

Rose-scented geranium (*Pelargonium* spp.) herbage yield, essential oil yield and composition as influenced by nitrogen nutrition and liming

by

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**Submitted in partial fulfilment of the requirements for the degree
Doctor of Philosophy: Horticultural Science**

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February 2012

DECLARATION

I, Hintsa Tesfamicael Araya declare that this thesis, which I hereby submit for the degree of Doctor of Philosophy in Horticultural Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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ACKNOWLEDGEMENTS

I thank God for His wisdom and guidance throughout my life and study.

This work would not have been complete without an encouragement and contribution of the many individuals and organizations.

I would like to express my sincere thanks and deepest gratitude to my supervisor Prof. P. Soundy and Co-supervisor Prof. J.M. Steyn for their support, patient guidance, encouragement and excellent advice throughout my studies. I am sincerely indebted to their constructive criticism, guidance and financial support from their projects after my scholarship halted. This work is the way it is today because of them.

My grateful acknowledgment goes to Technology and Human Resources for Industry Programme (THRIP), Biosys Plant Extracts (Pty) Ltd and Clive Teubes CC for funding this project. I would like to thank Mr R.A. Mojela for distillation of the plant material and for arranging analysis of the essential oils.

My special thanks goes to all my friends and colleagues, including Melake Kesete, Solomon Beyene, Eyob Habte, Micheal Ghebre-Meskel, Amanuel Bokhre, Goitom Teklay, Besrat Tesfagiorgis and Jon McLea for being there for me whenever I needed their support.

I am thankful to staff members of the Department of Plant Production and Soil Science, particularly Mrs JJ Herman and her family for all their support. I am also indebted to the managers, technicians and daily labourers at the Hatfield Experimental Farm, University of Pretoria, for their technical support.

To my wife Nadia and our daughter Sienna, thank you for all the love, encouragement, and support throughout the study. Last but not least, I would like to show my gratitude to my parents and my siblings who were so generous with their encouragement and prayers throughout the study.

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LIST OF SYMBOLS AND ABBREVIATIONS

$\text{CaMg}(\text{CO}_3)_2$	Dolomitic lime
CaO	Calcium oxide
CEC	Cation exchange capacity
CO_2	Carbon dioxide
cv.	Cultivar
e	Exponential function;
GDD	Growing degree days
GDU	Growing degree units
GC	Gas chromatography
G:C	Citronellol to geraniol ratio
HA	Harvested area
HClO_4	Perchloric acid
HNO_3	Nitric acid
IAH	Immediately after harvest
K	Potassium
KCl	Potassium chloride
K_2O	Potassium oxide
ℓ	Liter
LA	Leaf area
LAI	Leaf area index ($\text{m}^2 \cdot \text{m}^{-2}$)
LAN	Limestone ammonium nitrate (28% N)
La_2O_3	Lanthanum oxide
LAR	Leaf area ratio
LSD	Least significant difference
MgO	Magnesium oxide
N	Nitrogen
<i>n</i>	Sample number
NH_4^+	Ammonium
NH_4OAc	Ammonium acetate
NO_3^-	Nitrate
NUE	Nitrogen use efficiency

P	Phosphorus
P ₂ O ₅	Super phosphate
r	Correlation coefficient
r ²	Coefficient of determination
SAS	Statistical analysis system
SI	Sufficiency index
SLA	Specific leaf area (cm ² ·g ⁻¹)
SPAD	Soil Plant Analysis Development
THRIP	Technology and Human Resources for Industry Programme
WFD	Wetting front detectors
x	Weeks after cut back of rose-scented geranium
Y _n	Total biomass (t·ha ⁻¹) or essential oil (kg·ha ⁻¹) yield
Y _o	Total biomass (t·ha ⁻¹) or essential oil (kg·ha ⁻¹) yield for the zero N plot

ROSE-SCENTED GERANIUM (*PELARGONIUM* SPP.) HERBAGE YIELD, ESSENTIAL OIL YIELD AND COMPOSITION AS INFLUENCED BY NITROGEN NUTRITION AND LIMING

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ABSTRACT

Rose-scented geranium (*Pelargonium capitatum* x *P. radens*) belongs to the family Geraniaceae and it is a multi-harvest, high value, commercially important essential oil yielding aromatic plant. The essential oil extracted from the herbage of the plant is widely used in the fragrance and cosmetics industry and scenting of soaps. The essential oil is extracted by steam distillation.

South Africa is now producing significant quantities of geranium oil. However, previous experience by this research group showed that seedlings often take long to establish, resulting in high death rates and sometimes poor growth after establishment. Stunted growth and yellowing of leaves was also observed in some cases. Poor vegetative growth causes low herbage yield and, consequently, low total essential oil production per hectare. Poor growth is believed to be due to a combination of factors, including nutrient deficiencies and acidic soil conditions and has not been studied under South Africa condition. Production of the crop is also reported to respond differently to rate and source of nutrition in various agro-ecological regions of the world. Therefore, field trials were conducted at the Hatfield Experimental Farm, University of Pretoria, in order to investigate how the plant responds to agronomic practices, such as source and amount of nitrogen, time of N fertilizer application, season of N fertilization and liming.

Response of rose-scented geranium to source and amount of N showed that, at the first harvest (summer/autumn), there was no significant effect of conventional N on fresh herbage and oil yield, probably due to leaching of N by rainfall. However,

organic N at $100 \text{ kg} \cdot \text{ha}^{-1}$ increased fresh herbage and oil yields by 58% and 48% over the control, respectively. In the second harvest (spring/summer), fresh herbage yield increased by 46% (conventional N) and 60% (organic N) at $100 \text{ kg} \cdot \text{ha}^{-1}$ compared to the control. Compared to the control, $100 \text{ kg} \cdot \text{ha}^{-1}$ conventional and organic N also increased essential oil yields by 94% and 129%, respectively. For both N sources nitrogen use efficiency (NUE) and LAI decreased with an increase in N level, and organic N gave highest essential oil production efficiency and LAI. Essential oil content (% fresh mass basis) also varied between the harvests, being greater in the second harvest (September to December 2005; spring/summer) than the first harvest (February to May 2005; summer/autumn). This was due to environmental variations that occurred between the harvesting periods. N level and source were found to have no noticeable effect on essential oil composition. This study revealed that rose-scented geranium produced higher fresh herbage and essential oil yield when organic fertilizer was used as a source of N.

Nitrogen management in terms of rate and time of application is important in rose-scented geranium production. Delaying nitrogen topdressing (conventional N in the form of LAN; N 28%) after harvest to between the 7th and 9th week after cut back, was found to have a significant positive effect on biomass and essential oil production. Essential oil content of the plant did not show any response to a delay in nitrogen topdressing. A delay in nitrogen topdressing, in the first re-growth resulted into a lower citronellol to geraniol (C:G) ratio, which favour essential oil quality of the crop. Generally, the characteristics of the essential oil were within the internationally acceptable range for rose-scented geranium essential oil. In addition, production of rose-scented geranium during cooler periods is not advisable due to limited biomass production which might encourage leaching of nitrogen.

The net benefits from N application is dependent on the growing period and in the present study spring and summer were more beneficial than winter and autumn. Organic N at $100 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ increased herbage and essential oil yield of the crop in spring and summer but further increases in organic or conventional N levels had no significant effect. N application either in winter or autumn did not improve production of the crop. Application of more N than what is required for optimum growth of the plant had no positive effect on essential oil production. Application of organic N also

resulted in higher N use efficiency than conventional N. The essential oil contents (% fresh mass basis) achieved in the present study generally fell within the range of 0.04 to 0.2%. Citronellol and geraniol concentration (%), were at peak in spring season followed by summer and autumn and lowest in winter. The ratio between these two components (C:G ratio) is also used as an indicator of rose-scented geranium essential oil quality and most desirable (low C:G ratio) essential oil was attained in spring, summer and autumn harvesting seasons and least desirable oil was attained in winter. The relationship between SPAD-502 chlorophyll meter readings (SPAD units) and leaf N content (% dry weight basis) was a quadratic function. SPAD-502 chlorophyll meter readings (SPAD units) matched well with that of leaf N concentration data of rose-scented geranium. Regardless of the factors that affect the readings, this instrument can be used as an indicator of leaf N status of rose-scented geranium.

Soil pH above 5.5 and soil base saturation above 55% increased fresh herbage and essential oil yield (per ha), which corresponded in this case with 2 to 6 t·ha⁻¹ of lime application. Oil content (%) was not significantly affected by application of lime. Therefore, optimum growth of rose-scented geranium can be achieved by application of lime when plants are grown on acidic soils, but without any effect on oil content and essential oil composition. It can be concluded that N rate, source and season of production and soil pH should be considered to ensure optimal rose-scented geranium production.

Keywords: Base saturation; citronellol; citronellyl formate; citronellol to geraniol ratio (C:G); delaying nitrogen topdressing; essential oil content; Geraniaceae; geraniol; geranyl formate; guaia-6,9-diene; nitrogen use efficiency; oil content; organic N; *Pelargonium* spp.; rose-scented geranium; SPAD-502 chlorophyll meter; soil pH

CHAPTER ONE

GENERAL INTRODUCTION

Worldwide there are about 3000 plant species which are known for their essential oil production (Lintu, 1995). However, approximately only 300 of these species are commercially important to date (Lintu, 1995) and among these is rose-scented geranium (*Pelargonium capitatum* × *P. radens*), which belongs to the family Geraniaceae and genus *Pelargonium*. The genus *Pelargonium* L' Herit comprises about 250 species and these are distributed throughout the world such as in South Africa, Australia, Madagascar, the Middle East, other parts of Africa, Europe and Asia. The centre of origin for this genus is South Africa; about 600 wild scented geranium species are known to be native to the Cape Province of South Africa (Weiss, 1997; Lis-Balchin, 2002).

Botanically, rose-scented geranium is a male sterile, erect and perennial aromatic shrub, with a multi-harvest, high value, commercially important essential oil (Weiss, 1997; Lis-Balchin, 2002). It is a highly fragrant shrub, about 1 to 1.3 m in height, with soft, green to grey-green stem, which becomes woody and dark with age. Leaves are always fragrant with a rose essence, lobed with 5 to 7 palmate, which grow opposite to each other from the stem. The inflorescence is pink and flowering is in spring and early autumn. The root system is extensively spread and is believed to penetrate below 30 cm, especially under stress conditions (Weiss, 1997; van Wyk & Gericke, 2000; Demarne, 2002; Lis-Balchin, 2002; Miller, 2002).

The essential oil extracted from the herbage of the plant is widely used in the fragrance and cosmetics industry and scenting of soaps. The essential oil is extracted by steam distillation (Rajeswara Rao, Singh & Bhattacharya, 1990a,b; Ram, Ram & Roy, 2003). When tender shoots and abaxial and adaxial leaf surfaces of the plant are examined under electron microscope, densely populated special structures called granular and non-granular trichomes can be noticed. As the nomenclature indicates, the granular trichomes are the ones responsible for secretion and storage of the essential oil. While non-granular are ones believed to be there for creating discomfort

to insects and other pathogens during feeding, and to reduce moisture loss through evaporation under moisture limited conditions (Lis-Balchin, 2002). During the steam distillation process, membranes covering the glands (cuticles) get ruptured with heat and consequently the essential oil becomes released. This makes the above ground biomass (leaves and stem) an important yield factor in rose geranium production (Oosthuizen & Coetzee, 1984; Demarne & van de Walt, 1989; Weiss, 1997). The essential oil contents (% fresh mass basis) recovered during distillation is reported to vary within the range of 0.04 to 0.2% (Weiss 1997; Sabina Aiyanna *et al.*, 1998; Motsa *et al.*, 2006; Eiasu *et al.*, 2009). In addition, the essential oil is a composite of more than 120 components (Demarne & Van der Walt, 1993) but the main constituents of rose-scented geranium essential oil are citronellol, geraniol, isomenthone, citronellyl formate, geraniol formate (Swamy *et al.*, 1960; Weiss, 1997; Miller, 2002; Peterson *et al.*, 2006) and guaia-6,9-diene (Rajeswara Rao, 2000).

