAG SIZE ON YIELD AND OIL COMPOSITION OF ROSE GERANIUM (*Pelargonium graveolens* L.)

The effect of potassium and potting-bag size on yield and oil composition of rose geranium (*Pelargonium graveolens* L.)

Abstract

The effect of potassium (1.3, 3.3, 5.3 and 7.3 mmol L^{-1}) and potting-bag size (5 and 10 L) was evaluated on the yield and oil composition of rose geranium. Plants were grown in a controlled temperature plant house and treatments were arranged in a randomised complete block design assigned in a split plot layout. Potassium concentrations were allocated to the main plots and while the potting-bag sizes were allocated to the subplots, replicated three times. Plant height, potassium content, linalool, geraniol, geranyl formate and citronellol to geraniol ratio were the only parameters affected by the potassium. Plant height, number of branches, branch to height ratio, foliage mass and oil yield were affected by the potting-bags size. Plant foliar mass was significantly increased by the interaction between 5.3 mmol K L^{-1} and 5 L potting-bag. Rose geranium growers are advised to apply 5.3 mmol K L^{-1} concentrations and to use 5 L potting bags for increased yield.

4.1 Introduction

To improve yield and oil composition of rose geranium, studies have been conducted on the application of minerals such as nitrogen, phosphorus and sulphur as a means of improving yield and oil composition of rose geranium (Sedibe and Allemann 2012; Araya *et al*. 2006). However, potassium has been neglected in most rose geranium nutrition studies. Potassium is one of the most important mineral required by plants after nitrogen and phosphorus (Dibb 1998). Potassium acts as a catalysts enhancer and is involved in many activities of plant growth. It enhances the quality and also induces stress tolerance in plants (Wen Xu *et al*. 2011; Tounekti *et al*. 2010). Potassium is involved in the activation of more than 60 enzymatic reactions such as osmoregulation, water transport in the xylem, water assimilation, protein biosynthesis, osmotic adjustment, electrical neutralisation of anionic groups and control of cell membrane polarisation (Wen-Xu *et al*. 2011; Nguyen *et al*. 2010). Puttanna *et al*. (2010) reported that low quantity of potassium hinders nitrogen uptake. The application of potassium has been reported to increase the

yield and total herbage of patchouli (*Pogostemon cablin* [Blanco] Benth.) and palmarosa (*Cymbopogon martini* [roxb.] Wats. Var. Motia burk) plants (Singh and Ganesha-Rao 2009; Singh 2008), although no significant effect of potassium was shown on the growth of thyme (*Thymus vulgaris* L.) and basil (*Ocimum basilicum* L.) (Eryuce *et al*. 2012; Wierdak *et al*. 2012).

Rose geranium is cultivated under open field and little is known about the size of potting bag used in a plant house. The most preferred potting bag for greenhouse plants is made out of a plastic due to its cost effectiveness and durableness; however the appropriate size is unknown for rose geranium (Combrink 2005). The size of potting bag is an important aspect when selecting the cultivation container for cultivation along with type of root-media and available space in the tunnels (Geply *et al*. 2011). NeSmith and Duval (1998) indicated that an appropriate potting bag promotes leaf area, shoot biomass and root biomass, the ratio between roots and foliar shoots. It has been reported by NeSmith and Duval (1998) that bigger potting bag hold more root media than small pots, which allows better root penetration and root distribution. Moreover, bigger potting bag have a better moisture storage than smaller potting bag which is prone to drying out quickly at high temperatures and requires frequent irrigation. A significant reduction in maize (*Zea mays* L.) and sorghum (*Sorghum bicolor* L.) yield grown in smaller cultivation was reported by Yang *et al*. (2010). Pooter *et al*. (2012) reported an increase of 43% by doubling the potting bag size when growing water lilies (*Nympaea* spp.).

The objective of this study was to determine the effect of potassium and potting-bag sizes on the yield and oil composition of rose geranium.

4.2 Materials and methods

Chapter 3 describes all the material used and methods followed in this chapter; this section only describes the relevant procedures that are not included in the general materials and methods.

4.2.1 Field plan

Potassium and potting-bag size trial was carried out during 2011-2012 summer season (December-March). Figure 4.1 illustrate the experimental plan of the potassium and potting-bag size trial whereby the experiment was laid out in a randomised complete block design. The experimental plots were arranged in a split plot layout whereby the four concentrations of potassium (1.3, 3.3, 5.3 and 7.3 mmol L^{-1}) were allocated to the main plots, potting-bag sizes (5 and 10 L) were allocated to the subplots and the units were replicated three times. Each potassium concentration level contained 12 potted plants that were divided into two cultivation units (Figure 4.1). Within each cultivation unit, six potted plants were grown in different sizes of potting bags, 5 L is represented by shaded circles and 10 L represented in non-shaded circles. Pots within the units were placed randomly. In addition, the plant house layout had three blocks with two extraction fans on the entrance and a wet-wall in a way that the first block was placed closer to the wet walls; second block was placed in the middle of the tunnel and the third block placed closer to the two axial fans.

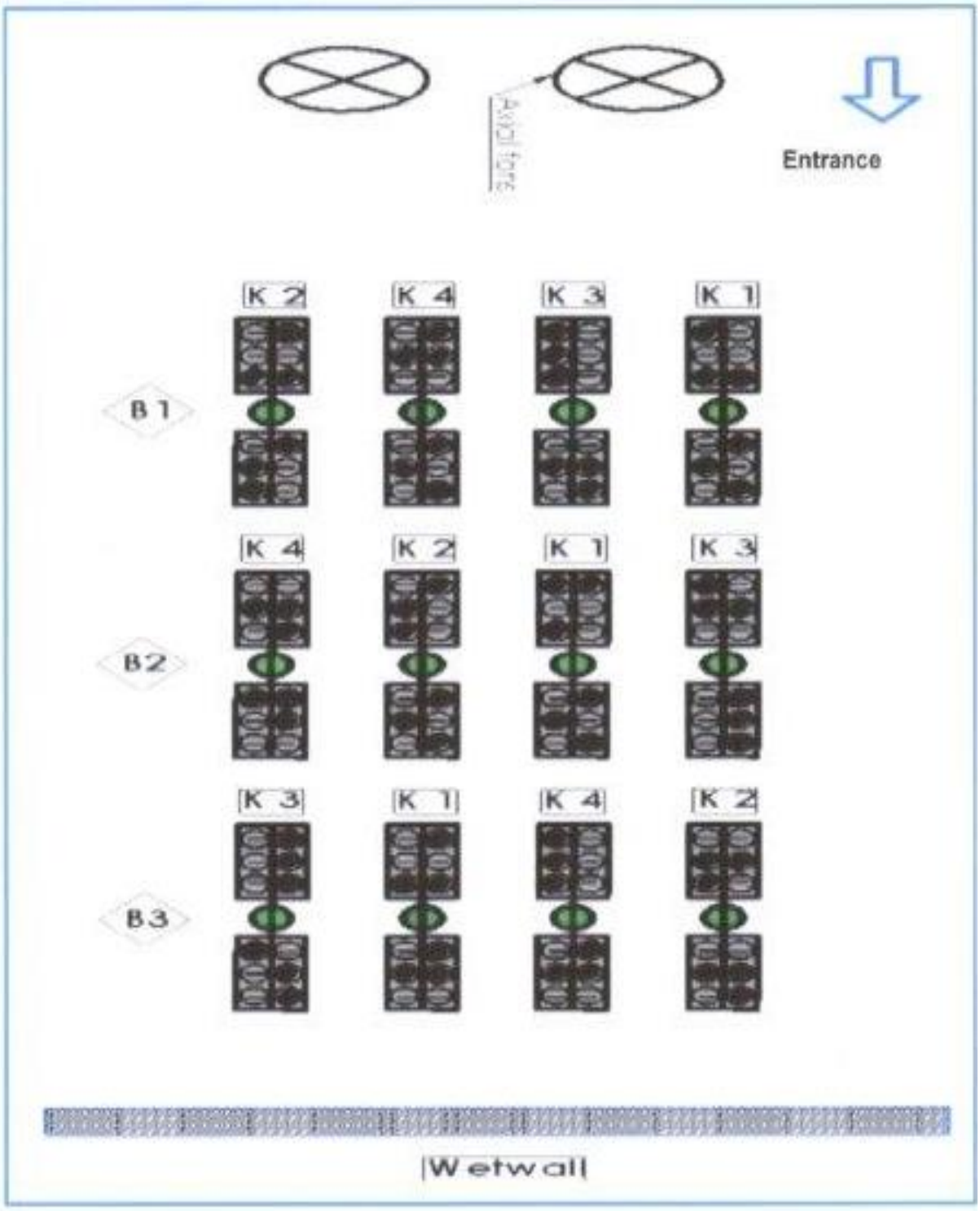

Figure 4.1 The experimental plan of potassium and potting-bag size experiment on rose geranium trial in the plant house. Keys: K 1-4 (potassium levels), B 1-3 (Blocks/Replicates), ● (5 L), ○ (10 L).

4.2.2 Experimental treatments

According to Steiner's universal nutrient solution, cations of the macronutrient should consist of 35% potassium, 45% calcium and 20% magnesium for a balanced nutrient solution (Steiner 1984). Potassium treatment consisted of 1.30, 3.30, 5.30 and 7.30 mmol L^{-1}, while potting-bag sizes were 5 and 10 L. Therefore, to maintain balanced nutrient solution in all treatment levels, calcium and magnesium had to be decreased proportionally to the increased potassium application (Table 4.1). Ammonium and the anions (nitrate, phosphate and sulphate) remained constant on all levels (Steiner 1984). The electric conductivity and pH of the nutrient solution were maintained at 1.63 mS cm^{-1} and 5.5, respectively.

Table 4.1 Nutrient concentrations (mmol L^{-1}) used in the potassium experiment

	Ions (mmol L^{-1})								
Potassium (mmol L^{-1})	NH_4^+	K^+	Ca^{2+}	Mg^{2+}	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	Cl^-	HCO_3^-
1.3	1.00	1.30	9.20	3.56	10.00	1.50	3.44	0.98	0.40
3.3	1.00	3.30	7.75	2.95	10.00	1.50	3.44	0.98	0.40
5.3	1.00	5.30	6.30	2.40	10.00	1.50	3.44	0.98	0.40
7.3	1.00	7.30	4.85	1.85	10.00	1.50	3.44	0.98	0.40

4.2.3 Parameters

Plant height and chlorophyll content were determined prior the harvesting. Foliar fresh mass, leaf area, number of branches, oil yield and mineral analyses were measured after harvesting.

4.2.3.1 Branch toheight ratio (B:H ratio)

Branch to height ratio was calculated by dividing the number of branches per plant by plant height (cm) to express B:H as branches cm^{-1}.

4.2.3.2 Minerals analyses

Plants were dried and milled by the procedure described by Jones (1991). Plant material were oven dried (ECOtherm labotec oven, RSA) at a temperature of 70^{o}C for 48 hours. After the drying process, leaves were mechanically milled to 0.25 mm diameter using a micro hammer mill (Culatti, Zurich) to produce suitable material for minerals analysis.

Nitrogen content analysis

Plant tissue nitrogen content was determined using the Dumas combustion nitrogen analyser (LecoCorp, FP-528 N Analyzer, St. Joseph, MI, USA) whereby sample of 200 mg was weighed into a gel capsule and dropped into an 850°C furnace purged with O_2 gas. Mixtures of gases released during combustion were catalytically converted to N_2. The nitrogen content was measured by a thermal conductivity cell against a helium background and the result was displayed as weight percentage of nitrogen (Matejovic 1995).

Potassium and phosphate content analysis

Dried leaf material (1 g) was placed in a 100 mL volumetric flask mixed with 10 mL of an acid mixture (Nitric-perchloric acid). The flask was placed on a hot plate heated to a temperature range from 90°C to 200°C. The mixture was heated until the production of red NO_2 fumes ceased. These contents were further heated until the mixture became colourless and its volume had reduced to 4 mL. After cooling, the 4 mL volume was mixed with distilled water and filtered through a filter paper (Whattman filter paper No.1). The prepared solution was used for analysis of potassium and phosphate (Medical Chemistry 2008).

Potassium and phosphate were measured using a high-resolution atomic absorption spectrometer (Perkin Elmer Shelton USA) equipped with a xenon short arc lamp set at wavelengths of 766.5 nm and 715 nm for potassium and phosphate, respectively (Medical Chemistry 2008).

Sulphur content

Sulphur content was measured following the procedure described by Zasoski and Burau (1977), ICP-Optical Emission Spectroscopy (JY Horiba Ultima USA) using an extract solution set at a wavelength of 181.9 nm. After analysis, the system was purged with nitrogen at wavelengths below 189.0 nm to prevent interference from atmospheric oxygen.

4.2.4 Data analysis

Collected data was analysed using the statistical procedure outlined in chapter 3.

4.3 Results and discussion

Plant height, number of branches, B:H ratio, leaf area, chlorophyll content, foliar fresh mass, oil yield and oil content results are shown in Table 4.2.

Table 4.2 The effect of potassium concentrations and potting-bag size on plant height, number of branches, B:H ratio, leaf area, chlorophyll, foliar fresh mass, oil yield and oil content of rose geranium

Treatments	Parameters							
	Plant height (cm)	**Number of branches (plant^{-1})**	**B:H ratio**	**Leaf area (cm^2)**	**Chloro-phyll (%)**	**Foliar fresh mass (g plant^{-1})**	**Oil yield (g plant^{-1})**	**Oil content (%)**
Potassium (mmol L^{-1})								
1.3	45.17^{ab}	34.78^{a}	0.74^{a}	29.13^{a}	27.68^{a}	333.05^{a}	0.74^{a}	0.23^{a}
3.3	40.50^{ab}	32.97^{a}	0.81^{a}	28.85^{a}	26.71^{a}	261.40^{a}	0.87^{a}	0.32^{a}
5.3	49.30^{a}	36.33^{a}	0.70^{a}	34.28^{a}	27.75^{a}	419.55^{a}	0.61^{a}	0.22^{a}
7.3	37.39^{b}	27.11^{a}	0.67^{a}	30.59^{a}	26.69^{a}	261.55^{a}	0.79^{a}	0.49^{a}
LSD $_{T(0.05)}$	9.30**	ns	ns	ns	ns	ns	ns	ns
CV%	2.44	2.44	2.44	2.44	2.44	2.44	2.44	2.44
Potting bag (L)								
5	49.22^{a}	44.30^{a}	0.89^{a}	31.61^{a}	27.16^{a}	463.75^{a}	1.10^{a}	0.30^{a}
10	36.96^{b}	21.89^{b}	0.57^{b}	29.82^{a}	27.26^{a}	174.03^{b}	0.41^{b}	0.33^{a}
LSD $_{T(0.05)}$	4.98*	8.71*	0.15*	ns	ns	101.25*	0.31*	ns
CV%	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30
Potassium x potting bag								
LSD $_{T(0.05)}$	ns	ns	ns	ns	ns	202.51**	ns	ns
CV%	2.30	2.30	2.30	2.30	2.30	2.30	2.30	2.30

Means followed by the same letter in the same column are statistically non-significant at $P<0.05$ according to the least significant difference, ($LSD_{T0.05}$). CV% = coefficient of variation, ns = not significant at $P<0.05$, * F-ratio probability of $P<0.01$, ** F-ratio probability of $P<0.05$

There was no significant effect of potassium concentrations on the number of branches, B:H ratio, leaf area, chlorophyll, foliar fresh mass (FFM), oil yield and oil content. However, plant height was significantly (*P*=0.05) affected by the potassium application. Tallest plants of rose geranium were produced at 5.3 mmol K L^{-1} concentration but not significantly taller than plants produced at 1.3 and 3.3 mmol K L^{-1}. Furthermore, plants produced at 1.3 and 3.3 mmol K L^{-1} were also not significantly different from those produced at 7.3 mmol K L^{-1}. Potassium activates enzymes that are responsible for plant growth, although this effect was not observed on rose geranium yield (Wen Xu *et al*. 2011). The results obtained on plant height were erratic and showed high level of inconsistency. A significant interaction (*P*=0.05) between potassium and potting-bag size affected the foliar biomass and was optimized by the ratio between 5.3 mmol K L^{-1} and 5 L potting bag (Figure 4.2). This interaction between 5.3 mmol K L^{-1} was applied and 5 L is attributed to the activation of enzymes which is associated with potassium as an enzyme activator and sufficient moisture in the 5 L potting bag. Plants roots grow towards moisture, therefore it is assumed from the study that; in smaller potting bags, roots grew towards the water film at the bottom of the potting bag and increased growth than in the bigger potting bags.

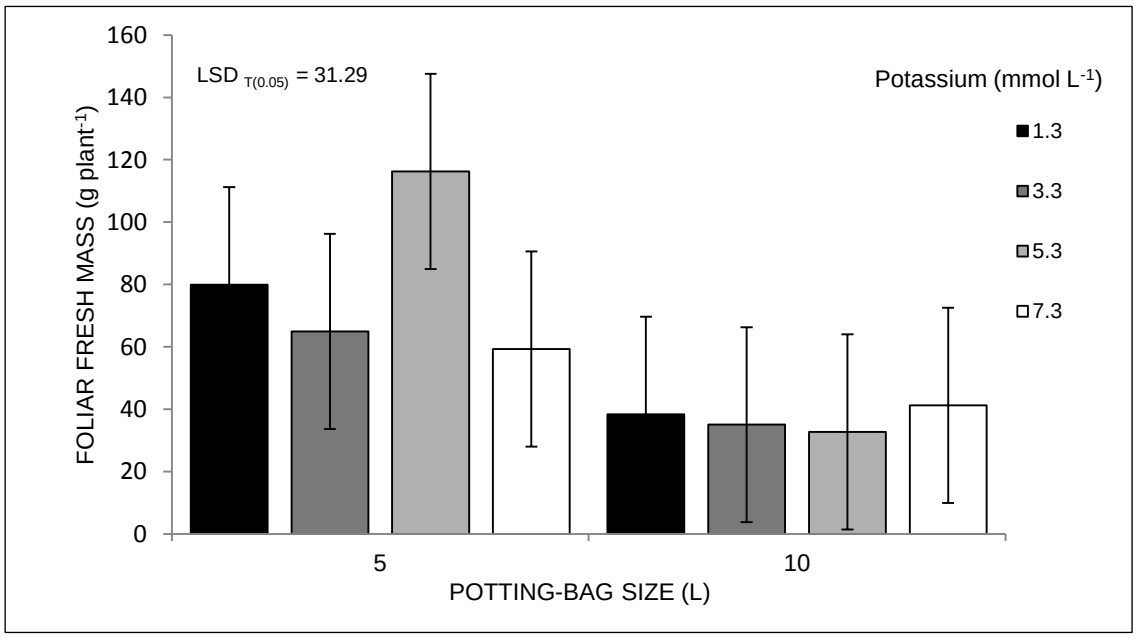

Figure 4.2 Effect of potassium concentrations and potting-bag size on foliar fresh mass of rose geranium.

Potting-bag size had a significant (*P*=0.01) effect on plant height, number of branches, B:H ratio, foliar fresh mass (FFM) and oil yield (Table 4.2). All this parameters were optimized where a 5 L potting bag was used. Plant height, number of branches, B:H ratio, FFM and oil yield are associated with yield components of rose geranium. Rose geranium is a perennial crop and the increase of yield in smaller potting bags is associated with the shorter cultivation period. Moreover, NeSmith and Duval (1998) reported that plants grown in potting bags tend have different root morphology compared to those grown in the field. Plants grown in potting bags have a root system that is exposed to moisture and tend to be short morphologically compared to field plants. Therefore, the optimal potting bag size selection should be selected along with appropriate root medium type (Geply *et al*. 2011).

Contrary to the current study, Watkinson and Wallace (2007) reported an increased leaf area, foliar biomass and root biomass when bigger cultivation bags were used on little bluestem (*Schizachyrium scoparium* [Michx.] Nash) and lanceleaf coreopsis (*Coreopsis lanceolata* L.) grass. Increased yield resulted to the use of bigger potting bags was also reported by Geply *et al*. (2011) on barbados nut (*Jatropha curcas* L.) and Al-Menaie *et al*. (2012) on water lilies (*Nymphaea* spp.), where larger potting bag size increased the amount of root-media pore spaces, which directly increased the availability of water in the root zone.

Potassium concentration and potting-bag size interaction had no significant effect on foliage tissue nitrogen; phosphorus and sulphur content (Table 4.3). However, plant tissue potassium was affected by the interaction between potassium and potting-bag size at 7.3 mmol K L^{-1} concentration, where 10 L potting bag was used (Figure 4.3). Potassium effect on tissue potassium contents was reported on field crops such as cotton (*Gossipium hirsutum* L.) and rosemary (*Rosmarinus officinal* L.), whereby high potassium application increased the potassium tissue content of cotton and rosemary (Gerardeaux *et al*. 2010; Puttanna *et al*. 2010).

Table 4.3 Effect of potassium concentrations and potting-bag size on nitrogen, phosphate, potassium and sulphate composition of rose geranium foliage

	Plant minerals (%)			
Treatments	**N**	**P**	**K**	**S**
Potassium (mmol L^{-1})				
1.3	3.89[a]	0.52[a]	2.54[d]	0.56[a]
3.3	3.71[a]	0.54[a]	2.94[c]	0.46[a]
5.3	3.71[a]	0.52[a]	4.19[b]	0.50[a]
7.3	3.88[a]	0.53[a]	4.56[a]	0.48[a]
LSD $_{T(0.05)}$	ns	ns	0.30*	ns
CV%	2.44	2.44	2.44	2.44
Potting bag (L)				
5	3.65[a]	0.51[a]	3.35[b]	0.48[a]
10	3.95[a]	0.55[a]	3.77[a]	0.52[a]
LSD $_{T(0.05)}$	ns	ns	0.26*	ns
CV%	2.30	2.30	2.30	2.30
Potassium x potting bag				
LSD $_{T(0.05)}$	ns	ns	0.52**	ns
CV%	2.30	2.30	2.30	2.30

Means followed by the same letter in the same column are statistically non-significant at $P<0.05$ according to the least significant difference, ($LSD_{T0.05}$), CV% = coefficient of variation, ns = not significant at $P<0.05$, * F-ratio probability of $P<0.01$, ** F-ratio probability of $P<0.05$

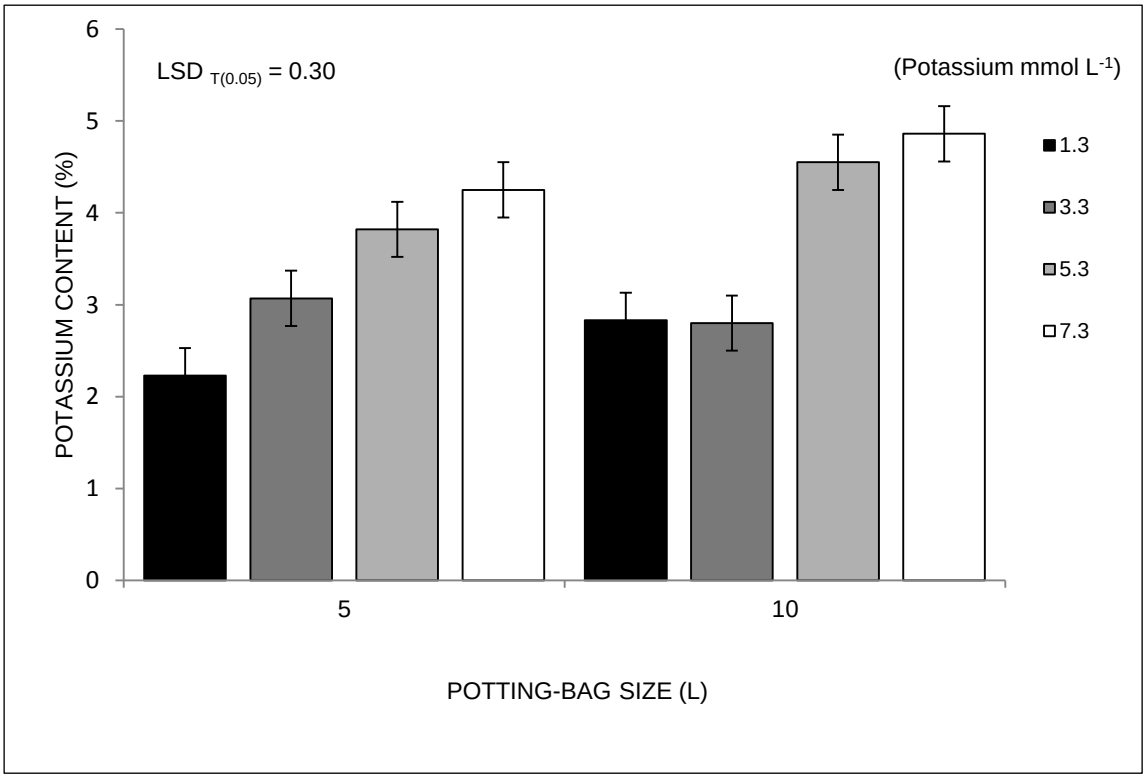

Figure 4.3 Effect of potassium concentrations and potting-bag size on potassium content of rose geranium.