Worldwide, demand of the essential oil is estimated to be around 600 ton per annum and South Africa is targeting to compete in the geranium oil market (TLV *et al.*, 2004). Historically, China, Egypt, Réunion Island and India are the leading rose geranium essential oil producing countries (Rajeswara Rao *et al.*, 1990a,b; Ram *et al.*, 2003). Oil from each region has a unique chemical composition and, therefore, a unique position in the market. Réunion oil (Bourbon) has typically demanded the highest price (Weiss, 1997).

Essential oil production of crops is reported to be influenced by several factors, including nitrogen rate, source and season of production (Rajeswara Rao *et al.*, 1990a; Araya *et al.*, 2006). For instance, rose-scented geranium essential oil production and oil composition was reported to be affected by change in temperature, humidity, rainfall (Weiss 1997; Kumar *et al.*, 2001), fertilization (Araya *et al.*, 2006), soil moisture availability (Eiasu, 2009), harvesting season and plant shoot age (Rajeswara Rao *et al.*, 1996; Motsa 2006), soil type and pH (Araya *et al.*, 2011; Weiss, 1997), photoperiod (Adams & Langton, 2006) and distillation method and techniques (Hey & Waterman, 1993; Coleman, 2003). Weiss (1997) also suggested that climatic factors which favour high herbage yield in rose-scented geranium, have the contrary effect on oil, and vice versa; and further suggested that if one of the products is reduced it can be balanced by increasing the other factor.

So far, environmental factors are known to affect the biomass, oil content and essential oil composition of the plant, which indirectly also influences its market value. Due to this, certain regions of the world are well known for the production as well as for their dominance in the world market of rose geranium essential oil. This is why the essential oil of the plant is characterized and marketed with an inclusion of its growing region, such as rose geranium oil from Grasse, France or Réunion Island 'Bourbon type' (Weiss, 1997; Lis-Balchin, 2002). South Africa is also a country with different growing regions and many of these are believed to have enough potential for rose geranium production. However, in order for production of the crop to be successful, research inputs are important. The country is already in the world map for citrus and eucalyptus essential oil production. For instance, in 2003 about 485 tons of citrus and 300 tons of eucalyptus essential oils were exported and this was 2% and 5% of the world market, respectively (TLV *et al.*, 2004). South Africa, despite being the centre of origin of the genus, rose geranium production is only at the start (about 3 ton per annum) but potentially it is estimated to contribute about 50 ton per annum of geranium essential oil to the world market (TLV *et al.*, 2004).

Rose-scented geranium responds well to application of fertilizers (Rajeswara Rao *et al.*, 1990a; Araya *et al.*, 2006). According to different studies (Rajeswara Rao *et al.*, 1990a; Weiss, 1997), herbal plants (such as rose-scented geranium) in general are reported to require larger quantities of the essential elements, in plant nutrition, such as nitrogen, phosphorus and potassium, for optimum growth. Among these essential elements, nitrogen plays an important role in yield and growth of rose-scented geranium especially after every harvest, and since most of the above ground biomass is removed at harvest, fertilization of the crop becomes essential. Traditionally, fertilization of the crop includes application of chemical fertilizers, addition of organic matter and liming (Weiss, 1997). However, understanding the advantages and disadvantages of this source of nutrients (conventional and organic fertilizers) is important. Commonly, farmers use chemical fertilizers (conventional fertilizers) sparsely because of their comparatively high costs (Chabalier, 1992).

Nitrogen is one of the essential elements that are required for healthy plant growth. In crop production it is most deficient of all nutrients. Its deficiency is characterized by yellowing and stunted growth, while its adequacy is associated with vigorous

vegetative growth and dark green leaves that result into high photosynthetic activity (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). At normal growth, plants contain 1 to 5% of N on a dry mass basis. Plants absorb N in the form of nitrate (NO_3^-), ammonium (NH_4^+) and urea. These elements are reserved in the soil, but the supply of the reservoir for this element is very limited and it can often not stay effective for a very long time, in cultivated soil. Therefore, for optimum crop production, additional N supply is important either in the form of organic or inorganic N fertilizers (Tisdale *et al.*, 1993; Brady & Weil, 2002; Taiz & Zeiger, 2002).

Nitrogen has a direct or indirect effect on crop production. Its direct effect might be through the influence on the photosynthetic activity of the crop, since the leaf is the major part where photosynthesis takes place and by the involvement of N in pigmentation (chlorophyll) of the crop. Chlorophyll is a plant pigment which is required for sunlight absorption. Therefore, discoloration of the leaves will result in less sunlight being trapped and as a result low synthesis of carbohydrate (sugar). This will lead to poor plant growth and low crop production (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). Its indirect effect could be related to its influence on essential oil producing plants. For instance, in rose-scented geranium (*Pelargonium capitatum* $\times$ *P. radens*) the essential oil is stored in structures called glandular trichomes (Weiss, 1997; Demarine, 2002). Though these structures are also present on young succulent stem parts of the plant, most of them are populated on both the abaxial and adaxial surfaces of the leaf (Mallavarapu *et al.*, 1997; Demarine, 2002). According to different researchers, more than 50% of the plant's essential oil comes from the leaves (Kumar *et al.*, 2001). Other studies also supported this finding by confirming the existence of a direct relation between plant leaf number and essential oil yield (Mallavarapu *et al.*, 1997). Therefore, loss of leaves from the plant will have a dual negative impact by influencing the biomass production and essential oil yield.

Studies on fertilization of rose geranium were started in 1894 (Demarkine, 2002). When there are no other limiting factors such as water supply, weed and pest infestation and deficiency of other nutrients such as phosphorus, positive response of the plant to applied N was reported. Analogous to the role of N in production, the studies described how important it is to know how much to apply (Weiss, 1997). Adequate N enhances leaf preservation (Salisbury & Ross, 1992; Taiz & Zeiger,

2002) and at the same time increases the chances of essential oil recovery through distillation (Mallavarapu *et al.*, 1997). However, if it is applied in excess, it might not result in high production by encouraging the development of rust, caused by *Puccinia pelargonii-zonali* due to shading and yellowing and loss of bottom leaves, as well as due to inadequate sunlight penetration (Weiss, 1997). In addition, excess N might leach either during irrigation or rainfall and cause environmental pollution. Therefore, proper N management of both rate and source to be used becomes highly essential for optimum growth and yield of the crop.

Nitrogen requirement of rose-scented geranium varies with growing area. A rate that was found advantageous for optimum growth in one area might not be appropriate for another area. In addition, essential oil from each region has a unique chemical composition and, therefore, a unique position in the market. Réunion oil (Bourbon) has typically demanded the highest price. The chemical characteristics of Réunion oil are well understood. South Africa is now producing significant quantities of geranium oil. The country is also well known for its diversity in agro-climatic regions and many of these are believed to have a potential for rose-scented geranium production. However, production is limited by lack in proper nutrition management, over fertilization (especially N) and inadequate liming. For instance, in addition to the naturally existing acidic soils of South Africa (16 million ha) more than 5 million ha of high potential land have been acidified due to inappropriate agronomic practices (Laker, 2005). The present study was therefore conducted with the following objectives:

- To investigate the response of rose-scented geranium, in terms of fresh herbage yield, essential oil yield, oil composition and nitrogen use efficiency (NUE), to different amounts of conventional and organic N fertilizers (Chapter 3);
- To understand the regrowth trend and to identify the most appropriate time for nitrogen topdressing, and the effect of delayed N application on rose-scented geranium biomass and oil yield, essential oil content and composition (Chapter 4);

- To identify the influence of N application in relation to harvesting season, source and rate on rose-scented geranium production and to estimate N uptake of the crop using a chlorophyll meter (SPAD 502) (Chapter 5); and
- To evaluate the fresh herbage and essential oil yield response of rose-scented geranium grown on acidic soils to different liming rates and consequent changes in base saturation and soil pH (Chapter 6).

Finally, recommendations of this study are presented in Chapter 7.

CHAPTER TWO

LITERATURE REVIEW

2.1 INTRODUCTION

Today our world's agricultural production does not only include edible, forage, timber and fibre producing plants but also herbal and medicinal plants as well. These herbal and medicinal plants are plants which have medicinal and culinary value, and most have aroma and chemicals important for the perfumery or chemical industries. Due to these characteristics, these plants brought the attention of different civilizations from domestication to their commercialisation. These plant species were domesticated and are cultivated for their secondary metabolites.

Secondary metabolites are organic compounds that are synthesized by plants through metabolic pathways but not essential for normal plant growth and development. The name secondary metabolite, secondary products or natural products was given in order to separate them from the primary compounds, i.e. compounds which have significant roles in development of plants such as sugar phosphates, amino acids and amides, proteins, nucleotides, nucleic acids, chlorophyll and organic acids (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). For many years these compounds were believed to be waste products of metabolic processes. Then recently, a new discovery confirmed their role as attractants in the interaction of plants and other organisms (eg. in pollination) and as antibiotics or toxins against pathogens and herbivores (Rost *et al.*, 1998; Taiz & Zeiger, 2002).

Essential oils fall also under this secondary metabolite category and are stored in special structures (granular trichomes) located in one or more parts of plants (leaves, flowers, stems or root) (Taiz & Zeiger, 2002). Throughout history these compounds which are also known as volatile oils have been used by different civilizations as folk medicine in and sacrifices during religious ceremonies (e.g. herbs and resins) then later as perfumery. As described in written history, the use of plants for perfumery is believed to have started in ancient China, India and Egypt. They were not used only in

religious ceremonies but were also used as fragrance indulgence in the upper classes of the civilizations. Pomade method was the common method of extraction, which is still practiced in France and third world countries for the extraction of volatile oils from delicate flowers. It works by leaving the plant parts for three to four days in oil and then changing them and by letting the process continues for months. Using this method, the Egyptians extracted volatile oils from lily, rose, violet, lotus, and jasmine; Indians from jasmine, a number of species, champaca, rose, and pandanus and Chinese from rose, jasmine, orange flower, osmanthus, and pitosporum. Then after the invention of the distillation method, which is believed to have been in Egypt, distillation of the extract from the pomade or the plant part itself was started (Richard, 2004). by the mid 19th century, direct distillation of plant parts for essential oils was well understood and alongside isolation of the essential oil components was also started. The first component to be isolated was rhodinol or also known as rose alcohol and was found to comprise about 30% of rose otto essential oil composition and 70% of geranium. This encouraged for the frequent distillation of geranium plants (Lis-Balchin, 2002; Richard, 2004). But nowadays, the escalating price of rose geranium oil resulted into synthetically extraction of rhodinol (Lis-Balchin, 2002).