As shown in Table 4.4, potassium concentration had no significant effect on rose oxide (*cis* and *trans*), iso-menthone, citronellol, citronellyl formate and guaia-6,9-diene contents. However, linalool (*P*=0.05), geraniol (*P*=0.01), geranyl-formate (*P*=0.01) and C:G ratio (*P*=0.01) were affected by potassium at 1.3 mmol K L^{-1} and these parameters were all not different from the results obtained at 5.3 mmol K L^{-1} (Table 4.4). Essential oil compositions results were erratic and showed high level of inconsistency. Beside its effect on yield, potassium also affects the synthesis of secondary metabolites such as phenolic acid, flavonoids and essential oil. The changes in the essential oil constituents were caused by potassium due to its effect on enzyme activities and metabolic activities during essential oil synthesis (Khalid 2013). Although the C:G ratio was significantly affected by potassium concentration, the ratios were higher than standards requirement by perfumery industry.

Table 4.4 Effect of potassium (mmol L^{-1}) concentrations on oil composition of rose geranium

Treatments	Oil composition (%)									
	Linalool	Rose oxide (*cis*)	Rose oxide (*trans*)	Iso-menthone	Citronellol (C)	Geraniol (G)	Citronellyl formate	Geranyl formate	Guaia -6,9-diene	C:G ratio
Potassium										
1.3	1.25[a]	0.09[a]	0.00[a]	1.00[a]	34.98[a]	9.03[a]	21.01[a]	5.02[a]	9.43[a]	3.89[b]
3.3	0.50[b]	0.15[a]	0.04[a]	1.24[a]	35.19[a]	6.22[b]	21.62[a]	3.53[b]	10.00[a]	5.69[a]
5.3	0.84[ab]	0.13[a]	0.03[a]	2.69[a]	31.32[a]	8.06[a]	20.96[a]	4.95[a]	10.31[a]	3.90[b]
7.3	0.55[b]	0.19[a]	0.08[a]	1.77[a]	33.70[a]	6.29[b]	22.13[a]	3.99[b]	10.18[a]	5.38[a]
LSD $_{T(0.05)}$	0.49**	ns	ns	ns	ns	1.46*	ns	0.74*	ns	0.87*
CV %	2.44	2.44	2.44	2.44	2.44	2.44	2.44	2.44	2.44	2.44

Means followed by the same letter in the same column are statistically non-significant at $P<0.05$ according to the least significant difference ($LSD_{T0.05}$), CV% = coefficient of variation, ns = not significant at $P<0.05$, * F-ratio probability of $P<0.01$, ** F-ratio probability of $P<0.05$

4.4 Conclusion

It is evident from the results obtained that potassium has no effects on oil composition of rose geranium. Rose geranium growers are advised to use 5.3 mmol K L^{-1} and to use 5 L potting bags to enhance oil yield of rose geranium.

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CHAPTER 5

YIELD AND OIL COMPOSITION RESPONSE OF ROSE GERANIUM (*Pelargonium graveolens* L.) TO WATER QUALITY AND ROOT MEDIA

Yield and oil composition response of rose geranium (*Pelargonium graveolens* L.) to water quality and root media

Abstract

A study was carried out in a climate-controlled plant house at the University of the Free State to determine the effect of water quality (NaCl) (1.68, 2.40, 3.20 and 4.0 mS cm^{-1}) on oil yield and oil composition of rose geranium (*Pelargonium graveolens* L.) grown in sand and sawdust growth media.. Treatments were arranged in a randomised complete block design assigned in a split plot layout. Water quality (salt) was allocated to the main plots and root media to the subplots; this was replicated three times. Increased salt (4.0 mS cm^{-1}) significantly reduced the number of leaves, plant height, number of branches, leaf area and chlorophyll content. Geranyl formate and citronellol:geraniol ratio were also affected by water quality. Furthermore, plants that were grown on sawdust had a better number of leaves, leaf area and foliar fresh mass. It is evident from the study that rose geranium is a moderately salt-sensitive crop and it is recommended to grow rose geranium where water has salt level is below 4 mS cm^{-1}.

5.1 Introduction

Agriculture in South Africa uses about 50% of the country's available water (DWAF 1996). Increasing water needs claimed for domestic, industrial and mining use may decrease agriculture's share to less than the allocated amount (Sedibe *et al*. 2013). Water quality concerns were neglected in the past because good quality water supplies were in abundance, but this situation is changing. The demand for good quality water is escalating (Sedibe *et al*. 2006) and water quality has become a key factor based on specific concentrations of ions, phytotoxic substances and the presence of micro-organisms (Schwarz *et al*. 2005; Tognoni *et al*. 1998). For soil-less production, growers use water sources of different origins (rivers, dams, lakes, boreholes and artificial ponds) and this has a huge impact on quality (Sedibe *et al*. 2013).

Irrigation water varies greatly in quality depending upon the type and quality of dissolved salts, pH and mineral content. Salts in the irrigation water originate from weathered rocks, fertilizers and soil. The suitability of irrigation water is determined not

only by the total amount of salt present but also by the kind of salts (Sedibe *et al*. 2013). High levels of salts in the feeding water induce physiological and metabolic activities in plants. The effect of salinity varies between plants species and also according to the type of production system used (Jouyban 2012; Sonneveld 2000). Said-Al Ahl and Omer (2011) reported that accumulation of salts in the root media was caused by rates of evapo-transpiration and poor leaching of water.

The objective of this was to determine the effect of water quality (NaCl) (1.68, 2.40, 3.20 and 4.0 mS cm^{-1}) on oil yield and oil composition of rose geranium (*Pelargonium graveolens* L.) grown in sand and sawdust growth media..

5.2 Material and methods

All the materials used and methods followed in this chapter was described in chapter 3.

5.2.1 Field plan

The water quality and root-media trial was carried out during 2012 autumn season (March-June). Figure 5.1 illustrate the experimental plan of the water quality and root-media trial. The experiment was laid out in a randomised complete block design, arranged in a split plot layout, where the main plots consisted of four different salt levels and the sub-plots consisted of two root media types and the units were replicated three times. Each salinity concentration level contained 12 potted plants that were divided into two cultivation units (Figure 5.1). In each unit, six potted plants were grown in different root media, silica sand represented in dark-shaded circles and sawdust represented in non-shaded circles.

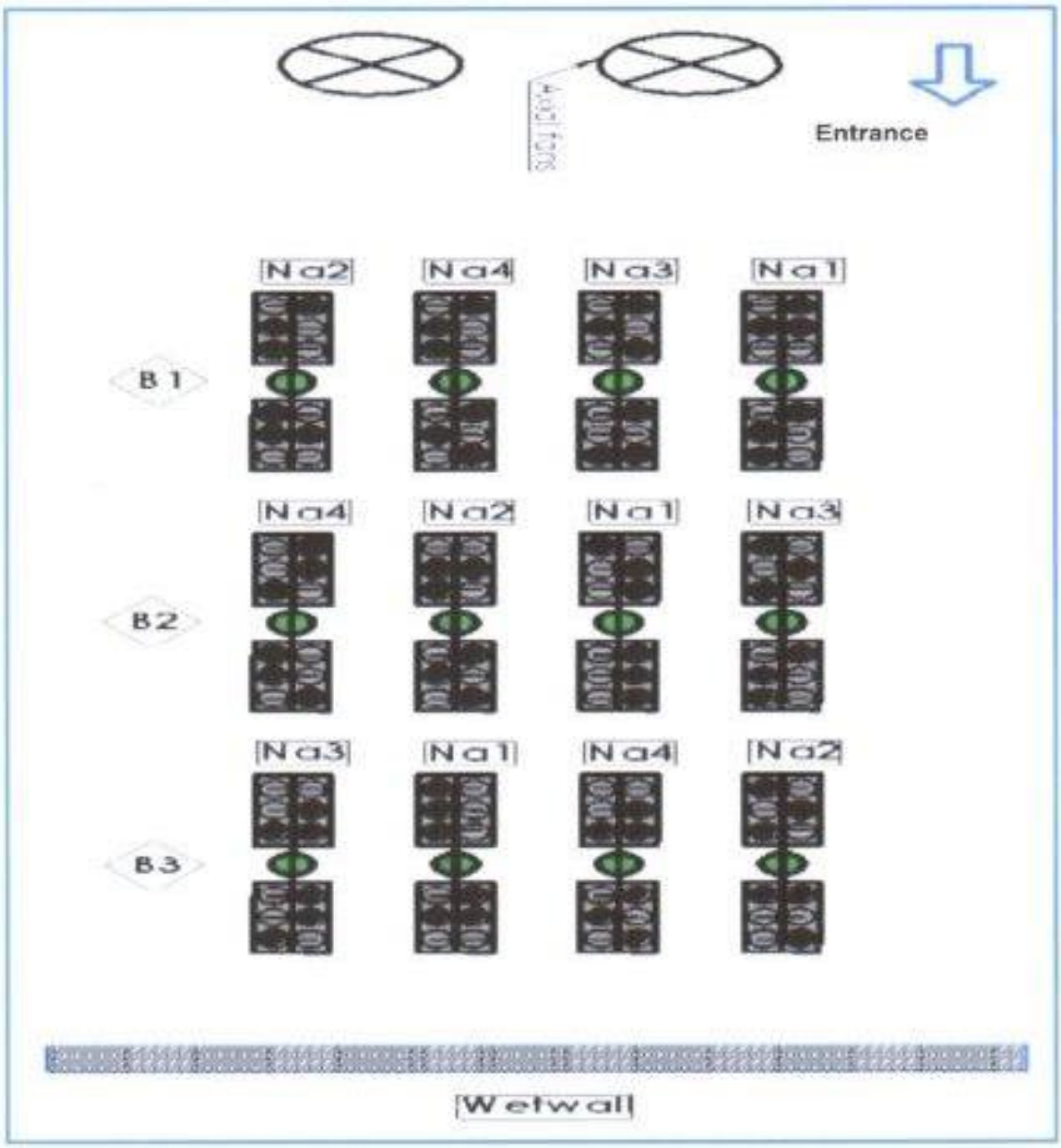

Figure 5.1 The experimental plan of water quality and root media experiment on rose geranium trial. Keys: Na 1-4 (Salt levels), B 1-3 (Blocks/Replications), ● (Sawdust), ○ (Sand).

Table 5.1 Nutrient solution concentrations used to study the response of rose geranium to water quality (1.6, 2.4, 3.2 and 4.0 mS cm^{-1})

	Ions															
Water quality (mS cm^{-1})	**Na^+**	**NH_4^+**	**K^+**	**Ca^+**	**Mg^{2+}**	**NO_3^-**	**$H_2PO_4^-$**	**SO_4^{2-}**	**Cl^-**	**HCO_3^-**	**Fe^{2+}**	**Mn^{3+}**	**B**	**Cu^{2+}**	**Mo^{2+}**	**Zn^{2+}**
1.6	1.31	1.00	5.5	6.50	2.5	11.04	0.10	3.80	1.31	0.40	1.12	0.54	0.03	0.02	0.05	0.18
2.4	8.49	1.00	5.5	6.50	2.5	11.04	0.10	3.80	8.49	0.40	1.12	0.54	0.03	0.02	0.05	0.18
3.2	16.5	1.00	5.5	6.50	2.5	11.04	0.10	3.80	16.5	0.40	1.12	0.54	0.03	0.02	0.05	0.18
4.0	24.5	1.00	5.5	6.50	2.5	11.04	0.10	3.80	24.5	0.40	1.12	0.54	0.03	0.02	0.05	0.18

5.2.2 Experimental treatments

Irrigation water of different qualities induced by salt (NaCl) was evaluated at 1.68, 2.40, 3.20 and 4.0 mS cm^{-1} (Table 5.1), while silica sand (2 mm) and sawdust (pine sawdust) were the selected root media. Salt shock was avoided by initiating salinity stress gradually in a weekly sequence in the third month after transplanting at 25, 50, 75 and 100% until constant levels were met in all treatment levels. All ions applied remained constant on all levels. The nutrient solutions received the same micro-nutrients in all salinity levels. Nutrients solution pH was maintained at 5.5 in the experimental units.

5.2.3 Parameters

Plant height, chlorophyll content, stomatal conductance was determined prior the harvesting. Foliar fresh mass, number of leaves, number of branches, leaf area, relative water content, and oil yield were measured after harvesting.

5.2.3.1 Stomatal conductance

Stomatal conductance was determined twice at 10:00 and 14:00 for four days before harvesting. Data were collected on clear days, using a leaf porometer (Decagon SC-1 USA). Measurements were taken on six selected mature leaves (Bunce 2006).

5.2.3.2 Relative water content

Three leaves on each plant were collected to determine relative water content (RWC). Leaf fresh mass (LFM) was determined immediately at harvesting and subsequently the leaves were immersed in distilled water for 12 hours to determine leaf turgid mass (LTM). Afterwards these samples were dried in an oven set at 60°C for 24 hours to determine the leaf dry mass (LDM). The relative water content was determined by procedure described by Sedibe (2012);

$$RWC\% = (LFM - LDM) \div (LTM - LDM) \text{ X } 100$$

5.2.4 Data analysis

Collected data was analysed using the statistical procedure outlined in chapter 3 section 3.5.

5.3 Results and discussion

The results on the number of leaves, plant height, number of branches, leaf area, relative water content (RWC), chlorophyll content, foliar fresh mass (FFM), oil yield and oil content are shown in Table 5.2.

Table 5.2 The effect of water quality (NaCl) and rooting media on the number of leaves, plant height, number of branches, leaf area, relative water content, chlorophyll, foliar fresh mass, oil yield and oil content of rose geranium

Treatments	Parameters								
	Number of leaves (plant^{-1})	Plant height (cm)	Number of branches (plant^{-1})	Leaf area (cm^2)	Relative water content	Chloro-phyll (%)	Foliar fresh mass (g plant^{-1})	Oil yield (g plant^{-1})	Oil content (%)
Water quality(mS cm^{-1})									
1.6	448.44^a	43.44^a	38.88^a	931.16^a	84.56^a	28.11^a	490.83^a	1.49^a	0.31^a
2.4	482.11^a	42.83^a	33.33^{ab}	811.05^{ab}	81.27^a	26.05^{ab}	430.27^a	1.44^a	0.35^a
3.2	425.44^a	43.49^a	32.11^b	686.11^b	86.57^a	25.87^{ab}	502.50^a	1.87^a	0.37^a
4.0	249.94^b	36.27^b	30.50^b	459.44^c	83.13^a	22.92^b	373.33^a	1.28^a	0.35^a
LSD $_{T(0.05)}$	88.64*	4.75**	5.91**	155.09*	ns	3.22**	ns	ns	ns
CV%	2.11	2.11	2.11	2.11	2.11	2.11	2.11	2.11	2.11
Root media									
Sand	351.13^b	40.10^a	32.66^a	644.80^b	83.62^a	24.92^a	387.22^b	1.34^a	0.35^a
Sawdust	451.83^a	42.91^a	34.75^a	799.08^a	84.15^a	26.55^a	511.24^a	1.70^a	0.34^a
LSD $_{T(0.05)}$	62.68*	ns	ns	109.66*	ns	ns	85.62*	ns	ns
CV%	2.11	2.11	2.11	2.11	2.11	2.11	2.11	2.11	2.11
Water qualityx root media									
LSD $_{T(0.05)}$	ns	ns	ns	ns	ns	ns	ns	ns	ns
CV%	2.11	2.11	2.11	2.11	2.11	2.11	2.11	2.11	2.11

Means followed by the same letter in the same column are statistically non-significant at $P<0.05$ according to the least significant difference, ($LSD_{T0.05}$). CV% = coefficient of variation, ns = not significant at $P<0.05$, * F-ratio probability of $P<0.01$, ** F-ratio probability of $P<0.05$

No significant effect of water quality was found on RWC, FFM, oil yield and oil content. However, the number of leaves (*P*=0.01), plant height (*P*=0.05), number of branches (*P*=0.05), leaf area (*P*=0.01), chlorophyll content (*P*=0.05) and FFM (*P*=0.01) were significantly decreased at 4 mS cm^{-1} salt level (Table 5.2). Yield of rose geranium was reduced at 4 mS cm^{-1} salt level and this is associated with the toxicity effect of salt within the plant cell. According to Ozturk *et al*. (2004), plants can tolerate salt up to a certain threshold without any yield reduction. High salt conditions reduced the number of branches, plant height and number of flowers of chamomile (*Matricaria chamomilla* L.) (Dadkhah 2010). High salt conditions induce severe ion toxicity by depositing high levels of sodium in plant cells, causing the plant membrane to disorganise and thereafter inhibit cell division and cell expansion (Zhani *et al*. 2012). Vacuole is an antiporter that regulates sodium uptake; excess sodium ions will be transported and stored in the vacuole. High sodium ions disrupt the activities of enzymes in the plant cell (Blumwald 2000; Sonneveld 2000).

Plant height, number of branches, RWC, chlorophyll content, oil yield and oil content were also not significantly affected by the root media used (Table 5.2). However, root media affected the number of leaves (*P*=0.01), leaf area (*P*=0.01) and FFM (*P*=0.01) of rose geranium. The number of leaves, leaf area and FFM were increased to 451.83, 799.08 m^2 and 511.24 g $plant^{-1}$ where sawdust was used as a root medium, respectively. No significant interaction was found between salt levels and root media on the number of leaves, plant height, number of branches, leaf area, RWC, chlorophyll content, FFM, oil yield and oil content (Table 5.2). Sawdust is a common root medium used by most soil-less growers in South Africa, followed by peat, sand and gravel. Proper cultivation can increase the yield of plants when grown on a good substrate, using a balanced nutrient solution (Sedibe and Allemann 2012). Better response of rose geranium grown in sawdust is ascribed to sufficient root-zone moisture, suitable temperature and lower bulk density. According to Combrink (2005) and Raviv *et al*. (2002), sawdust offers better drainage and good aeration and it also provides plants with sufficient moisture, aeration and an optimum ratio between elements in the root zone. Alii fig (*Ficus binnendijkii* L.) and gypsophila (*Gypsophila paniculata* L.) developed better sprouts when sawdust was used as a substrate (Wahome *et al*. 2011; Shah *et al*. 2006).

Table 5.3 Effect of water quality, root media and time on stomatal conductance between days on rose geranium

Treatments	Stomatal conductance			
	Day 1	**Day 2**	**Day 3**	**Day 4**
Water quality (mS cm^{-1})				
1.6	59.53^{a}	45.89^{a}	57.46^{a}	60.76^{a}
2.4	57.56^{a}	53.74^{a}	48.25^{a}	55.47^{a}
3.2	43.59^{a}	41.57^{a}	56.35^{a}	48.73^{a}
4.0	40.70^{a}	48.44^{a}	44.79^{a}	49.00^{a}
LSD $_{T(0.05)}$	ns	ns	ns	ns
CV%	2.13	2.13	2.13	2.13
Root media				
Sand	43.36^{b}	47.99^{a}	49.35^{a}	53.15^{a}
Sawdust	57.33^{a}	46.83^{a}	54.07^{a}	53.83^{a}
LSD $_{T(0.05)}$	10.55**	ns	ns	ns
CV%	2.08	2.08	2.08	2.08
Time				
10h00	66.24^{a}	55.04^{a}	64.20^{a}	65.27^{a}
14h00	34.45^{b}	39.77^{b}	39.22^{b}	41.71^{b}
LSD $_{T(0.05)}$	11.98*	13.99**	11.43*	10.03*
CV%	2.02	2.02	2.02	2.02
Water quality x root media				
LSD $_{T(0.05)}$	ns	ns	25.08*	ns
CV%	2.08	2.08	2.08	2.08
Root media x time				
LSD $_{T(0.05)}$	16.95*	ns	ns	ns
CV%	2.02	2.02	2.02	2.02
Water quality x time				
LSD $_{T(0.05)}$	ns	ns	ns	ns
CV%	2.02	2.02	2.02	2.02
Water quality x root media x time				
LSD $_{T(0.05)}$	ns	ns	32.33*	ns
CV%	2.02	2.02	2.02	2.02

Means followed by the same letter in the same column are statistically non-significant at $P<0.05$ according to the least significant difference, ($LSD_{T0.05}$). CV% = coefficient of variation, ns = not significant at $P<0.05$, * F-ratio probability of $P<0.01$, ** F-ratio probability of $P<0.05$

There was no significant effect of salinity and root media on the stomatal conductance of rose geranium. Time of the day had a significant effect on stomatal conductance recorded on day 1 (P=0.01), day 2 (P=0.05), day 3 (P=0.01) and day 4 (P=0.01) (Table 5.3). Stomatal opening occurred mostly in the morning when light intensity was relatively low. The opening of stomata at 10:00 is directly associated to better gas exchange and low light fluxes (Sharkey and Raschke 1981). Besides the opening of the stomata, light intensity has an effect on the size of internodes, chlorophyll content, photosynthesis and CO_2 assimilation (Combrink 2005).

Table 5.4 Effect of water quality induced by NaCl and nutrient solution and root media on oil composition of rose geranium

Treatments	Oil composition (%)							
	Linalool	Iso-menthone	Citronellol	Geraniol	Citronelyll formate	Geranyl formate	Guaia-6, 9-diene	C:G ratio
Water quality(mS cm^{-1})								
1.6	1.23^{a}	1.78^{a}	31.51^{a}	13.71^{a}	21.21^{a}	7.98^{a}	9.37^{a}	2.35^{a}
2.4	1.46^{a}	1.95^{a}	31.84^{a}	12.70^{a}	21.55^{a}	7.61^{a}	9.37^{a}	2.54^{a}
3.2	1.48^{a}	2.18^{a}	32.48^{a}	12.62^{a}	21.66^{a}	7.63^{a}	9.51^{a}	2.60^{a}
4.0	1.10^{a}	1.48^{a}	35.19^{a}	11.10^{a}	22.73^{a}	6.53^{b}	9.49^{a}	3.18^{b}
LSD $_{T(0.05)}$	ns	ns	ns	ns	ns	0.94**	ns	0.40*
CV%	2.26	2.36	2.20	2.17	2.20	2.20	2.22	2.20
Root media								
Sand	1.04^{a}	2.54^{a}	32.58^{a}	12.57^{a}	21.97^{a}	7.53^{a}	9.71^{a}	2.66^{a}
Sawdust	1.39^{a}	1.66^{b}	32.98^{a}	12.50^{a}	21.60^{a}	7.32^{a}	9.16^{a}	2.68^{a}
LSD $_{T(0.05)}$	ns	0.79**	ns	ns	ns	Ns	ns	ns
CV%	2.26	2.36	2.20	2.17	2.20	2.20	2.22	2.20
Water qualityx root media								
LSD $_{T(0.05)}$	ns	ns	ns	ns	ns	Ns	ns	ns
CV%	2.26	2.36	2.20	2.17	2.20	2.20	2.22	2.20

Means followed by the same letter in the same column are statistically non-significant at $P<0.05$ according to the least significant difference, ($LSD_{T0.05}$). CV% = coefficient of variation, ns = not significant at $P<0.05$, * F-ratio probability of $P<0.01$, ** F-ratio probability of $P<0.05$

As shown in Table 5.4, salt had no significant effect on the linalool, iso-menthone, citronellol, geraniol, citronelyll formate and guaia-6,9-diene contents of rose geranium. Furthermore, root media had no significant effect on linalool, citronellol, geraniol, citronelyll formate, geranyl formate and guaia-6,9-diene. However, significant results were found on geranyl formate (*P*=0.05) and C:G ratio (*P*=0.01) at high salt level (4.0 mS cm^{-1}). Geranyl formate content was reduced at a high level of salt; consequently the C:G ratio was increased, thus lowering the oil quality. With the exception of iso-menthone (*P*=0.05), there was no significant interaction between salt and root media was recorded either. Most essential oil biosynthesis activities occur inside the cell and are stored in the vacuole (Blumwald 2000). Salt affect the biosynthesis of essential oil in the palisade cells. Sodium at high levels accumulates in the plant cell and interferes directly with the biosynthesis of essential oil in the vacuole. Literature was not found that explains in detail how salt affects the synthesis of geranyl formate. However, salt has favourable effects on the oil quality and disease resistance of plants (Stutte 2006; Shannon and Grieve 1999). The linalool content of basil and coriander was increased to respectively 57% and 45% at high salinitycontent (Attia *et al*. 2010). Good oil quality was reported on sage (*Salvia officinalis* L.), where high salt content increased oil quality (Taarit *et al*.2010).

5.4 Conclusion

It is evident from the study that rose geranium can only be grown using a nutrient solution that has an electric conductivity that is lower than 4 mS cm^{-1} and use sawdust root medium.