Rhodinol is a mixture of geraniol, citronellol and linalool from rose geranium. It is important in high grade perfumes and cosmetics (Qinghua, 1993; Rajeswara Rao *et al.*, 2002). Rose geranium (*Pelargonium capitatum* × *P. radens*) belongs to the family Geraniaceae and genus *Pelargonium*. The genus *Pelargonium* L' Herit comprises about 250 species which are distributed throughout the world. The first discovery of the genus was 1672 (*P. cucullatum*) at Cape region (Table Mountain) of South Africa. Then when the British controlled the Cape of Good Hope of South Africa, the interest on the genus increased and resulted into the introduction of the species into most British colonized areas. In addition, the easy to grow characteristics is also believed to contribute toward the global spreading of the plant (Weiss, 1997; Lis-Balchin, 2002).

South Africa despite being the centre of origin, of the genus rose geranium production is only at the start. Like all agricultural crops, rose geranium production is dependent on environmental factors (Weiss, 1997; Lis-Balchin, 2002). So far, these factors are known to affect the biomass, oil content and essential oil composition of the plant, which indirectly influences its market value. Due to this, certain regions of the world

are well known for the production as well for their dominance in the world market of rose geranium essential oil. This is why the essential oil of the plant is characterized and marketed with an inclusion of its growing region, such as rose geranium oil from Grasse, France or Réunion Island ‘Bourbon type’ (Weiss, 1997). South Africa is also a country with different growing regions and many of these are believed to have enough potential for rose geranium production. However, in order for production of the crop to be successful, research inputs are important. The country is already in the world map for citrus and eucalyptus essential oil production. For instance, in 2003 about 485 tons of citrus and 300 tons of eucalyptus essential oils were exported and this was 2% and 5% of the world market, respectively (TLV *et al.*, 2004).

2.2 Secondary metabolites and plants

Plants are known to be super chemists due to their ability to synthesize more complex compounds than animals (Salisbury & Ross, 1992; Rost *et al.*, 1998). They synthesize chemical compounds which are more than what is required for their growth in quality and quantity (Rost *et al.*, 1998). In the plant kingdom, certain plant species are known for the synthesis of two metabolic products. These are primary and secondary metabolites. Secondary metabolites are organic compounds that are synthesized by plants through metabolic pathways (Fig. 2.1) but not essential for normal growth and development of the plant. The nomenclature secondary metabolite, secondary products or natural products was given in order to separate them from the primary compounds, i.e. compounds which have a significant role in development of plants such as sugar phosphates, amino acids and amides, proteins, nucleotides, nucleic acids, chlorophyll and organic acids (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). For many years they were believed to be waste products of metabolic processes. Then in the nineteenth and early twentieth century a study of these compounds was initiated by organic chemists who were interested in their extraction for medicinal drugs, poisons, flavours and industrial materials (Taiz & Zeiger, 2002). Recently, the magnitude of these compounds in plants was discovered and some are known to play important roles as attractants in the interaction of plants and other organisms (e.g. in pollination) and some are antibiotics or toxins against pathogens and herbivores (Rost *et al.*, 1998; Taiz & Zeiger, 2002).

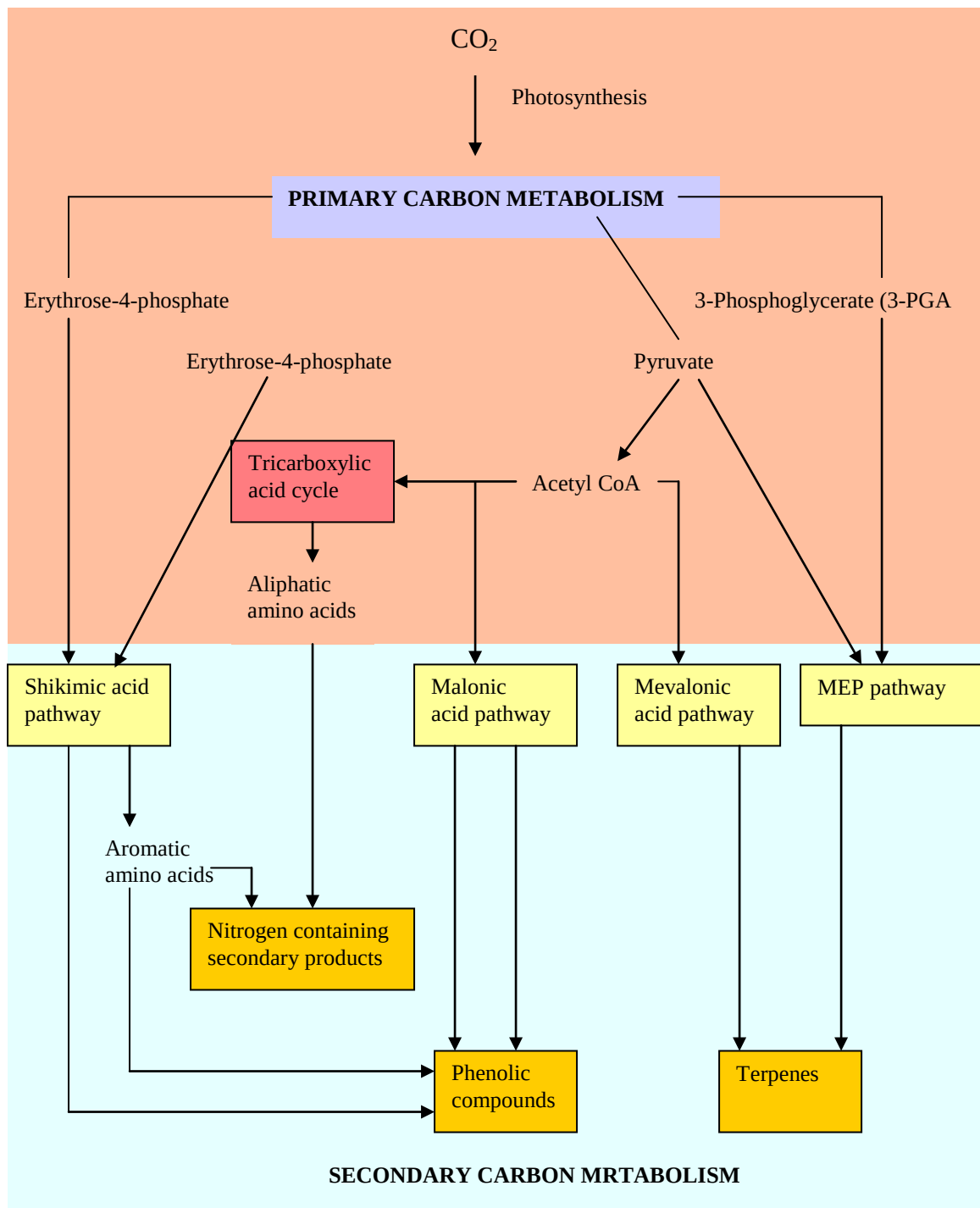


Figure 2.1: Secondary metabolite pathways and their relationship with primary metabolites (after Taiz & Zeiger, 2002)

Secondary compounds comprise three major groups called terpenes, phenolics and nitrogen-containing compounds (Fig. 2.1) (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). Terpenoids, also known as isoprenoids are compounds that contain carbon atoms of 10 (*monoterpenoids*), 15 (*sesquiterpenoids*), 20 (*diterpenoids*) or 30 (*triterpenoids*). Terpenoids that contain 10 or 15 carbon atoms are known as essential

oils, volatile oils or a name of the plant species from where the extraction was made followed by oil (e.g. rose geranium oil). This is to indicate, they reflect the essence of the used plant material (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). The essential oils are stored in the outer surface of the epidermal cells, in special structures, called granular trichomes, and these are the structures which are responsible for the secretion and storage of the compounds (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). The extraction could be through steam or water distillation (Fig. 2.2) (Weiss, 1997) and it can be from the leaves, flowers, roots, barks, rhizomes, fruits and woods (Salisbury & Ross, 1992; Sangwan *et al.*, 2001; van Wyk & Gerick, 2000; Taiz & Zeiger, 2002). These extracts can be used in perfumery, aromatherapy, cleansing or disinfecting, cosmetics and skin care (Doane, 2004).

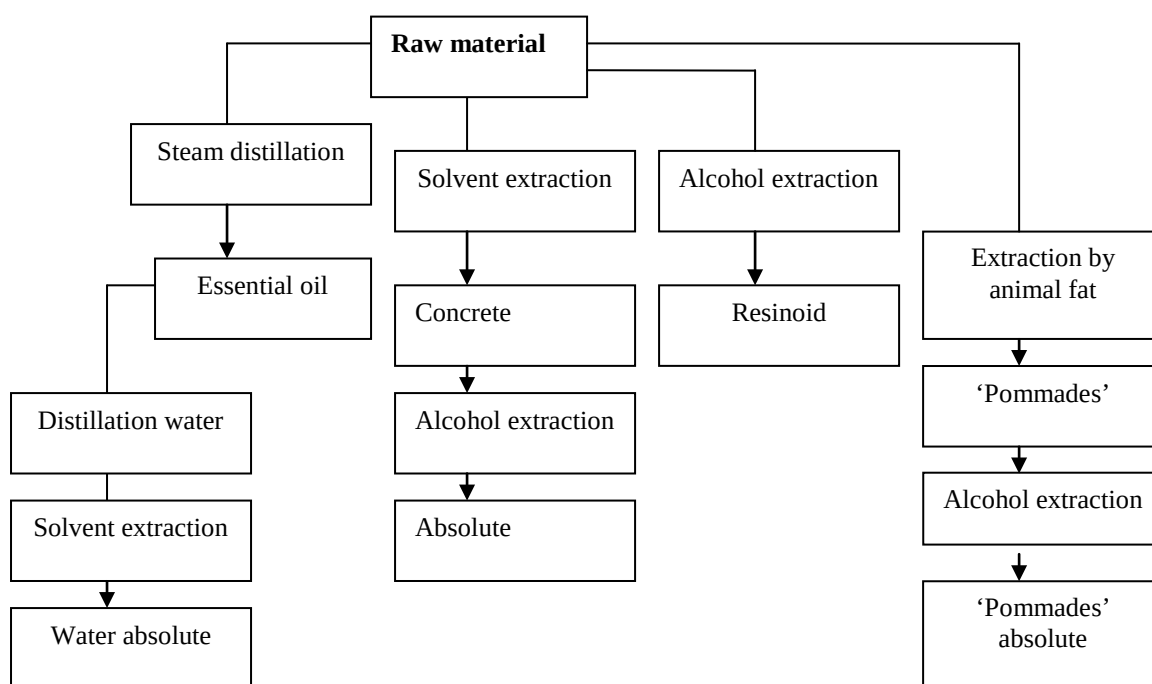


Figure 2.2: Methods of obtaining essential oils or derivatives (after Weiss, 1997)

Essential oils are highly concentrated and mixtures of large number of chemical components. These components play an important role in determining the essential oil quality and marketing value. The synthesis and quality is reported to be influenced by biotic and abiotic factors. A number of abiotic factors such as temperature, light, moisture and soil appear to control the essential oil content and composition of different plants. For instance, the effect of environmental factors on *Mentha* species

oil composition (Farooq *et al.*, 1999), *Anmopsis californica* essential oil content and composition (Medina-Holguín *et al.*, 2007), rose-scented geranium essential oil composition (Motsa *et al.*, 2006) were reported. Recent research on plant species such as corn, cotton and wild tobacco also indicated that the plants start synthesizing or emitting insect or pathogen repellent chemicals only when attacked. This might either be a defence mechanism or for seeking help from other organisms (Taiz & Zeiger, 2002).