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CHAPTER 6

SALINITY EFFECTS ON EXTERNAL AND INTERNAL MORPHOLOGY OF ROSE GERANIUM (*Pelargonium graveolens* L.) LEAF

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Salinity effects on external and internal morphology of rose geranium (*Pelargonium graveolens* L.) leaf

Abstract

Salinity causes stress on plants, especially when soil salt levels are high. It limits crop metabolic activities and hampers plant growth and the synthesis of secondary metabolites. It also affects osmotic potential in the plant root zone. A complete randomised block design was used to evaluate the effect of salinity applied at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} on external and internal morphology of rose geranium (*Pelargonium graveolens* L.) leaf and treatments were replicated three times. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to evaluate the morphology of the leaf. Salinity levels induced the development of capitate trichome. The abaxial leaf position had a higher number of trichomes than the adaxial leaf position. A strong polynomial (r^2=0.97) relationship was found between capitate trichome and salinity. High densities of capitate trichomes were found when salinity conditions were high. Although the development of asciiform trichome was induced, it was at an insignificant level. Trichome densities are therefore not affected by salinity. It was thus concluded that rose geranium have some degree of tolerance to salinity.

6.1 Introduction

Salinity is an environmental condition that causes stress in plants, especially when soil salt levels are high. It limits crop metabolic activities and hampers plant growth and the synthesis of secondary metabolites. It also affects the osmotic potential in the root zone of plants (Baatour *et al*. 2012). It induces ion toxicity due to excessive uptake of sodium ions (El-Baz *et al*. 2003). The storage and pathway of essential oil in oil glands of sweet marjoram (*Origanum majorana* L.) and pennyroyal (*Mentha pulegium* L.) were affected by salinity. These effects take place in the plant cell, leaf surface and the trichomes. Salts present in irrigation water originate from dissolution or weathering of rocks and soil (lime, gypsum and other slowly dissolved soil minerals). The suitability of water for irrigation is determined not only by the total amount of salt present, but also by the kind of salt (Ayers and Westcot 1994).

Rose geranium produces two types of glandular trichomes, namely; brevicollate and asciiform trichomes, as well as non-glandular trichomes. Brevicollate trichomes are referred to as large trichomes and asciiform trichomes are referred to as small trichomes (Sedibe 2012; Motsa *et al*. 2006). Most of these trichomes are found on the adaxial and abaxial areas of a leaf surface (Sedibe 2012). Essential oils are stored in brevicollate and asciiform trichomes (Sedibe 2012; Motsa *et al*. 2006). Moreover, oil yield is correlated to leaf size and to foliar mass (Sedibe 2012).

Synthesis of the essential oils occurs in the plants cell, especially the palisade cells. Plant cells contain organelles such as bladder cells, which appear as huge vacuole and other cell organelles that are pushed onto the plant cell wall. Plant species have a specific genetic mechanism that controls the trichome morphology and density (Roy *et al*. 1999; Payne 1978). Most essential oil synthesis activities occur inside the cell and the results are stored in the vacuole (Blumwald 2000).

Environmental stress is said to have an effect on trichome density, as was found on different wormwood (*Artemisia annua* L. and *Artemisia campertris* L.) plants by Kjaer *et al*. (2012) and Ascensao and Pais (1987), respectively. This effect was never tested on rose geraniums; therefore, the objective of this study was to determine the effect of salinity applied at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} salt levels on the internal and external morphology of the rose geranium leaf.

6.2 Materials and methods

The materials and methods in chapter 3 outline all the chapter's materials and methods. Salinity levels, the field plan and harvest dates are outlined in chapter 5. This section of the material and methods describes only specific procedures used in this chapter.

6.2.1 Parameters

6.2.1.1 Leaf external morphology

Leaf microscopic data measurements were taken using SEM as was described by Eiasu (2009) and Motsa *et al*. (2006). Pieces of leaf samples (1 cm^2) were collected

from the leaf apex and fixed in a 3% glutardialdehyde (sodium phosphate buffer 0.1 M at pH 7.0) and post-fixed for two hours in osmium tetraoxide (1%), prior to rinsing with distilled water. These samples were dehydrated once with a series of ethanol concentrations of 50, 70, 95 and 100% (twice) for 15 minutes each, followed by drying in a Tousimis critical point drier apparatus (Bio-Rad E300 Rockville). Dried samples were mounted on aluminium stabs using double-sided adhesive tape and thereafter coated with gold using a vacuum unit (BIO-RAD Microscience Division coating system UK). The critical point dryer was pressured with CO_2 liquid at 37°C to replace ethanol. These samples were examined using a Shimadzu SSX-550 (Kyoto Japan) scanning electron microscope set at magnifications of x300 and x400 µm. The sizes and morphology of the glandular and non-glandular trichomes were distinguished by the description characteristics described by Sedibe (2012), Motsa *et al*. (2006) and Payne (1978).

6.2.1.2 Leaf internal morphology analysis

A sample of 1 cm^2 was collected from the apex of the leaf and fixed in a 0.1 M sodium phosphate buffer solution at pH 7, containing 3% of glutardialdehyde, and post-fixed for two hours in osmium tetraoxide (1%), followed by a wash with distilled water. The dehydration processes were followed with acetone in a series of 30, 50, 70 and 95% for 10-30 minutes in each stage. Acetone (100%) was used twice in a final dehydration process and each process lasted for 15-30 minutes. The dehydration process was followed by embedding the dehydrated samples with epoxy (100% for eight hours at 70°C in vacuum desiccators overnight) to make thin sections for the microscopy study. Sections were cut with Leica glass knives (EM KMR3) using a Leica ultra-microtome (EM UC7 [Vianna Austria] between 60-90 nm [1000 nanometer [nm] = 1 microtome]). Sections were stained with 6% uranyl acetate and lead citrate and rinsed with water. Philips (FEI The Netherlands) CM100 TEM was used to examine the sections (Zhang *et al*. 2012). The epidermal cell morphology was determined using digital images obtained from a computer mounted on the system (Eiasu 2009).

6.2.2 Data analysis

Collected data was analysed using the statistical procedure outlined in chapter 3 and the correlation was compared using Microdoft Excel software 2007.

6.3 Results and discussion

6.3.1 Leaf external morphology analysis

Three types of trichomes (asciiform, brevicollate and non-glandular trichomes) were described on rose geranium leaf by Sedibe (2012) and Motsa *et al*. (2006). Payne's (1978) description of trichomes was followed in this study. Four types of trichomes were observed on the leaves of rose geranium, i.e. asciiform, brevicollate, non-glandular trichomes and the type of trichomes not commonly found on rose geranium, capitate trichome. Capitate trichomes are characterised by elongated segment flask-shaped bodies that incorporate a round head (Figure 6.1 micrograph a).

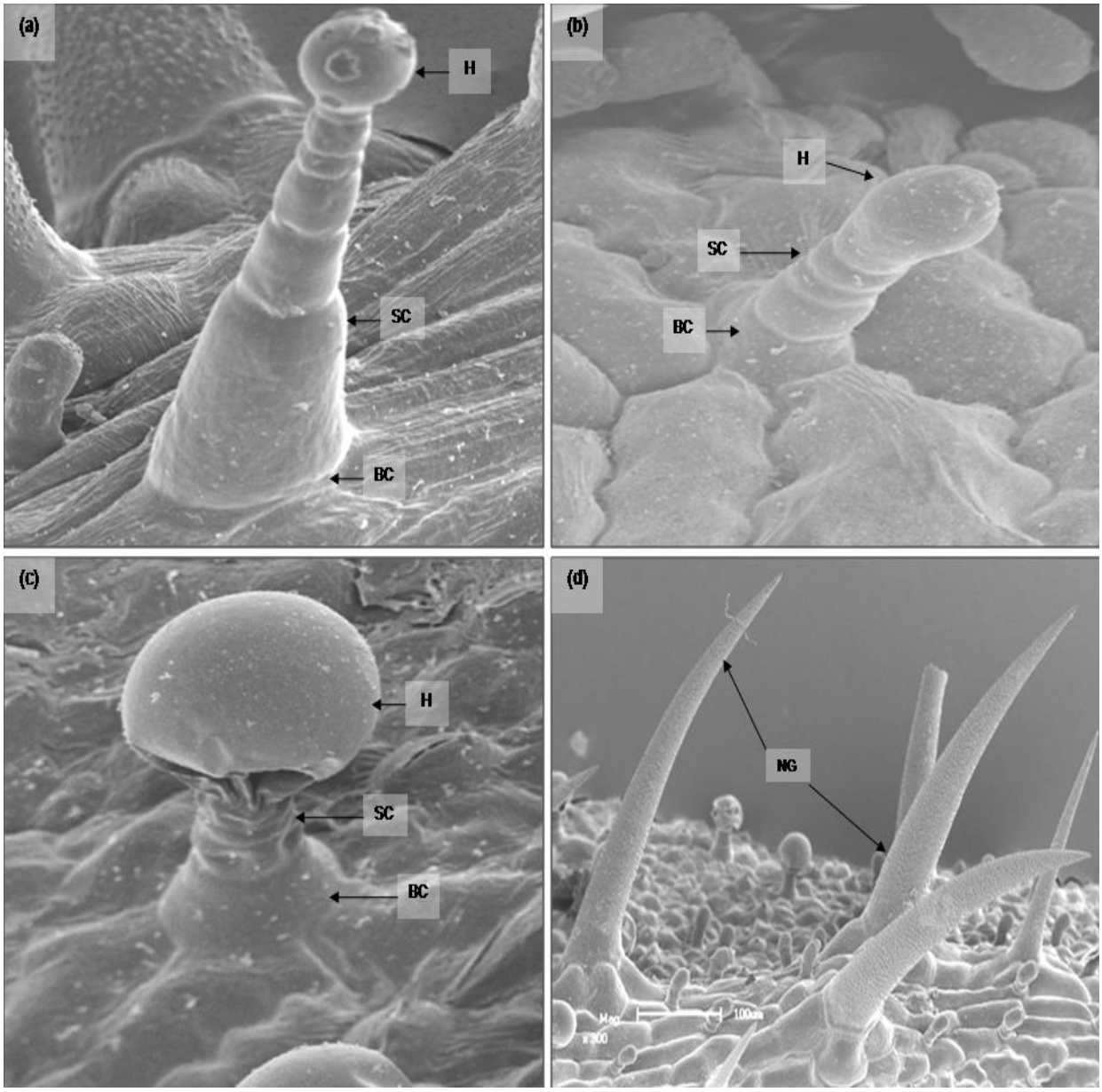

Figure 6.1 Different types of trichomes found on the leaf of rose geranium subjected to salinity levels , observed under scanning electron microscope (300x and 400x magnifications), Key: a) Capitate trichome b) Asciiform trichome c) Brevicollate trichome d) Non-glandular trichome (NG), Basal cell (BC), Unicellular stalk cell (SC) and Head (H) are relevant to all of a, b, c and d.

The densities of most trichomes were not affected by salinity. However, a strong polynomial (r^2=0.97) relationship was found between capitate trichome and salinity (Figure 6.2). Capitate trichomes' density was significantly increased at a high salinity

level of 4.0 mS cm^{-1} (Table 6.1), but this increase did not affect the total trichome density (data not presented). The development process of capitate trichome occurs through elongation of asciiform trichome (Tissier 2012). Trichome development is initiated by a hypertrophy process and an anti-clinal division of the protodermal cells followed by the development of glandular head cells. Growth on the epidermis occurs through a basal cell (Sugiyama *et al*. 2006; Berta *et al*. 1993). Although the developmental stages of this parameter were not measured in this study, the formation of asciiform trichome is attributed to the effect of salinity during the development stage of this trichome.

The trichome head grows round during the first stage of development; thereafter, oil accumulation occurs (Sugiyama *et al*. 2006). To date, no literature has been found that shows the effect of salinity during cell division of trichomes during developmental stages. However, increased trichome density due to salinity was reported on pennyroyal and sweet marjoram by Baatour *et al*. (2012) and Karray-Bouraoui *et al*. (2009), respectively.