2.3 Historical background and geographic distribution of rose-scented geranium

Rose-scented geranium (*Pelargonium capitatum* × *P. radens*) belongs to the family Geraniaceae and genus *Pelargonium*. The genus *Pelargonium* L' Herit comprises about 250 species and these are distributed throughout the world such as in South Africa, Australia, Madagascar, the Middle East, other parts of Africa, Europe and Asia. The centre of origin for this genus is South Africa; about 600 of wild scented geranium species are known to be native in the Cape Province of South Africa (Weiss, 1997; Lis-Balchin, 2002). The first discovery was in 1672 (*P. cucullatum*) from Table Mountain. When the British controlled the Cape of Good Hope, South Africa, the interest on the genus augmented and it was introduced in most British colonized areas. The easy to grow characteristics of the plant also contributed towards its spreading (Weiss, 1997; Lis-Balchin, 2002).

The genus *Pelargonium* contains 15 subgenera (sections) which include the subgenus *Pelargonium* with about 25 species and the species in this subgenus are characterised by variegated or fragrant leaves. Of the 25 species, only four cultivars (*P. graveolens*, *P. odoratissimum*, *P. capitatum* and *P. radens*) are believed to have the potential for essential oil production. With species from South Africa, by the year 1690, a cross breeding between the species was started in England, at a botanical garden known as Kew Garden and resulted into the commercially important essential oil producing cultivar Rose, a hybrid between *P. capitatum* × *P. radens* (Wiess, 1997; Lis-Balchin, 2002).

By the middle of the nineteenth century, the essential oil demand in France (Paris) of ‘Rose of the Levant’, *Rosa damascena* Mill. (Rosaceae) increased and as a result the price soared and became unaffordable for consumers. This forced the perfumer industries to look for an alternative essential oil yielding crop. By the year 1819 a distillation research was done on rose geranium and the potential of the species for perfumery production was confirmed (Weiss, 1997). In the year 1847, the first commercial cultivation of the hybrid was started in Grasse, France and it remained as the centre of production until the Second World War, when due to an increase in cost of production and industrialisation, the commercial production of the crop was exterminated. Flow of the plant material in the French colonised areas was then started using the same plant materials from Grasse and the production area was shifted into countries such as Algeria, Morocco and the Réunion Island. These areas also stayed productive and steady for sometime but then started declining (Wiess, 1997; Lis-Balchin, 2002).

The main reason for shifting and spreading of rose geranium production during the early times was not only due to increasing interest in the crop for its highly commercially available essential oil but also due to other constraints as well, such as lack of enough machinery for all the agronomic practices to cultivate the plant, expensive labour and so on. Due to this, production in areas such as Grasse could not cope with the highly escalating market demand of the essential oil. This resulted in shifting or looking for other growing regions with cheap labour and favourable soil and climate for production of the crop. First, it was introduced to Algeria in 1865 and then to Réunion Island in 1880 but after the twentieth century it was introduced into different parts of our world. Among these were: Corsica, Italy, Morocco, Tunisia, Egypt, Russia, Congo, Kenya, Madagascar, India, Spain, Portugal, Brazil, Comoro Islands and most recent development into China (Wiess, 1997; Lis-Balchin, 2002).

At the time of introducing the crop, there was lack of machinery and growing of this crop by traditional agricultural practises like cutting preparation, weeding, harvesting, firewood distillation and in addition sheltering of the plantation during the cold season were expensive. All these factors summed up and lead to the shifting of the plantations to other areas where cheap labour was available and favourable environments for the growth of the crop were available, since geranium requires areas

with good sunshine, well drained soils with high organic matter. The crop is sensitive to frost and temperatures below 2 °C because this may affect the growth or could also be lethal to the crop. When North Africa and Indian Ocean were under the French colony, these areas were found to be favourable for the growth of the crop (Weiss, 1997; Lis-Balchin, 2002). The current international demand of about 600 t geranium oil is being met by China, Morocco, Egypt, Reunion Island and South Africa (Qinghua, 1993; Anon., 1996/1997). There are also small producing countries like Spain, Malaysia, Belgium, Congo, Kenya and India (Weiss, 1997).

South Africa despite being the centre of origin of the genus, rose-scented geranium production is still at its infancy. Like all agricultural crops, rose geranium production is dependent on environmental factors (Weiss, 1997; Lis-Balchin, 2002). So far, these factors are known to affect the biomass, oil content and essential oil composition of the plant, which directly influences its market value. Due to this, certain regions of our world are well known for the production as well as for being dominant in the world market for rose geranium essential oil. Thus, the essential oil of the plant is characterized and marketed including its growing region, such as rose geranium oil from Grasse (France), Réunion Island 'Bourbon type', Africa 'the Egyptian oil or Moroccan oil' or China 'China oil' (Weiss, 1997; Lis-Balchin, 2002). Oil from each region has a unique chemical composition and, therefore, a unique position in the market. For instance, Réunion oil (Bourbon) has typically demanded the highest price and the chemical characteristic of the oil is well understood (Rajeswara Rao *et al.*, 1990a,b; Ram *et al.*, 2003).

South Africa is well known for its diversity in agro-climatic regions and many of these are believed to have a potential for rose-scented geranium production. These regions are also known to be suitable for the production of other essential oil producing plants such as lavender, peppermint and lemon grass. But primarily, farmers are more encouraged to grow rose geranium than other essential oil crops, due to high demand of the world market and escalating price and good return of the oil (South Africa Info., 2003).

2.4 Botanical description of rose-scented geranium

The genus *Pelargonium* L'Herit is one of the indigenous plants to South Africa; contains 15 subgenera and among this the subgenus *Ciconium*, *Dibrachya* and *Pelargonium* can be mentioned (Weiss, 1997). As described by Van der Walt (1985), the species from the subgenus *Pelargonium* are characterised by highly branched stems, aromatic or multicoloured leaves and most woody shrubs or sub-shrubs.

Rose-scented geranium belongs to the subgenus *Pelargonium* and it is male sterile with haploid somatic number of $2n = 77$. It is an erect and perennial aromatic shrub, with a multi-harvest, high value, commercially important essential oil (Weiss, 1997; Lis-Balchin, 2002). It is a hybrid between *P. capitatum* and *P. raden*. It is a highly fragrant shrub, about 1 to 1.30 m in height, with soft, green to grey-green stem, which become woody and dark with age. Leaves are always fragrant with a rose essence, lobed with 5 to 7 palmate, which grow opposite to each other from the stem. The inflorescence is pink and flowering is in spring and early autumn. The root system is extensively spread and is believed to penetrate below 30 cm especially under stress conditions (Weiss, 1997; van Wyk & Gericke, 2000; Demarne, 2002; Lis-Balchin, 2002).

When tender shoots and abaxial and adaxial leaf surfaces of the plant are examined under electron microscope, densely populated special structures called granular and non-granular trichomes can be noticed. As the nomenclature indicates, the granular trichomes are the ones responsible for secretion and storage of the essential oil. While non-granular are ones believed to be there for creating discomfort to insects and other pathogens during feeding, and to reduce moisture loss through evaporation under moisture limited conditions (Lis-Balchin, 2002). During the steam distillation process, membranes covering the glands (cuticles) gets ruptured with heat and consequently the essential oil becomes released. This makes the above ground biomass (leaves and stem) as an important yield factor in rose geranium production (Oosthuizen & Coetzee, 1984; Demarne & van de Walt, 1989; Weiss, 1997). The development of both these structures is still being debated; some researchers consider their number as fixed throughout the growing stage of the plant (Oosthuizen & Coetzee, 1984), while

others assume an increase in their number with growth and development of the plant (Motsa *et al.*, 2006).

2.5 Cultivation of rose-scented geranium

Growing rose-scented geranium under commercial production is described by many authors as a difficult job. This is because in order for one has to get good returns different steps have to be followed. First, the plantation has to stay as perennial for 3 to 5 years (Lis-Balchin, 2002). Second, land preparation has to be done attentively such as land clearing, ploughing, removal of former plantation and debris. This is because so far the plant is known to be susceptible to root diseases caused by fungi and bacteria, and leaving any of this on site might enhance the risk of infection. Third, once the plant is established weeding might be difficult without disturbing the root system of the establishing seedlings. Therefore to minimize, the risk well removal of former weeds is important. In addition, rose geranium seedlings easily get prone to competition at an early growth stage (Weiss, 1997; Lis-Balchin, 2002). According to Kothari, Singh & Singh (2002) after planting, rooted cuttings of geranium require 30 to 35 days to establish and 40 to 45 days to cover the ground with their canopy and to start competing with weeds. However, when growing geranium under non-commercial conditions, minimum cultivation can be enough (Weiss, 1997).

Soil and climatic requirements

Geranium requires deep well drained and slightly sandy soils, with high organic matter and a pH ranging from 5.5 to 7.0 has been found to be suitable for optimum geranium production. Heavy clay soils with poor drainage and soils with alkaline or acidic pH (generally soils with pH below 5) are not suitable for geranium growth. However, there are some exceptional local cultivars, which grow in soil with low to moderate amount of sodium chloride (Weiss, 1997). As supplementary to rainfall in some areas such as Australia, it was reported that geranium could grow with irrigation water with high EC, which could inhibit the growth of other crops; whereas in Armenian positive response of the plant was reported from saline soil when combined with optimum agronomic practices (Weiss, 1997). A research done in India also

confirmed that the cultivar Bourbon can tolerate sodicity up to 16.0% for herbage yield and 7.0% for oil content of the crop. Certain components of the essential oil also increased when sodicity was between 16 to 24% but increasing the sodicity percentage (> 28) reduced their concentration (Prasad, Chattopadhyay, Chand, Naqvi & Yadav, 2006). Generally, the soil requirement of geranium is similar to vegetable crops. The plant is known for its adaptability to a wide range of soils and slopes; the plant is even recommended to grow in hillsides, ridges or contours to reduce soil erosion. The altitude requirement of geranium was found to differ with countries where the crop is grown commercially; among these are southern France with 300 to 1200 m, Algeria and Reunion with a maximum of 1400 m; Kenya between 2000 to 2500 m and India between 1500 to 2000 m can be mentioned (Weiss, 1997).

Geranium grows well in warm-temperate regions and is more productive if it is grown in areas with Mediterranean climate, with warm winter and an average summer temperature of 30 to 35°C. Areas with relatively low humidity and an annual rainfall of 1000 to 1500 mm, well distributed throughout the growing season, are favorable for optimum production of the plant. The plant is poorly resistant to areas or conditions with high humidity and high rainfall (Weiss, 1997; Lis-Balchin, 2002). These conditions are known to hasten susceptibility of the plant to fungal diseases and consequently reduce leaf growth of the plant. In addition, the plant is also not tolerant to water-logging; for instance, a report from Reunion Island indicated that mortality rate of the plant was increased when the soil remained saturated for sometime. Similarly, though geranium is one of the drought tolerant crops, prolonged drought was found to retard growth, reduce oil content and affect the characteristics of the oil (Weiss, 1997; Lis-Balchin, 2002). Due to the above mentioned reasons irrigation is mostly not recommended but if applied, good management is advisable (Lis-Balchin, 2002). Oil content of the plant is also inversely related to the plant moisture content (Sanderovich *et al.*, 1983) and similar findings were reported in *Officinal* sp. by Yaniv & Palevitch (1982).