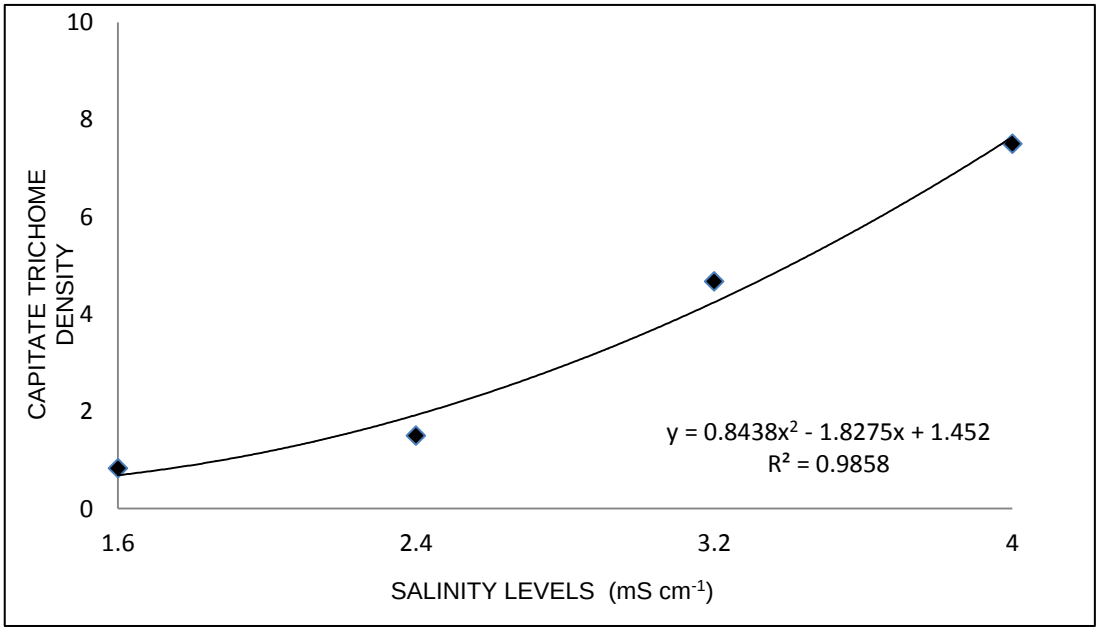

Figure 6.2 Polynomial relationship between capitate trichome density and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm^{-1}).

Table 6.1 The effect of salinity on trichome density of rose geranium

Treatments	Trichome density			
	Brevicollate	**Asciiform**	**Capitate**	**Non-glandular**
Salinity (mS cm^{-1})				
1.6	74.83^{a}	86.50^{a}	0.83^{c}	55.67^{a}
2.4	55.83^{a}	105.00^{a}	1.50^{bc}	51.67^{a}
3.2	60.83^{a}	92.00^{a}	4 .67^{ab}	39.83^{a}
4.0	59.83^{a}	108.00^{a}	7.50^{a}	50.83^{a}
LSD $_{T(0.05)}$	ns	ns	3.27*	ns
CV%	2.14	2.14	2.14	2.14

Means followed by the same letter in the same column are statistically non-significant at $P<0.05$ according to the least significant difference, (LSDT0.05), CV% = coefficient of variation, ns = not significant at $P<0.05$, * F-ratio probability of $P<0.01$

As shown in Figure 6.3, the density of trichomes on the leaf surface varies according to the trichome type. Most glandular and non-glandular trichomes occurred on the abaxial leaf surface of rose geranium. The asciiform trichome and capitate trichome were not significantly influenced by the leaf position. Most brevicollate and non-glandular trichome densities were significantly increased on the abaxial leaf surface. A similar trend on the density of trichomes was reported on wormwood subjected to external stress (Kjaer *et al*. 2012; Ascensao and Pais 1987).

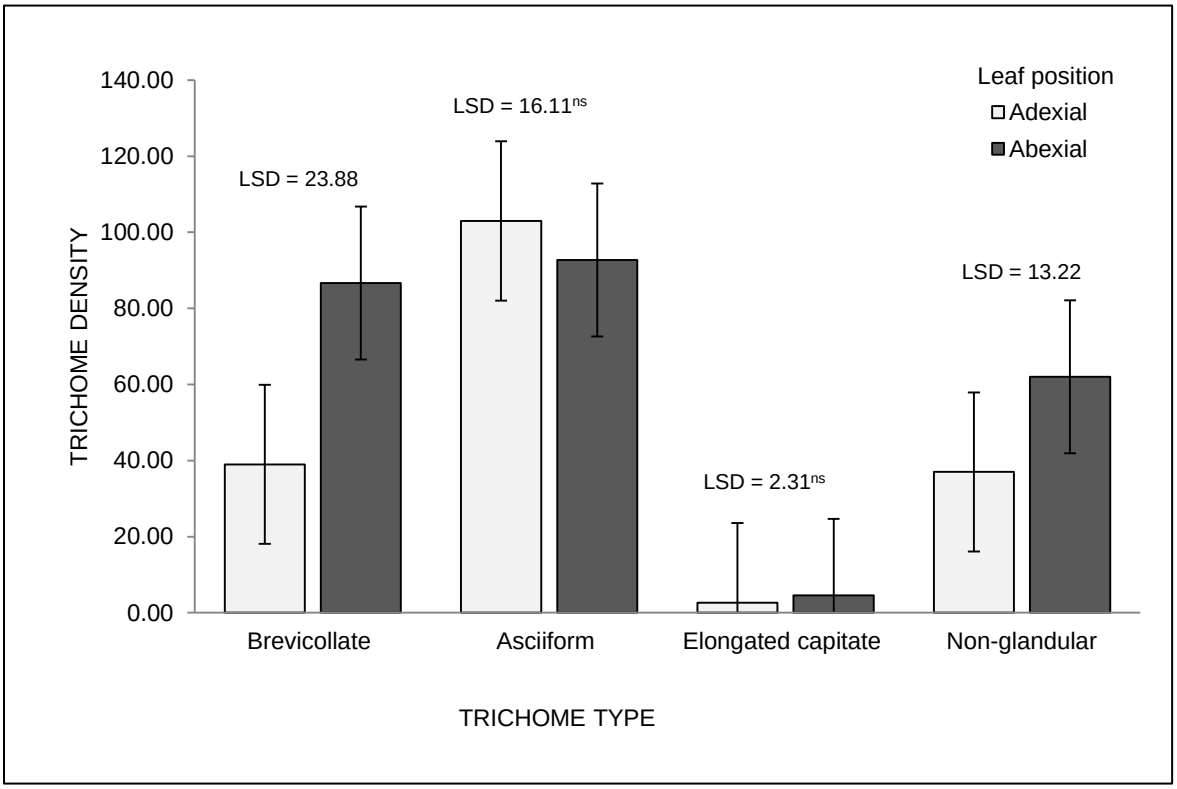

Figure 6.3 Trichome densities measured on the adaxial and abaxial leaf surface of rose geranium subjected to salinity at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} salt levels.

6.3.2 Leaf internal morphology

The micrographs in Figure 6.4 (micrographs a to d) show the effect of salinity on epidermal cell morphology of rose geranium leaf. Plant leaves develop a thicker upper

palisade, spongy parenchyma, a thicker lower epidermis and densely spaced trichomes on plants subjected to stress (Ennajeh *et al*. 2010).

In Figure 6.4 (micrograph a) the section illustrated with darker coating is associated with stored essential oils (EO) on the surface of the tonoplast. Micrograph b, illustrates a sac associated with EO in the vacuole, which was visible where salinity was applied at 2.4 mS cm^{-1} salt level. Furthermore, on micrograph b the edge of the palisade cell, an organic cellular activity (OCA) demonstrates activities associated with the biosynthesis of EO on the surface of the plasma membrane (Kjaer *et al*. 2012; Marty 1999). At level 3.2 and 4.0 mS cm^{-1} salt level, PALs show a decay of the EO compounds on the surface of tonoplast and this effect is attributed to increased salt levels micrograph a to d which is similar to results reported by Horie and Schroeder 2004.

Most activities are associated with biosynthesis of EO and take place within a specialised section located in the PALs, cell organelles, cytosol, mitochondria (MIT) and chloroplasts (CHL) (Kjaer *et al*. 2012; Marty 1999). Accumulation of EO in the trichome head occurs during the third stage of trichome formation, whereby EO accumulates in the trichome head directly from the vacuole of the palisade mesophyll cell (Kjaer *et al*. 2012; Sugiyama *et al*. 2006). The accumulated EOs are identified by dark patches around the tonoplast of the PAL micrograph a to d.

The effect of salinity on the internal morphology of the trichome was not investigated; only the cross-sections of rose geranium leaf internal morphology were observed to illustrate the accumulation of EO on the epidermal cells. The synthesis and storage of EO has been outlined; salinity has proven to have an effect on the storage of EO rose geranium leaf cells micrograph a to d.

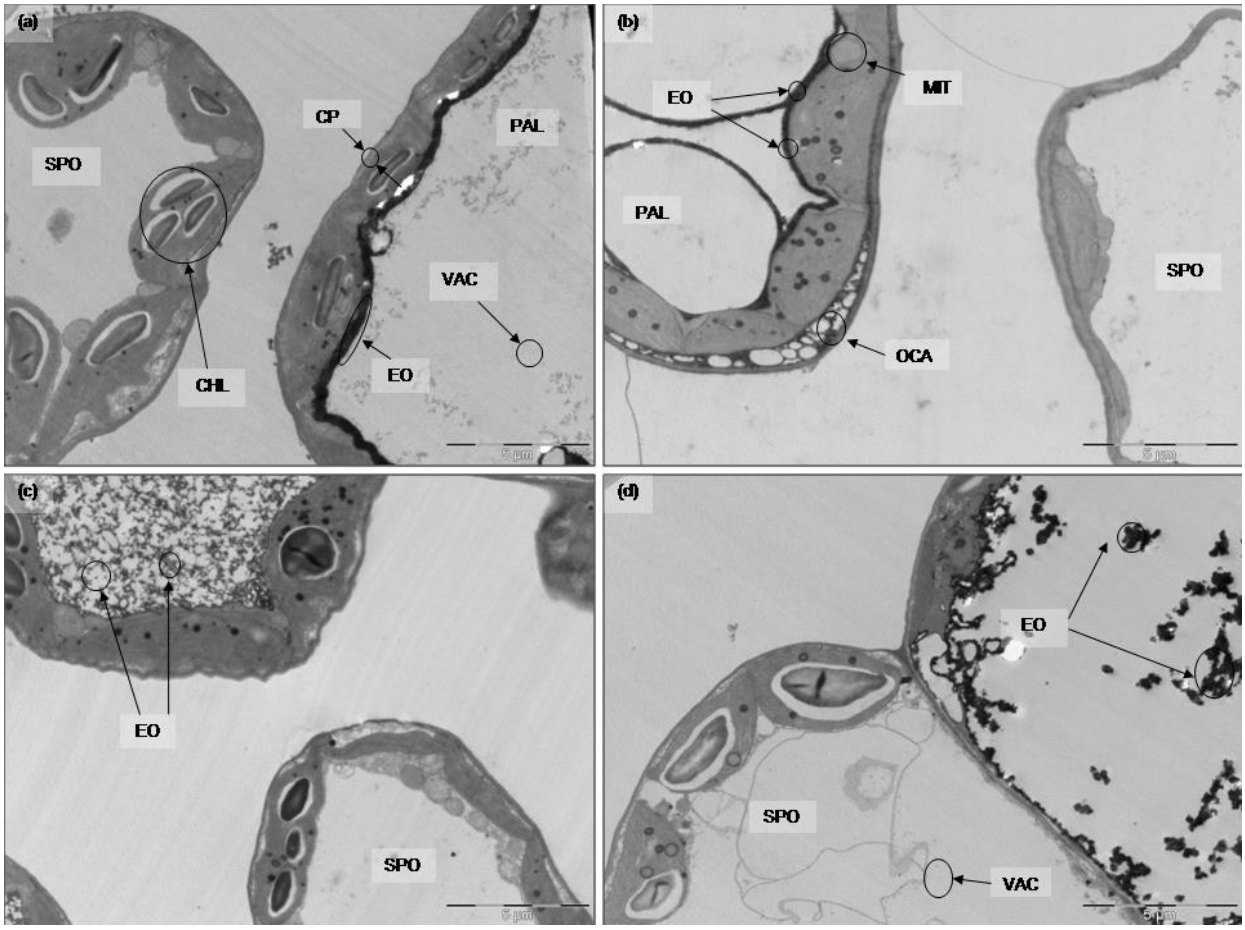

Figure 6.4 Internal leaf morphology of rose geranium subjected to salinity at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} observed under transmission electron microscopy (5 µm) showing SPO (spongy mesophyll cell), PAL (palisade mesophyll cell), CHL (chloroplast with starch grains), MIT, VAC (vacuole), EO, OCA and CP (cell wall and plasma membrane).

The cell vacuole regulates cytoplasmic ions and pH in the plant cell (Marty 1999). Therefore, the essential oil observed on the surface of the tonoplast where salts were applied at 1.6 and 2.4 mS cm^{-1} in Figure 6.4 (micrograph a and b) explains vacuole functions as an anti-porter that has been reported to play a significant role in salt regulation (Xu *et al*. 2009). Vacuole regulates sodium uptake, whereby excess sodium ions are transported and stored in the vacuole (Blumwald 2000). Moreover, micrograph c and d shows the decayed EO compound as a result of increased salt levels found at 3.2 and 4.0 mS cm^{-1} and this is associated with plant nutrient deficiency stress (El-Baz *et al*. 2003).

6.4 Conclusion

Salinity induced the development and density of capitate trichome. However, the density of most trichomes was not affected; it was therefore concluded that rose geranium trichomes' morphology are not detrimentally affected by salinity.

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CHAPTER 7

GENERAL CONCLUSIONS AND RECOMMENDATIONS

General conclusion and recommendations

Field experiments with various potassium concentrations need to be carried out in future. The current recommendations are for growth of rose geranium in hydroponic systems by growing rose geranium in a nutrient solution that has an electric conductivity that is lower than 4 mS cm^{-1}. Potassium should be applied at 5.3 mmol K L^{-1} (pH 5.5) using 5 L potting bag and sawdust as a root medium. Optimum growth conditions for high quality essential oil production in open fields may differ with the use of potassium.

Appendix A

Table A1: Summary of ANOVA table for plant height, number of branches, B:H ratio, leaf area, chlorophyll, foliar fresh mass, oil yield and oil content of rose geranium

Treatments	DF	*P*=value of parameters							
		Plant height (cm)	Number of branches (plant^{-1})	B:H ratio	Leaf area (cm^2)	Chloro-phyll (%)	Foliar fresh mass (g plant^{-1})	Oil yield (g plant^{-1})	Oil content (%)
Main plot									
Potassium (mmol L^{-1})	**3**	**	ns	ns	ns	ns	ns	ns	ns
Error	**6**								
Sub plot									
Potting bag (L)	**1**	*	*	*	ns	ns	*	*	ns
Potassium x potting bag	**3**	ns	ns	ns	ns	ns	**	ns	ns
Error	**8**								

Means followed by the same letter in the same column are statistically non-significant at *P*<0.05 according to the least significant difference, ($LSD_{T0.05}$), ns = not significant at *P*<0.05, * *F*-ratio probability of *P*<0.01, ** *F*-ratio probability of *P*<0.05.

Table A2: Summary of ANOVA table for nitrogen, phosphate, potassium and sulphate composition content of rose geranium

		P=value of plant minerals (%)			
Treatments	**DF**	**N**	**P**	**K**	**S**
Main plot					
Potassium (mmol L^{-1})	**3**	ns	ns	*	ns
Error	**6**				
Sub plot					
Potting bag (L)	**1**	ns	ns	*	ns
Potassium x potting bag	**3**	ns	ns	**	ns
Error	**8**				

Means followed by the same letter in the same column are statistically non-significant at *P*<0.05 according to the least significant difference ($LSD_{T0.05}$), , ns = not significant at *P*<0.05, * *F*-ratio probability of *P*<0.01, ** *F*-ratio probability of *P*<0.05

Table A2: Summary of ANOVA table for oil composition of rose geranium

Treatments	DF	*P*=value of oil composition (%)									
		Linalool	Roseoxide (*cis*)	Roseoxide (*trans*)	Iso-menthone	Citronellol (C)	Geraniol (G)	Citronellyl ɔrmate	Geranyl formate	Guaia -6,9-diene	C:G ratio
Potassium	**3**	**	ns	ns	ns	ns	*	ns	*	ns	*
Error	**6**										

Means followed by the same letter in the same column are statistically non-significant at $P<0.05$ according to the least significant difference ($LSD_{T0.05}$), , ns = not significant at $P<0.05$, * *F*-ratio probability of $P<0.01$, ** *F*-ratio probability of $P<0.05$

Table B1: Summary of ANOVA table for number of leaves, plant height, number of branches, leaf area, relative water content, chlorophyll, foliar fresh mass, oil yield and oil content of rose geranium

Treatments	DF	*P*=value of parameters								
		Number of leaves (plant^{-1})	Plant height (cm)	Number of branches (plant^{-1})	Leaf area (cm^2)	Relative water content	Chloro - phyll (%)	Foliar fresh mass (g plant^{-1})	Oil yield (g plant^{-1})	Oil content (%)
Main plot										
Salt (mS cm^{-1})	**3**	*	**	**	*	ns	**	ns	ns	ns
Error	**16**									
Sub plot										
Root media	**1**	*	ns	ns	*	ns	ns	*	ns	ns
Salt x root media	**3**	ns	ns	ns	ns	ns	ns	ns	ns	ns
Error	**16**									

Means followed by the same letter in the same column are statistically non-significant at *P*<0.05 according to the least significant difference ($LSD_{T0.05}$), ns = not significant at *P*<0.05, * *F*-ratio probability of *P*<0.01, ** *F*-ratio probability of *P*<0.05

Table B2: Summary of ANOVA table for on stomatal conductance between days on rose geranium

Treatments		*P*=value of stomatal conductance			
	DF	**Day 1**	**Day 2**	**Day 3**	**Day 4**
Salt level (mS cm^{-1})	**3**	ns	ns	ns	ns
Root media	**1**	**	ns	ns	ns
Time	**1**	*	**	*	*
Salt x root media	**3**	ns	ns	*	ns
Root media x time	**1**	*	ns	ns	ns
Salt x time	**3**	ns	ns	ns	ns
Salt x root media x time	**3**	ns	ns	*	ns
Error	**40**				

Means followed by the same letter in the same column are statistically non-significant at $P<0.05$ according to the least significant difference ($LSD_{T0.05}$), ns = not significant at $P<0.05$, * *F*-ratio probability of $P<0.01$, ** *F*-ratio probability of $P<0.05$

Table B3: Summary of ANOVA table for oil composition of rose geranium

Treatments	DF	*P*=value of oil composition (%)							
		Linalool	Iso-menthone	Citronellol	Geraniol	Citronelyll formate	Geranyl formate	Guaia-6, 9-diene	C:G ratio
Main plot									
Salt level (mS cm^{-1})	**3**	ns	ns	ns	ns	ns	**	ns	*
Error	**11**								
Sub plot									
Root media	**1**	ns	**	ns	ns	ns	ns	ns	ns
Salt x root media	**3**	ns	ns	ns	ns	ns	ns	ns	ns
Error	**9**								

Means followed by the same letter in the same column are statistically non-significant at *P*<0.05 according to the least significant difference ($LSD_{T0.05}$), ns = not significant at *P*<0.05, * *F*-ratio probability of *P*<0.01, ** *F*-ratio probability of *P*<0.05

Table C1: Summary of ANOVA table for trichome density of rose geranium

Treatments	DF	*P*=value of trichome density			
		Brevicollate	**Asciiform**	**Capitate**	**Non-glandular**
Salt level (mS cm^{-1})	**3**	ns	ns	*	ns
Error	**14**				

Means followed by the same letter in the same column are statistically non-significant at $P<0.05$ according to the least significant difference ($LSD_{T0.05}$), ns = not significant at $P<0.05$, * *F*-ratio probability of $P<0.01$, ** *F*-ratio probability of $P<0.05$

Appendix B

Salinity Effects on External and Internal Morphology of Rose Geranium (*Pelargonium graveolens*L.) Leaf

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Abstract: Salinity is an environmental condition that induces stress on plants especially under high soil salts levels. It limits crop metabolic activities, hampers plant growth and synthesis of secondary metabolites. It also affects osmotic potential in the plant root zone. A complete randomized block design was used to evaluate the effect of salinity applied at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} on external and internal morphology of rose geranium (*Pelargonium graveolens* L.) leaf and treatments were replicated three times. Scanning electron microscopy (SEM) and transmission electron microscopy (TEM) were used to evaluate the morphology of the leaf. Salinity levels induced the development of capitate trichome. Abaxial leaf position had the highest number of trichomes than the adaxial leaf position. A strong polynomial (r^2=0.97) relationship was found between capitate trichome and salinity. High densities of capitate trichomes were found at a high salinity level. Although the development of asciiform trichome was induced, it was in an insignificant level, trichomes densities are therefore not affected by salinity. It was therefore concluded that rose geranium might have some degree of tolerance to salinity.
[Sedibe MM, Khetsha ZP, Malebo N**. Salinity Effects on External and Internal Morphology of Rose Geranium (*Pelargonium graveolens* L.) Leaf.** *Life Sci J* 2013;10(11s):99-103] (ISSN:1097-8135). http://www.lifesciencesite.com. 18

Keywords: Rose geranium, salinity, SEM, TEM, trichome

1. Introduction

Salinity is an environmental condition that induces stress on plants especially under high soil salts levels. It limits crop metabolic activities, hampers plant growth and synthesis of secondary metabolites. It also affects the osmotic potential in the root zoneof plants (Baatour et al. 2012). It induces ion toxicity due to excessive uptake of sodium ions (El-Baz et al. 2003). The storage and pathway of essential oil to the oil glands synthesis of sweet marjoram (*Origanummajorana*L.) and pennyroyal (*Menthapulegium*L.) were affected by salinity. These effects take place in the plant cell, leaf surface and the trichomes. Salts present in irrigation water originate from dissolution or weathering of the rocks and soil (lime, gypsum and other slowly dissolved soil minerals). The suitability of water for irrigation is determined not only by the total amount of salt present but also by the kind of salt (Ayers and Westcot 1994).

Rose geranium produces two types of glandular trichomes; brevicollate, asciiform trichomes and the non-glandular trichomes. Brevicollatetrichomes are classified as large trichomes and asciiform trichomes are classified as small trichomes (Sedibe 2012; Motsa et al. 2006). Most of these trichomes are found on the adaxial and abaxial of a leaf surface (Sedibe 2012). Essential oil are only stored on brevicollate and asciiform trichomes (Sedibe 2012; Motsa et al. 2006). Moreover, oil yield is correlated to leaf size and to foliage yield (Sedibe 2012).

Synthesis of the essential oils occurs within the plants cell, especially the palisade cells. Plant cells contain organelles such as bladder cells which appear as huge vacuole and other cell organelles which are pushed onto the plant cell wall. Most essential oil synthesis activities occur inside the cell and stored in the vacuole (Blumwald 2000). Plants species have a specific genetic mechanism that control the trichome morphology and density (Roy et al. 1999; Payne 1978).

Environmental stress are reported to have an effect on trichomes density as was found on wormwood (*Artemisia annua* L.)and wormwood (*Artemisia campertris*L.) plants by Kjaer et al. (2012) andAscensao and Pais (1987), respectively. This effect was never tested on rose geraniums, therefore, the objective of this study was to determine the effect of salinity applied at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} salts levels on internal and external morphology of rose geranium leaf.

2. Material and Methods

The experimental plots were laid out in a randomized complete block design. Four levels of salinity induced by NaCl were evaluated at 1.68, 2.40, 3.20 and 4.0 mS cm^{-1} salts levels (Table 1). Salinity was initiated gradually on a weekly sequence on the third month after transplanting at 25, 50, 75 and 100% until constant levels were met for all the levels. The pH was maintained at 5.5 for all the experimental units. The study was conducted under a

corrugated fiber glass plant house described by Sedibe and Allemann (2012).

Table 1 Macro-nutrients concentrations and micro-nutrients used in the salinity study subjected to 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} salinity level

Cations					
Salinity	Na^+	NH_4^+	K^+	Ca^+	Mg^{2+}
1.6	1.31	1	5.5	6.5	2.5
2.4	8.49	1	5.5	6.5	2.5
3.2	16.5	1	5.5	6.5	2.5
4.0	24.5	1	5.5	6.5	2.5
Anions					
Salinity	NO_3^-	$H_2PO_4^-$	SO_4^{2-}	Cl^-	HCO_3^-
1.6	11.04	0.1	3.8	1.31	0.4
2.4	11.04	0.1	3.8	8.49	0.4
3.2	11.04	0.1	3.8	16.5	0.4
4.0	11.04	0.1	3.8	24.5	0.4
Micro					
Salinity	Fe^{2+}	Mn^{3+}	B	Cu^{2+}	Mo^{2+}
1.6	1.12	0.54	0.03	0.02	0.05
2.4	1.12	0.54	0.03	0.02	0.05
3.2	1.12	0.54	0.03	0.02	0.05
4.0	1.12	0.54	0.03	0.02	0.05

Rooted rose geranium cuttings were transplanted during autumn and pieces of leaf samples were harvested in summer. Irrigation system was scheduled using methods of Sedibe (2012) in a custom built small scale growing units (450 x 800 x 215 mm). The irrigation systems had six dripper tubings with a flow rate of 4 L h^{-1}; these drippers were allocated to 6 potted plants.