Geranium prefers clear sunny days for maximum leaf growth and high oil content, rather than cold temperatures or cloudy days. Daily temperature has to be between 20 to 25 °C with $\pm 5^{\circ}\text{C}$ for optimum growth and temperatures below 6°C normally inhibit growth, while frost or a temperature below 3°C is often lethal (Weiss, 1997). Climatic

factors such as wind has no significant effect after establishment of the plant but at an early stage it can damage or uproot the plants, while hail and harsh storms can cause terrible damage to plantations (Swamy *et al.*, 1960; Demarne, 1984; Weiss, 1997).

Propagation and planting

In literature, rose geranium is described as male-sterile and, therefore, can not produce seeds. This is due to its unusual chromosome number ($x = 11$; $'2n' = 7x = 77$) and hybrid characters (Weiss, 1997; Lis-Balchin, 2002). Therefore, the only means to propagate this plant is by asexual method, mostly stem cuttings (Lis-Balchin, 2002). Stem cuttings from the terminal as well as the hard stem cuttings can propagate geranium and both cuttings were found to produce similar essential oil (Rajeswara Rao, 1999). Top cuttings root best followed by medial and basal cuttings in sand. Rooted cuttings, suckers or splits are also reported to be equally effective to stem cuttings even though they require a longer time to grow. Vegetative propagation through tissue culture is also possible and an effective method of producing large numbers of plants, but it is not mostly used because it is expensive compared to other propagation methods (Brown & Charlwood, 1986; Yue *et al.*, 1993; Satyakala *et al.*, 1995).

Plant materials for the propagation can be obtained either from an existing plantation or from a nursery. A plant in good growth condition can provide 20-25 cuttings and normally 30,000 to 50,000 cuttings are required for one hectare. Cuttings with a length of 15 to 20 cm and 4 to 6 nodes are suitable for propagation (Weiss, 1997). Propagation materials should be taken from the current season's growth, and taken only from healthy, vigorous, bushy plants (Hartman *et al.*, 1997). Application of rooting hormones such as Seradix have been found to improve rooting but usually unnecessary if cuttings have been properly prepared as the strike-rate is high. Dipping the bottom of the cuttings in fungicide like 0.03% Benlate helps to prevent against *Fusarium* wilt. It is important also to keep the nursery cool and the bed should have to be kept moist by regular application of water (Hartmann *et al.*, 2002; Weiss, 1997; Lis-Balchin, 2002). However, when cuttings are directly planted, the bottom leaves have to be clipped (leaving two to three leaves), and base of the cutting treated with

fungicide before planting into moist soil either mechanically or manually (Weiss, 1997).

Prior to planting, the field is ploughed and harrowed several times to produce a fine seedbed. Then geranium is planted, by planting cuttings in holes or in furrows dug manually and mostly farmers add manure, some fertilizers, lime and slow released insecticides at the bottom (Weiss, 1997; Lis-Balchin, 2002). Cuttings must be planted in to moist soil or irrigation should be applied immediately after planting and frequent irrigation is required in the initial stages of establishment of cuttings, as it is usually not feasible to water-in large-scale plantings. Dead or diseased plants have to be replaced with new plants and this operation has to continue until the lifetime of the plantation. If the area where the plantation is with heavy rain, ridging the field will encourage the drainage of the excess water and on the other hand in areas with low rainfall it helps to conserve maximum moisture. Fertilizers can be added together if machinery is used for ridging (Weiss, 1997). Plant population and profit have a direct relationship. But plant population depends on certain factors, such as type of the soil and climatic conditions of the growing area. Based on this the spacing between plants could be 80 x 30 cm and 100 x 60 cm and the optimum plant population for most areas is 35,000 plants ha⁻¹ (Lis-Balchin, 2002).

Weed control

In geranium plantations, controlling weed growth is important (Rajeswara Rao & Bhattacharya, 1997; Weiss, 1997). Rooted cuttings require 30 to 35 days to establish in the field and another 40 to 45 days for the canopy to cover the space between the rows. That is, the crop is susceptible to weed competition especially at an early stage (Singh, Chandrashekhara, Ganesha & Pakasa, 1996; Rajeswara Rao & Bhattacharya, 1997). Especially under tropical and subtropical climates, weed growth is very fast and fields are invaded very quickly causing competition for space, light water and nutrients with the crop. However, if weeds are not controlled, then they smother the young cuttings, which limit growth and branching of the cuttings and result in poor yield (Rajeswara Rao & Bhattacharya, 1997). Essential oil yield loss up to 65% has been reported depending on the density and type of the weeds (Kothari, Singh &

Singh, 2002). In addition, some weeds with odour may get harvested together with the crop and distilled and affect the scent of the essential oil (Lis-Balchin, 2002).

In many geranium producing countries, manual weeding is practiced even when herbicides are applied in the inter-rows. Weeding the rows is always done manually and since this operation is labour consuming, 60 to 125 labour·ha⁻¹·year⁻¹ are required (Lis-Balchin, 2002). During the early growth or at the critical growth stage three to four weedings, which considerably increase the cost of production, are required (Kothari *et al.*, 2002). In this practice weed control is a combination of manual hoeing, herbicide application, cultural practices and suitable crop rotations or co-cultivation with soil-cover plants (Lis-Balchin, 2002). Weeding must be as shallow as possible to avoid damaging the plant roots (Weiss, 1997) and using hoeing to control weed might also lead to drying of the soil and as a result cause increases in erosion (Lis-Balchin, 2002).

Even though manual weeding is common, herbicides have been used successfully in geranium (Weiss, 1997; Kothari *et al.*, 2002). The following have been used in geranium with no major damage to plants when correctly applied: atrazine, dalapon, glyphosate, linuron and simazine (Weiss, 1997). Systemic or contact herbicides can be used as pre-emergence weed control but it is important to check their tracing ability later in the essential oil (Lis-Balchin, 2002). Kothari *et al.* (2002) reported pre-emergence application of pendimethaline (0.75·1.00 kg⁻¹ AI per ha) or oxyfluorfen were as effective as three hand weedings or successive weeding and no variation in quality of the oil with or without herbicide was reported. Similarly, no influence in oil quality of menthol mint (*Mentha arvensis* L.) and coriander (*Coriandrum sativum* L.) from herbicide application was reported (Kothari, Singh & Singh, 1989; Kothari & Singh, 1994).

Fertilization

Like any crop geranium responds well to the application of fertilizers (Rajeswara Rao *et al.*, 1990a) and as stated by Lis-Balchin (2002) fertilization trial on geranium started from 1894. Fertilization of geranium traditional includes application of

chemical fertilizers, addition of organic matter and liming. Farmers use chemical fertilizers sparsely because of their comparatively high costs (Weiss, 1997; Demarne, 2002). Geranium can produce a biomass of 25 to 40 t·ha⁻¹ annually and since most of the crop biomass is removed it is advisable to add fertilizers after harvesting. When the nutrient content of the harvested geranium plant is analysed, 0.98% of nitrogen was found in the leaves while 1.13% and 0.80% of phosphate and potassium were found in the petioles, respectively (Weiss, 1997).

When there is a fertilizer balance or when no other factors are limiting geranium is found to respond well to nitrogen fertilizer application (Demarne, 2002). When nitrogen is deficient, while phosphorous, potassium and moisture are available, it was found only to affect the biomass yield of the crop but had little or no effect on the quality of the oil (Rajeswara Rao *et al.*, 1990a,b). To get faster growth, it is advisable to apply nitrogen fertilizer to cuttings before planting and after harvesting of the crop. This encourages new growth but it is also important to know the exact amount and application level. Application of nitrogen fertilizer at a total of 170 kg·ha⁻¹ ammonium sulphate (36 kg N) was not found to increase yield in Kenya but on the other hand 100 kg·ha⁻¹ was found to increase biomass in India (Rajeswara Rao *et al.*, 1990a,b). In Israel, high level of ammonium sulphate, 500 kg·ha⁻¹ prior to planting and 300 kg·ha⁻¹ as top dressing, before and after first harvest, yields 70 t·ha⁻¹ biomass and 185 kg·ha⁻¹ oil from three harvests in one year (Fleisher & Fleisher, 1985). High amounts of nitrogen may yield high biomass through its direct or indirect effect on the plant and it may also result in rust caused by *Puccinia pelargonii-zonalis* due to shading. But generally, little or no difference in biomass of the crop is observed with high application of different nitrogen fertilizers and therefore it is better to use the normal amounts (Weiss, 1997). However, there is no clear information on how much N is required for optimum growth and yield of the crop under South African conditions or areas with similar agro-climatic conditions.

Phosphorous plays a great role in rooting of cuttings as well as in successive biomass production but its effect on oil content and oil characteristics is little (Weiss, 1997). On the other hand, Lis-Balchin (2002) reported that, essential oil yield from geranium is directly related to the content of phosphorous of the plant. This was found when application of high levels of superphosphate (2 t·ha⁻¹) doubled the herbage yield of

rose geranium. Similar results were also reported in Kenya by applying $200 \text{ kg}\cdot\text{ha}^{-1}$ of P_2O_5 after the fifth harvest of the crop.

In India, growers commonly use $25\text{--}40 \text{ kg}\cdot\text{ha}^{-1}$ superphosphate and often apply throughout the life time of the plantation (Kumar *et al.*, 1985). In Réunion a compound fertilizer at a rate of 50:130:100 NPK at 600 to $800 \text{ kg}\cdot\text{ha}^{-1}$ was found to increase yield. More commonly, growers use the standard sugarcane fertilizer rate of 15:7:24 or 18:7:30 NPK and this is related to the average oil yield obtained from a hectare; that is, at 20 kg oil it is $200 \text{ kg}\cdot\text{ha}^{-1}$, at 30 to 40 kg oil it is 400 to $600 \text{ kg}\cdot\text{ha}^{-1}$ and above 50 kg oil it is 700 kg of 9.5:14:30 NPK plus 60 kg ammonium nitrate or 300 kg urea $\cdot\text{ha}^{-1}$ (Chablier, 1992). South African soils under commercial agricultural production are reported to have excessive P levels due to continuous fertilization (Laker, 2005). However, undisturbed soils throughout the country are reported to be low in available P ($<10 \text{ mg}\cdot\text{kg}^{-1}$). Phosphorous fertilization in the present study was based on the practices of commercial rose-scented geranium farmers in South Africa ($90 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$).

Rose geranium can withstand low levels of potassium before showing symptoms of deficiency. Symptoms and deficiencies appear when the K content of the crop drops down to 0.3% of the dry matter (Chablier, 1992). Research done in India showed that in many geranium growing areas lacked potassium. Mostly potassium chloride (KCl) is recommended annually at a rate of $35\text{--}70 \text{ kg}\cdot\text{ha}^{-1}$ ($18\text{--}36 \text{ kg}$ K) (Weiss, 1997). Potassium deficiency is not common for most commercial crops in South Africa . However according to the Fertilizer Hand Book of South Africa (2003), under intensive cultivation supplemental K is necessary.