Leaf external morphology analysis

Leaf microscopic data measurements were conducted using a scanning electron microscopy (SEM) a described by Eiasu (2009) and Motsa et al. (2006). Pieces of leaf samples (1 cm^2) were collected from the leaf apex and fixed in a 3% glutardialdehyde (sodium phosphate buffer 0.1 M at pH 7.0) and post fixed for 2 hours in osmium tetraoxide (1%), prior rinsing with distilled water. These samples were dehydrated once with a series of ethanol concentrations of 50, 70, 95 and 100% (twice) for 15 minutes each, followed by drying in a Tousimis critical point drier apparatus (Bio-Rad E300 Rockville). Dried samples were mounted on aluminium stabs using double-sided adhesive tape and thereafter coated with gold using a vacuum unit (BIO-RAD Microscience Division coating system United Kingdom). The critical point dryer was pressured with CO_2 liquid at 37°C to replace ethanol. These samples were examined using a Shimadzu SSX-550 (Kyoto Japan) scanning electron microscope set at the magnifications of x300 and x400 µm. Sizes and morphology of the glandular and non-glandular trichomes were distinguished by the description characteristics described by Sedibe (2012), Motsa et al. (2006) and Payne (1978).

Leaf internal morphology analysis

A sample of 1 cm^2 was collected from the apex of the leaf and fixed in a 0.1 M sodium phosphate buffer solution at pH 7 containing 3% of glutardialdehyde and post fixed for 2 hours in osmium tetraoxide (1%), followed by a wash with distilled water. The dehydration processes were followed with acetone in a series of 30, 50, 70 and 95% for 10-30 minutes in each stage. Acetone (100%) was conducted twice for a final dehydration process and each process lasted for 15-30 minutes. The process was followed by embedding the dehydrated samples with epoxy (100% for 8 hours at 70°C in vacuum desiccators overnight) to make thin sections for the microscopy study. Sections were cut with Leica glass knives (EM KMR3) using a Leica ultra-microtome (EM UC7 [Vienna, Austria] between 60-90 nm (1000 nanometer [nm] = 1 microtome)). Sections were stained with 6% uranyl acetate and lead citrate and rinsed with water. A Philips (FEI Netherlands) CM100 transmission electron microscopy (TEM) was used for examination of the sections (Zhang et al. 2012). The epidermal cell morphology was determined using digital images obtained from a computer mounted on the system (Eiasu 2009).

Analysis of variance was determined using the general linear model (GLM) of SAS statistical software version 9.2 (SAS 2008). Significant results were compared using Tukey's least significant difference (LSD_T), described by Steel and Tourie (1980). Statistically difference between treatment means was determined at the 5% level of significance.

3. Results and Discussion

Leaf external morphology

Three types of trichomes (asciiform, brevicollate and non-glandular trichomes) were described on rose geranium leaf by Sedibe (2012) and Motsa et al. (2006). Payne (1978) described different types of trichomes which were specifically described in this study. Four types of trichomes were observed on the leaves of rose geranium i.e. asciiform, brevicollate, non-glandular trichomes and the type of trichomes not commonly found on rose geranium, capitate trichome. Capitate trichomes are characterized by an elongated segment flask-shape body that consist of a round head (Figure 1a).

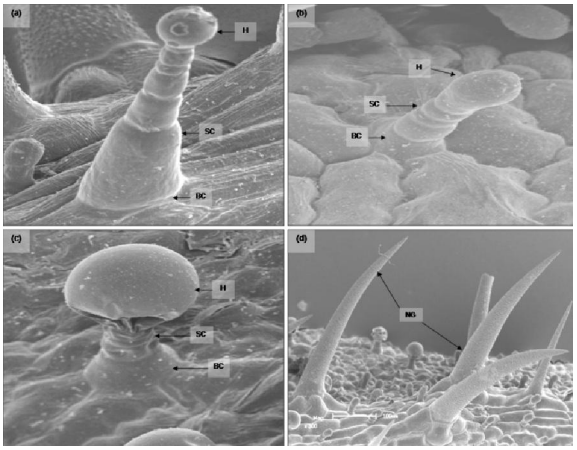

Figure 1. Different types of trichomes found on the leaf of rose geranium subjected to salinity at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} salt levels , observed under scanning electron microscope (300x and 400x magnifications) **a)** Capitate trichome **b)** Asciiform trichome **c)** Brevicollate trichome **d)** Non-glandular trichome (NG), Basal cell (BC), Unicellular stalk cell (SC), Head (H)

The densities of most trichomes were not affected by salinity. However, there was a strong polynomial (r^2=0.97) relationship found between capitate trichome and salinity (Figure 2). Capitate trichomes density were significantly increased at a high salinity level of 4.0 mS cm^{-1}, but this increase did not affect the total trichome density (data not presented) (Table 2). The development process of capitate trichome occurs through elongation of asciiform trichome (Tisser 2012). Trichome development is initiated by a hypertrophy process and an anti-clinal division of the protodermal cells followed by the development of glandular head cells. Growth on the epidermis occurs through a basal cell (Sugiyama et al. 2006; Berta et al. 1993). Although the developmental stages of this parameter was not measured in this study. The formation of asciiform trichome is attributed to the effect of salinity during the development stage of this trichome. The trichome head grow round during the first stage of development, thereafter, followed by oil accumulation (Sugiyama et al. 2006). To date, no literature found has shown the effect of salinity during cell division of trichomes during developmental stages. However, increased trichomes density due to salinity was reported on pennyroyal and sweet marjoram by Baatour et al. (2012) and Karray et al. (2009), respectively.

As shown in Figure 3 the density of trichomes on the leaf surface varied according to the trichome type. Most glandular and non-glandular trichomes occurred on the abaxial leaf surface of rose geranium (Figure 3). The asciiform trichome and capitate trichome were not significantly influenced by the leaf position. Most brevicollate and non-glandular trichomes densities were significantly increased on the abaxial leaf surface (Figure 3). Asimilar trend on density of trichomes was also reported on wormwood subjected to external stress (Kjaer et al. 2012; Ascensao and Pais 1987).

Table 2 The effect of salinity (1.6, 2.4, 3.2 and 4.0 mS cm^{-1}) on trichome density of rose geranium

Salinity	Trichome density			
	Brevicollate	Asciiform	Capitate	Non-glandular
1.6	74.83^{a}	86.50^{a}	0.83^{c}	55.67^{a}
2.4	55.83^{a}	105.00^{a}	1.50^{bc}	51.67^{a}
3.2	60.83^{a}	92.00^{a}	4.67^{ab}	39.83^{a}
4.0	59.83^{a}	108.00^{a}	7.50^{a}	50.83^{a}
P value	***ns***	***ns***	***0.01****	***ns***
LSD $_{T(0.05)}$	***33.77***	***22.78***	***3.27***	***18.69***
CV%	***2.14***	***2.14***	***2.14***	***2.14***

Means followed by the same letter in the same column are statistically non-significant at P<0.05 according to the least significant difference, ($LSD_{T0.05}$), CV% = coefficient of variation, ns = not significant at P<0.05, *F-ratio probability of P<0.01

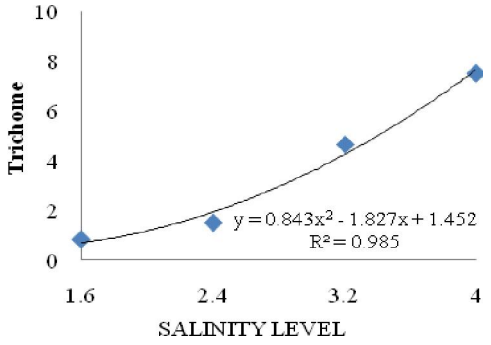

Figure 2. Polynomial relationship between capitate trichome density and salinity levels (1.6, 2.4, 3.2 and 4.0 mS cm^{-1})

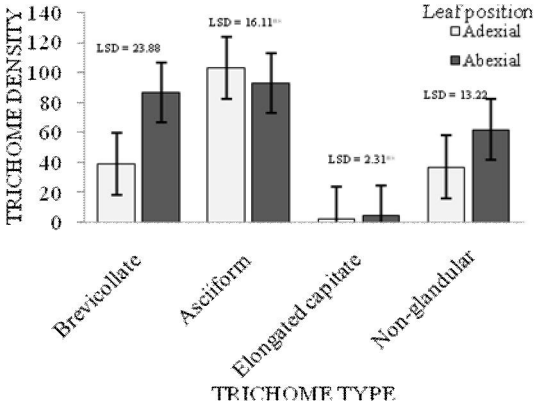

Figure 3.Trichome density measured on the adaxial and abaxial leaf surface of rose geranium subjected to salinity at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} salt levels

Leaf internal morphology

Plant leaves develop thicker upper palisade, spongy parenchyma, a thicker lower epidermis and densely spaced trichomes on plants subjected to stress (Ennajeh et al. 2010). The transmission electron micrograph in Figure 4 (a-d) shows the effect of salinity on epidermal cells morphology of rose geranium leaf. In Figure 4 (micrograph a) 1.6 mS cm^{-1} illustrated with darker coating is associated with stored essential oil (EO) on the surface of the tonoplast. Figure 4 (micrograph b), illustrates a sac associated with EO in the vacuole, which is visible where salinity was applied at 2.4 mS cm^{-1} salt level. Furthermore, on Figure 4 (micrograph b) the edge of the palisade cell, an organic cellular activity (OCA) demonstrates biosynthesis of EO on the surface of the plasma-membrane (Kjaer et al. 2012; Marty 1999). In level 3.2 and 4.0 mS cm^{-1} salt level, palisade cells shows a decay of the EO compounds on the surface of tonoplast and this effect is attributed to increased salt levels (Figure 4 micrograph a to d) (Horie and Schroeder 2004).

Most activities are associated with biosynthesis of EO and they take place within a specialized section located in the palisade cells (PAL), cell organelles, cytosol, mitochondria (MIT), and chloroplasts (CHL) (Kjaer et al. 2012; Marty 1999). Accumulation of EO in the trichome head occur during the third stage of trichome formation, whereby, EO accumulate in the trichome head directly from the vacuole of the palisade mesophyll cell (Kjaer et al. 2012; Sugiyama et al. 2006). The accumulated EO are identified by dark patches around the tonoplast of the palisade cells (Figure 4 micrograph a-d).

Although the effect of salinity on internal morphology of trichome was not investigated in this study, only the cross section of rose geranium leaf internal morphology was observed to illustrate the accumulation of EO on the epidermal cells. The synthesis and storage of EO has been outlined, salinity has shown to have an effects on the storage of EO rose geranium leaf cells (Figure 4 micrograph a to d).

Cell vacuole regulates of cytoplasmic ions and pH within the plant cell (Marty 1999). Essential oil observed on the surface of the tonoplast where salts was applied at 1.6 and 2.4 mS cm^{-1} in Figure 4 (micrograph a and b) give explanation of vacuole functions as an anti-porter and has been reported to play a significant role in salt regulation (Xu et al. 2009). Vacuole regulates Na uptake, whereby excess Na ions are transported and stored in the vacuole (Blumwald 2000). Moreover, Figure 4 (micrograph c and d) shows the decayed EO compound as a result of increased salt levels found at 3.2 and 4.0 mS cm^{-1} and this is associated to plant nutrient deficiency stress (El-Baz et al. 2003).

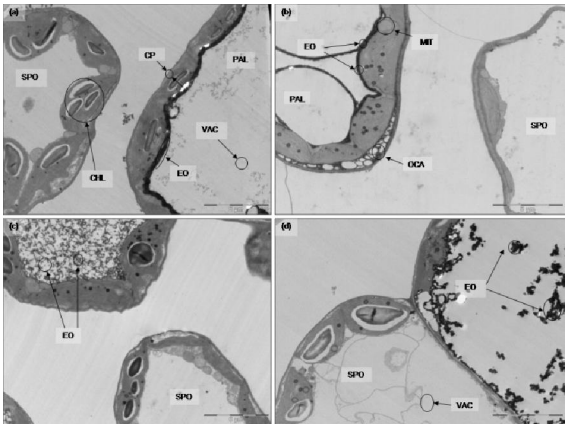

Figure 4. Internal leaf morphology of rose geranium subjected to salinity at 1.6, 2.4, 3.2 and 4.0 mS cm^{-1} salt levels observed under transmission electron microscopy (5 µm) showing SPO (Spongy mesophyll cell), PAL (palisade mesophyll cell), CHL (chloroplast with starch grains), MIT (mitochondria), VAC (vacuole), EO (essential oil), OCA (organic cellular activity), CP (cell wall and plasma membrane)

Conclusions

Salinity induced the development and density of capitate trichome. However, the density of most trichomes types were not affected, it was therefore concluded that rose geranium might have some degree of tolerance to salt stress.

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