Calcium is also required in small amounts by the crop since geranium requires slightly acidic soils. Application of Ca in the seedbed was found to increase biomass yield when there was enough of the major nutrients and it is mostly applied together with magnesium as dolomite lime stone or locally available ground magnesium limestone (Weiss, 1997). Application of micronutrients (B, Cu, Zn, Mo, etc.) is sometimes recommended since; under certain conditions these trace elements can favour the oil production (Lis-Balchin, 2002). This was supported when frequent applications of magnesium to potted geranium as well as in the field was found to

increase biomass but had no effect on oil content and quality. Similarly, molybdenum and copper increased biomass and oil yield in well fertilized plantations. On the other hand, in some experiments it was observed that the deficiency of micronutrients had not much effect on geranium plantations (Weiss, 1997).

The type of fertilizer used can be dependent on the conditions of the soil and the cropping system and the amount depends on the level of intensification of the plantation. For an intensive cropping system, the theoretical fertilization is 150 to 200 kg·ha⁻¹ of N, 60 to 80 kg·ha⁻¹ of P₂O₅ and 150 to 200 kg·ha⁻¹ of K₂O (Lis-Balchin, 2002). A 7 ton dry matter·ha⁻¹·year⁻¹ can produce a high yielding geranium crop, when 100 kg·ha⁻¹ of N, 32 kg·ha⁻¹ of P₂O₅, 165 kg·ha⁻¹ of K₂O, 250 Kg·ha⁻¹ of CaO, 28 kg·ha⁻¹ of MgO, 15 kg·ha⁻¹ of Na and 10 kg·ha⁻¹ of S are applied in Reunion (Weiss, 1997). Similar results were also reported in India (Prakasa Rao *et al.*, 1988).

At the time of planting, N, P and K can be applied together as a complete fertilizer, followed by once a year application at the end of the rainy season and in intensive cultivation nitrogen (urea or calcium ammonium nitrate) can be applied by splitting it into 2 to 4 doses. If fertilizer is put in holes during planting, it is important not to have contact between the fertilizer and the cuttings in order to avoid burning (Lis-Balchin, 2002). At the time of planting, addition of organic manure together with fertilizer is supported (Weiss, 1997). This is because organic matter is well known for its beneficial effect on the beneficial microbial activities and in improving soil structure (Tisdale *et al.*, 1993; Brady & Weil, 2002). Better root development and a two-fold increase in essential oil yield were reported when 5 to 10 tons of distillation residues were applied to geranium intercropped with legumes than the geranium crop grown as a single crop (Chabalier, 1992).

Irrigation

Rose geranium prefers to grow well in moderate rainfall areas. When it grows as a rainfed crop and in areas where there is risk of waterlogging, planting cuttings in ridges will insure good drainage (Lis-Balchin, 2002). When geranium grows in areas with harsh environmental conditions such as India (Singh *et al.*, 1996; Sabina Aiyanna *et al.*, 1998; Singh, 1999) supplementing the plantation with different

irrigation techniques and fertigation will increase both herbage and essential oil yield per hectare. Other than the result of the irrigation treatment negative correlation was found between the water content and essential oil content (Eiasu *et al.*, 2009).

To get good production, it is important to provide geranium plantation with enough amount of water and the soil has to have 50 to 65% available moisture throughout the growing period. To grow geranium commercially under irrigation is highly profitable because irrigating to a large extent will help to have high biomass and high total oil yield per hectare. But it was also reported to reduce the oil content of the leaves especially if overhead irrigation system was used (Weiss, 1997).

Harvesting, processing and world market

In the plant, geranium oil is contained in glandular trichomes (Demarne & der Walt, 1989). These glands are numerous on both surfaces of young leaves, on young stems, on the buds and on different parts of the inflorescences. The essential oil is therefore obtained from the top young parts of the plant and, because of the perennial character of the crop; harvesting geranium must fulfil two opposing objectives: the maximum production of herb and the preservation of the subsequent shoot development ability (Demarne, 1993). Harvesting of geranium is done 4 to 6 months after planting when the colour of the leaves turn from green to light green and when the odour of the leaves turn from lemon to rose. Depending on the factors like regrowth of the crop, weather condition, crop management programs and labour availability the following harvest could be after 3 to 5 months interval (Rajeswara Rao 2002; Weiss, 1997).

Harvesting of the crop should not be done before it is well established but normally the first harvest is done between 6 to 8 months after planting. This is because harvesting at an early stage may kill the plant or regrowth may be slow. Even though harvesting time can be affected by weather or other farming operations, maximum harvesting time can be determined by analysing the oil content. This can be through taking samples and when the plant reaches certain maturity stages like desired height or at flowering. An increasing in oil content was observed when the plant started to flower and was highest at the full bloom stage and thereafter decreased rapidly (Weiss, 1997). In general, geranium is harvested in high latitude areas at the flowering

stage when the crop is with well-developed foliage at 7 to 8 months after planting and in plain areas when the crop reaches full growth at 4 to 6 months after planting (Weiss, 1997; Rajeswara Rao, 2000; Demarne, 2002). Oil composition was also found to differ with time and this was related to the growth period of the plant and harvesting at the optimum time gave high oil content and quality (Weiss, 1997) and harvesting must be done on clear sunny days (Ram *et al.*, 1997). Manual harvesting with secateurs, knives or sickles is the best way for harvesting geranium because it prioritises an individual approach of the plant architecture and tends to optimise both the herb yield and the regrowth ability (Ram *et al.*, 1997).

Harvesting at a height of 12 to 20 cm above ground was reported to be most profitable in relation to distillation time and oil yield per tonne of material. To see the significance of cutting height, trials were conducted in India and Israel. Similar oil contents were obtained from the leaf blades, petioles and flowers, while little oil was obtained from the stem and none from 15 cm below the tip (Weiss, 1997; Demarne, 2002). Weiss (1997) also reported that the whole stem contains little essential oil and, therefore, it is a waste to distil it, but at present there is no practical way of harvesting only the leaves. For smallholders in India who always harvest by hand, only green leaves are selected and harvested and pruning is suggested after 5 years of such selective harvesting. In areas where many harvests are possible, only the top 15 to 20 cm length of the shoot is harvested and this leaves enough biomass for the re-growth (Rajeswara Rao *et al.*, 1996a).

Harvests should be loaded directly into a cart or trailer but should not be placed on the ground especially in plantations with tropical red earth soil, because these soils are high in iron or aluminium content and the adhering soil can affect oil quality (Weiss, 1997). Steam distillation is reported to give better quality of oil than hydrodistillation (Kual, Rajeswara Rao, Bhattacharya, Singh & Singh, 1995). Yield of essential oil was found to vary from 0.1 to 0.15% but hybrids developed in southern Russia yield up to 4%. Generally an average of 26 kg of essential oil can be obtained from one hectare and under intensive care up to 50 kg·ha⁻¹ can be attained (Weiss, 1997).

Annual oil yield obtained varied from 5 to 20 kg·ha⁻¹ depending on the cultivar and the number of harvests made. For example, in India oil yield increased to 45 kg·ha⁻¹ from 8

to $15 \text{ kg}\cdot\text{ha}^{-1}$, while 70 to $80 \text{ kg}\cdot\text{ha}^{-1}$ was recorded by cross selected high yielding strains in Georgia. The annual oil yield was suggested to be not less than 0.2 to 0.25% of the total herbage by volume for commercial plantations. This shows that in commercial production it is important to focus on agronomic as well as on management methods in increasing oil production (Weiss, 1997; Lis-Balchin, 2002). The life span of geranium plantation depends mainly on the management practices and it can be up to 10 years but for commercial production considering the cost of re-establishment it should not be less than 6 to 8 years (Weiss, 1997).

Currently, countries such as China, Egypt, Morocco, Reunion Island and India are known for their high production of rose geranium essential oil (Lawrence, 1985; Prakasa Rao *et al.*, 1985; Scheffer *et al.*, 1993; Weiss, 1997). Among these, China is the leading producer in the world market and has dominated this position for several years. Production is estimated to be 80 to 110 t per year, of this 20 to 30 t is consumed locally and the rest exported to the world market; followed by Egypt which produces 50 to 55 t per year; Réunion Island 6 t per year then India with ± 2 t per year (Demarne, 2002). However, still the world market demand could not be satisfied (210 t per year) (Demarne, 2002) and our world's major essential oil consumers are USA (40%), Western Europe (30%) and Japan (7%) (TLV *et al.*, 2004).

This creates an opportunity for countries such as South Africa for rose geranium production. Today, South African rose geranium production is expanding and is spreading into areas from George in the South-Eastern Cape to far north of the country, up to Malawi. There is a strong believe that the crop can bring trade into the country and that South Africa has the capacity to play a major role in the world market of rose geranium and to dominate the world US\$ 5-billion essential oil market per year (SouthAfrica Info., 2003). Other essential oils which already put the country into the international market are citrus and eucalyptus. For instance, in 2003 about 485 tons of citrus and 300 tons of eucalyptus essential oils were exported and this was 2% and 5% of the world market, respectively. In the same year, South Africa exported half a ton of rose geranium but the production can be increased as proposed by TLV *et al.* (2004) into a turnover of R125 million per annum from 150 tons of essential oil production from 3000 ha rose geranium plantations.

2.6 Essential oil content and quality of rose-scented geranium

Essential oil production and oil composition can be affected by change in temperature, humidity and rainfall. According to Weiss (1997) climatic factors which favour the production of high herbage yield, have the contrary effect on essential oil production, and vice versa; understanding this process can help that if one of the products (herbage or essential oil yield of the crop) is reduced it can be balanced by increasing or decreasing the climatic factors. Essential oil composition was also found to differ with harvesting time even in the same day and the same is also true in the harvesting height of the plant (Rajeswara Rao *et al.*, 1996). In India rainy/monsoon and autumn seasons were found conducive for good crop growth. This resulted in high biomass ($\text{t}\cdot\text{ha}^{-1}$), concentration (%) and essential oil yield ($\text{liter}\cdot\text{ha}^{-1}$) whereas low value was recorded in dry summer season. Winter and spring were also found to support good growth if the crop was irrigated (Rajeswara Rao *et al.*, 1996). On the other hand, crops harvested in the spring (semi-dry period) also had greater oil content than those cut after monsoon rains (Mani & Sampath, 1981; Prakasa Rao *et al.*, 1995), whereas oil from plants grown in semi-arid conditions showed similar variation (Bhattacharya *et al.*, 1993). In the Northern Hemisphere where there are no distinct seasons, high herbage production was mostly related with the flowering season (spring) and in Israel (Weiss, 1997) and Fleisher & Fleisher (1985) reported high herbage yield in May and high oil content in August.

The value of the oil extracted from this plant in the market is determined by its terpenoid composition in the market. The composition of terpenoid in rose-scented geranium was found to be affected by different factors. These were, locations of the crop where it was grown (Rajeswara Rao *et al.*, 1990a) and age of the leaves (Rajeswara Rao *et al.*, 1993, Motsa *et al.*, 2006). In other aromatic crops, terpenoid composition was found to be affected by photoperiod, light intensity, temperature (Rajeswara Rao *et al.*, 1996; Farooqi *et al.*, 1999) and harvesting month or season (Rajeswara Rao *et al.*, 1996; Motsa, 2006).

Even though there is no much information specifically about each environmental factor on the amount of oil produced, the characteristics of the oil were found to differ with location. Geranium essential oil of Grasse France has mostly rose like odour,

Reunion minty odour with high content of citronellol and this is the standard by which the quality of geranium oils is measured, Morocco oil is similar to that of France whereas Russian is frequently less strongly scented and oil in East African countries is also good based on their own grouping (Weiss, 1997). As cited by Weiss (1997) percentage of geraniol and citronellol also were found to differ with countries such as Spanish oil around 45% geraniol and 25% citronellol; Japan not more than 22% geraniol; Israeli oils have little geraniol but 45% citronellol. Oil produced from *P. radens* in Zimbabwe was dark with a pronounced minty note. This makes difficult to predict the quality of oil to be produced when a cultivar is introduced into a new area (Weiss, 1997).

CHAPTER THREE

RESPONSE OF ROSE-SCENTED GERANIUM HERBAGE YIELD, ESSENTIAL OIL YIELD AND OIL COMPOSITION TO CONVENTIONAL AND ORGANIC NITROGEN FERTILIZERS

3.1 INTRODUCTION

Rose-scented geranium (*Pelargonium capitatum* x *P. radens*) belongs to the family Geraniaceae and it is a multi-harvest, high value, commercially important essential oil yielding aromatic plant. The essential oil extracted from the herbage of the plant is widely used in the fragrance and cosmetics industry and scenting of soaps. The essential oil is extracted by steam distillation. China, Egypt, and Réunion Island are historically the leading rose geranium essential oil producing countries (Rajeswara Rao *et al.*, 1990a; Ram *et al.*, 2003). Currently, the worldwide demand for essential oil is estimated to be around 600 ton per annum (Bhan, Dhar, Choudhary, Rekha, Balyan, Khan, Agarwal & Shawl, 2006). Oil from each region has a unique chemical composition and, therefore, a unique position in the market. Réunion oil (Bourbon) has typically demanded the highest price. The chemical characteristics of Réunion oil are well understood (Weiss, 1997). The demand for especially organically produced South African rose-scented geranium is also increasing. However, despite being the centre of origin, South African rose geranium production is still very limited (about 3 ton per annum), but it is estimated that the country could potentially contribute about 50 ton per annum of geranium essential oil to the world market (TLV *et al.*, 2004). Therefore, research to improve rose geranium oil yield and quality is important.

To increase production, fertilizer inputs are becoming increasingly important; especially N. In South Africa, the demand for N fertilization is significantly increasing and therefore, understanding its management becomes important. Nitrogen is one of the essential elements that are required for healthy plant growth. In crop production it is most deficient of all nutrients. Its deficiency is characterized by yellowing and stunted growth, while its adequacy is associated with vigorous

vegetative growth and dark green leaves that result into high photosynthetic activity (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). At normal growth, plants contain 1 to 5% of N on a dry mass basis. Plants absorb N in the form of nitrate (NO_3^-), ammonium (NH_4^+) and urea. These elements are reserved in the soil, but the supply of the reservoir for this element is very limited and it can often not stay effective for a very long time, in cultivated soil. Therefore, for optimum crop production, additional N supply is important either in the form of organic or inorganic N fertilizers (Tisdale *et al.*, 1993; Brady & Weil, 2002; Taiz & Zeiger, 2002).

Nitrogen has a direct or indirect effect on crop production. Its direct effect might be by the influence on the photosynthetic activity of the crop, since the leaf is the major part where photosynthesis takes place and by the involvement of N in pigmentation (chlorophyll) of the crop. Chlorophyll is a plant pigment which is required for sunlight absorption. Therefore, discoloration of the leaves will result in less sunlight being trapped and as a result low synthesis of carbohydrate (sugar). This will lead to poor plant growth and low crop production (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). Its indirect effect could be related to its influence on essential oil producing plants. For instance, in rose-scented geranium (*Pelargonium capitatum* x *P. radens*) the essential oil is stored in structures called glandular trichomes (Weiss, 1997; Demarine, 2002). Though these structures are also present on young succulent stem parts of the plant, most of them are populated on both the abaxial and adaxial surfaces of the leaf (Mallavarapu *et al.*, 1997; Demarine, 2002). According to different researchers, more than 50% of the plant's essential oil comes from the leaves (Kumar *et al.*, 2001). Other studies also supported this finding by confirming the existence of a direct relation between plant leaf number and essential oil yield (Mallavarapu *et al.*, 1997). Therefore, loss of leaves from the plant will have a dual negative impact by influencing the biomass production and essential oil yield.

Studies on fertilization of rose geranium were started in 1894 (Demarkine, 2002). When there are no other limiting factors such as water supply, weed and pest infestation and deficiency of other nutrients such as phosphorus, positive response of the plant to applied N was reported. Analogous to the role of N in production, the studies described how important it is to know how much to apply (Weiss, 1997). Adequate N enhances leaf preservation (Salisbury & Ross, 1992; Taiz & Zeiger,

2002) and at the same time increases the chances of essential oil recovery through distillation (Mallavarapu *et al.*, 1997). However, if it is applied in excess, it might not result in high production by encouraging the development of rust, caused by *Puccinia pelargonii-zonalis* due to shading and yellowing and loss of bottom leaves, as well as due to inadequate sunlight penetration (Weiss, 1997). In addition, excess N might leach either during irrigation or rainfall and cause environmental pollution.

Nitrogen requirement of rose-scented geranium varies with growing area. A rate that was found advantageous for optimum growth in one area might not be appropriate for another area. For instance in India, Singh (1999) reported positive response of the crop with N application and biomass and essential oil yield were the highest at 200 kg·ha⁻¹ N. However, essential oil composition was not significantly influenced by N application. When conventional N was applied at a rate of 160 kg·ha⁻¹ together with organic mulch, a significant effect on biomass, essential oil yield and composition was reported, compared to when conventional N was applied the same rate solely (Ram *et al.*, 2003). Under South African conditions, there is not sufficient information available on N management in terms of optimum rate and source for rose geranium oil production. Current fresh biomass (t·ha⁻¹) and essential oil yields (kg·ha⁻¹) in South Africa are also reported to be low and therefore, research on this was highly important (Learmonth, 2006, personal communication).

Previous field experiments by this research group (unpublished data) showed that the plant usually grows well from establishment until the first harvest, but re-growth can, under certain circumstances, be poor. Poor growth results in low herbage yield and, consequently, low total essential oil production per hectare. Poor growth is believed to be due to a combination of factors, including nutrient deficiencies. Other authors (Prakasa Rao *et al.*, 1985; Singh *et al.*, 1996; Singh, 1999; Ram *et al.*, 2003) have reported that faster growth of the crop can be attained by application of N before planting and after harvesting. Knowing the exact amount of N for each growing region and how the plant responds to source of N, is important for economically viable production of this crop. Furthermore, market demand for organically certified rose geranium from South Africa is increasing, requiring a better understanding of how geranium responds to organic fertilization.

The objective of this study was to investigate the response of rose-scented geranium, in terms of fresh herbage yield, essential oil yield, oil composition and nitrogen use efficiency (NUE), to different amounts of conventional and organic N fertilizers. The following hypotheses were consequently tested:

- (i) Rose-scented geranium biomass and oil yields will respond positively to nitrogen top dressings after harvest.
- (ii) Rose-scented geranium will respond similarly or better to organic N than conventional N sources.

3.2 MATERIALS AND METHODS

Experimental site, experimental design and treatment details

A field experiment was conducted at the University of Pretoria's Hatfield Experimental Farm, Pretoria. The experimental site is situated at 25° 45'S latitude, 28° 16'E longitude and altitude of 1372 m. The soil was classified as a sandy clay loam Hutton (The Soil Classification Working Group, 1991). The 0 to 30 cm soil layer contained 22.7 % clay and had a pH 6.3 (1:2.5, soil: water ratio), 0.6% total carbon, 0.04% total N, 12 kg·ha⁻¹ P and 152 kg·ha⁻¹ exchangeable K. The experimental site has a warm, temperate, summer-rainfall climate. It receives an average of 670 mm rain annually, mostly during the months of October to March (Annandale *et al.*, 1999). Climatic data during the trial period (Appendix A) were collected using an automatic weather station (Campbell Inc., Nebraska, USA) installed close to the experimental field.

Healthy and rooted cuttings (45 days old) of rose geranium (*Pelargonium capitatum* x *P. radens*) were planted in the experimental plots on 29 January 2004, at a spacing of 0.625 m between plants and 1 m between rows, which gave a total of 16 000 plants·ha⁻¹. Before treatment application commenced, the plants were allowed to establish for twelve months and during this period all the necessary agricultural practices were taken. In early February 2005, plants were cut back to a height of 20 to 25 cm above ground level and at the same time treatment applications were started. Two sources of N, namely conventional (limestone ammonium nitrate or LAN, 28%

N) and organic fertilizers (blend of four organic fertilizers) (Table 3.1) at three levels (100, 200 and 300 kg·ha⁻¹ N·year⁻¹) and a control (no N) were applied. Treatments were replicated four times in a randomised complete block design. Phosphorous (P) and potassium (K) fertilizers were applied at 90 and 60 kg·ha⁻¹·year⁻¹ respectively, to all the plots. For conventional N treatments, super phosphate (P₂O₅, 11.5% P) and potassium chloride (KCl, 50% K) were used as sources of P and K, respectively. The yearly P and K were split into three applications, with one third applied before planting, followed by equal splits after each harvest. Similarly, the yearly N was applied in six equal splits at two monthly intervals (in order to minimize leaching) and harrowed in manually. For organic N treatments, the yearly amount was applied in three splits, one portion before planting, followed by equal splits after each cut back of the plants. Application of organic N treatments was designed to achieve comparable levels to conventional N treatments, while at the same time ensuring P and K levels of 90 and 60 kg·ha⁻¹·year⁻¹, respectively.

Table 3.1: Nitrogen (N), phosphorous (P) and potassium (K) content of the organic fertilizers used for the trial

N: P: K	N (g·kg ⁻¹)	P (g·kg ⁻¹)	K (g·kg ⁻¹)
7:2:2 (11)	70	20	20
2:3:2 (16)	46	69	46
3:1:5 (18)	60	20	100
4:1:1 (15)	100	25	25

Each experimental plot consisted of four rows of 5.6 m long, with a 1 m path between plots, giving a total area of 22.5 m²·plot⁻¹. From the four rows per plot, the two centre rows were used for collection of fresh herbage yield (t·ha⁻¹) and essential oil yield (kg·ha⁻¹) and oil composition analysis, while the two outer rows on either side were used as borders to reduce treatment overlap. Rainfall was supplemented with drip irrigation. Prior to irrigation, soil water content was measured to a depth of 60 cm using a neutron probe (Neutron water meter, Model 503 DR, CPN Corporation, CA, USA) on a weekly basis. Whenever the plant available water in the soil reached the 60% level, the profile was refilled to field capacity.

Data collection and analysis

At each harvest, fresh herbage yield, oil yield and oil composition were determined. The crop was harvested at four-monthly intervals. For the first harvest the growth period was from February to May (summer/autumn) and for the second harvest from September to December (spring/summer). Plants were cut to a height of 20 to 25 cm above ground level and herbage yield per hectare was computed. Oil content of the foliage (a sub-sample of 10 kg from each treatment) was determined by steam distillation using a custom-made unit. The total essential oil yield per hectare was computed per treatment according to the oil content and harvested area. The oil samples were analysed by gas chromatography (GC). For GC analysis, an Agilent GC (FID) model 6890N fitted with 30 m x 0.25 mm fused silica capillary column and film thickness of 0.25 μm was used, with helium as carrier gas. The temperature programming was 50 $^{\circ}\text{C}$ – 200 $^{\circ}\text{C}$, with ramp amount of 5 $^{\circ}\text{C min}^{-1}$ and with detector and injector temperatures of 220 $^{\circ}\text{C}$. The compounds were identified by comparing their retention times and Kovat indices (Adams, 2004) to standard values. After each harvest, soil samples were taken for N, P and K analyses and based on this, nutrients used by plants and lost via leaching and volatilisation were replaced so that the re-growth was started with the same amount of nutrients as for the previous harvest. Standard cultivation and plant protection practices were followed to control weeds, insects and diseases during the experimental period.

At each harvest, two plants per treatment per replication were taken for growth parameter measurements. Each plant was separated into leaves and stems, whereafter fresh and dry mass (after drying in an oven for 72 hrs at 70 $^{\circ}\text{C}$) were determined. Total leaf area per plant was determined using a belt driven LI 3100 leaf area meter (LiCor Inc., Lincoln, Nebraska, USA). For each treatment, number of branches per plant, plant spread and plant height was also determined.

Nitrogen use efficiency (NUE) was calculated as described by Rajeswara Rao *et al.* (1990c). The equation used to compute the NUE of the plant is described in Eq. [3.1], where, Y_n is the total biomass ($\text{t}\cdot\text{ha}^{-1}$) or essential oil ($\text{kg}\cdot\text{ha}^{-1}$) yield, Y_o is the total biomass ($\text{t}\cdot\text{ha}^{-1}$) or essential oil ($\text{kg}\cdot\text{ha}^{-1}$) yield for the zero N plot (control). Specific

leaf area ($\text{cm}^2 \cdot \text{g}^{-1}$) and leaf area index were calculated according to Eqs. [3.2] and [3.3], respectively.

$$\text{NUE} = \frac{Y_n - Y_o}{N} \quad [3.1]$$

$$\text{Specific Leaf Area (cm}^2 \cdot \text{g}^{-1}) = \frac{\text{Leaf area per plant}}{\text{Leaf weight per plant}} \quad [3.2]$$

$$\text{Leaf area index (cm}^2 \cdot \text{cm}^{-2}) = \frac{\text{Leaf area per plant}}{\text{Soil area per plant}} \quad [3.3]$$

Statistical analysis and interpretations were based on comparison of treatment means as well as comparison between cumulative harvests. The collected data were subjected to analysis of variance (ANOVA) and analysis was carried out using the SAS package (Statistical Analysis System Institute Inc. 1999-2001). To determine the relationship between essential oil yield and N levels (oil yield response curve), a quadratic curve was fitted to the data (Hyams, 1997). The used equation was: $Y = a + bx + cx^2$; where Y is the essential oil yield ($\text{kg} \cdot \text{ha}^{-1}$); while a,b and c were constants.

3.3 RESULTS AND DISCUSSION

Herbage and essential oil yields

The data showed that application of conventional N had no significant effect on fresh herbage and essential oil yields of rose-scented geranium, compared to the control, for the first harvest (summer/autumn) (Table 3.2). However, application of organic N at $100 \text{ kg} \cdot \text{ha}^{-1}$ increased fresh herbage and essential oil yields by 57.5% and 49.9% compared to the control, respectively. The lack of response of the plant to the applied conventional N could be due to leaching of N by heavy rainfall that occurred during this growing cycle (Appendix A).

Table 3.2: Influence of amount and source of N on fresh herbage ($\text{t}\cdot\text{ha}^{-1}$) and essential oil yield ($\text{kg}\cdot\text{ha}^{-1}$) of rose-scented geranium, i.e. first harvest from February to May 2005 (summer/autumn) and second harvest from September to December 2005 (spring/summer)

Source of N	Treatments	Herbage yield ($\text{t}\cdot\text{ha}^{-1}$) ^z			Oil yield ($\text{kg}\cdot\text{ha}^{-1}$)		
		Harvest			Harvest		
		1	2	Total	1	2	Total
	N ($\text{kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$)						
Control	0	14.83 ^a	20.91 ^a	35.74 ^a	7.27 ^a	13.59 ^a	20.86 ^a
Conventional	100	15.63 ^a	30.59 ^b	46.22 ^b	9.53 ^{ab}	26.31 ^b	35.84 ^{bc}
	200	17.00 ^a	32.54 ^b	49.54 ^{bc}	7.99 ^a	27.01 ^{bc}	35.00 ^{bc}
	300	16.43 ^a	30.80 ^b	47.23 ^{bc}	7.88 ^a	21.25 ^b	29.13 ^b
Organic	100	23.36 ^b	33.52 ^b	56.88 ^{cd}	10.90 ^b	31.17 ^c	41.92 ^{cd}
	200	23.78 ^b	35.23 ^{bc}	59.01 ^d	11.89 ^b	32.76 ^{cd}	44.65 ^d
	300	23.80 ^b	41.48 ^c	65.28 ^d	10.95 ^b	38.15 ^d	49.10 ^d
LSD ($\alpha = 0.05$)		6.08	7.96	9.72	2.45	6.82	7.22
<i>Conventional (over all N levels)</i>		16.35 ^a	31.31 ^a	47.66 ^a	8.47 ^b	24.86 ^a	33.33 ^a
<i>Organic (over all N levels)</i>		23.65 ^b	36.74 ^b	60.38 ^a	11.20 ^b	34.03 ^a	45.23 ^b
LSD ($\alpha = 0.05$)		2.88	4.56	5.21	1.48	4.07	4.18

^z statistical comparison is among values within the same column; Different letters within a column indicate significant difference means at the 95% confidential level using Tukey's test.

In the second harvest, application of N from both sources (conventional and organic) increased fresh herbage as well as essential oil yields (Table 3.2). The increases in fresh herbage yield over the control due to conventional and organic N at $100 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ were 46.3% and 60.3%, respectively. The $100 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ level of N also increased essential oil yield by 93.6% and 129.4%, compared to, the control for the conventional and the organic N, respectively.

Increasing N level beyond $100 \text{ kg} \cdot \text{ha}^{-1}$ with both N sources (conventional or organic) did not contribute to further significant increases in the fresh herbage yields for either harvests or the cumulative yields over the two harvests (Table 3.2). For essential oil yield, further N increments also provided no advantage for the first harvest. For the second harvest, further positive responses to increased N levels above $100 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ were observed, with a significantly higher oil yield at $300 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ organic N.

Rajeswara Rao *et al.* (1990a) also reported an increase of 19.1% in fresh herbage yield and 24.1% in essential oil yield at $100 \text{ kg} \cdot \text{ha}^{-1}$ N, but no response with higher N levels. However, Prakasa Rao *et al.* (1985) reported an increment in fresh herbage and essential oil yield of geranium with each increment of applied N. Similarly, in India, application of $200 \text{ kg} \cdot \text{ha}^{-1}$ N increased fresh herbage and essential oil yield over no N and $100 \text{ kg} \cdot \text{ha}^{-1}$ N (Singh *et al.*, 1996; Singh, 1999).

The average effect of all levels of organic N was compared to the average effect of all levels of conventional N. In all instances, organic N produced higher herbage and oil yields (Table 3.2). As described by different authors (Weiss, 1997; Demarine, 2002), geranium plants prefer to grow in well-drained soils and with high organic matter content for optimum herbage and essential oil yield. As it is also known, application of organic matter in the form of either organic fertilizer (plant or animal residues) or manure is well known for its beneficial effect on the beneficiary microbial activities and in improving the soil structure and water holding capacity of the soil (Tisdale *et al.*, 1993; Brady & Weil, 2002). Better root development and a two-fold increase in essential oil yield were reported when 5 to 10 tons of distillation residues was applied to geraniums intercropped with legumes, than geranium grown as a single crop (Chabalier, 1992).

Interaction between nitrogen amount and source

For the second harvest, there was a significant ($p \leq 0.05$) interaction between amount and source of N for essential oil yield (Figure 3.1). This implies that essential oil yield responded differently to an increase in N levels for the two sources of N. As organic N increased, essential oil yield increased, while it did not alter significantly when conventional N increased. Application of $300 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ in the form of organic N increased the essential oil yield by 180.7%, compared to the control and by 22.4% compared to $100 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ organic N (Table 3.2; Figure 3.1).

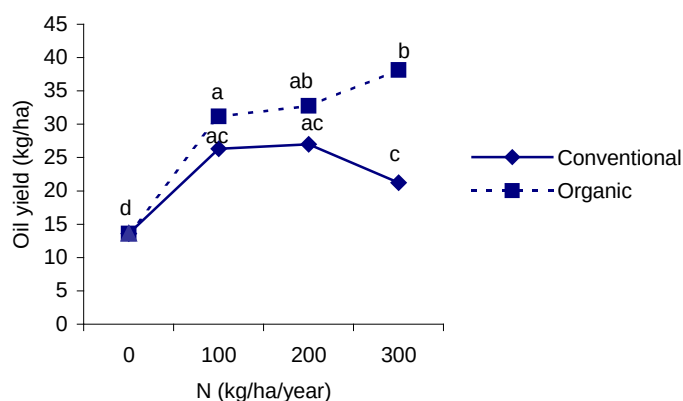


Figure 3.1: Interaction of amount and source of N on essential oil yield of rose scented geranium. Values presented are means ($n = 16$)

Scheffer *et al.* (1993) also reported that application of organic fertilizer (composed of cattle manure + straw) significantly increased essential oil yield of *Achillea millefolium* L. The positive response of the plant to organic N could be due to the nature of organic fertilizers in improving soil structure, water holding capacity and soil microbial activity (Tisdale *et al.*, 1993; Rajeswara Rao, 2001). Furthermore, the possibility of lower N loss through leaching and their slow nutrient releasing nature, compared to conventional fertilizers, may be the reason for increased herbage and essential oil yield (Tisdale *et al.*, 1993).

Oil yield response curve

Fertilizer recommendation has to take into account optimum yield, quality of the plant material to be produced, profit or return to be gained and the impact to the environment after application (Bock & Sikora, 1990; Angus *et al.*, 1993; Bullock & Bullock, 1994). This can be done by estimating the optimum N requirement of the plant for optimum growth and development through different statistical models, for instance through curve fitting (Cerrato & Black, 1990; Isfan *et al.*, 1995). In the present study, a simple model was developed to estimate the essential oil yield of rose-scented geranium with respect to N application rate ($\text{kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$) (Fig. 3.2). The essential oil yield was plotted against N ($\text{kg}\cdot\text{ha}^{-1}$) and the values were correlated using MS excel. After identifying the appropriate equation for the fit, essential oil yield values (with minimum initial essential oil yield values at $0 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ of N, control treatment, per harvest and source) were further extrapolated in order to identify the optimum N requirement of the crop (Fig. 3.3) using the fitted function. Based on this, the essential oil yield of the plant was affected by the source of N (conventional or organic) applied during the growing periods. In both harvests, the plant responded more to organic than to conventional N (Fig. 3.3).

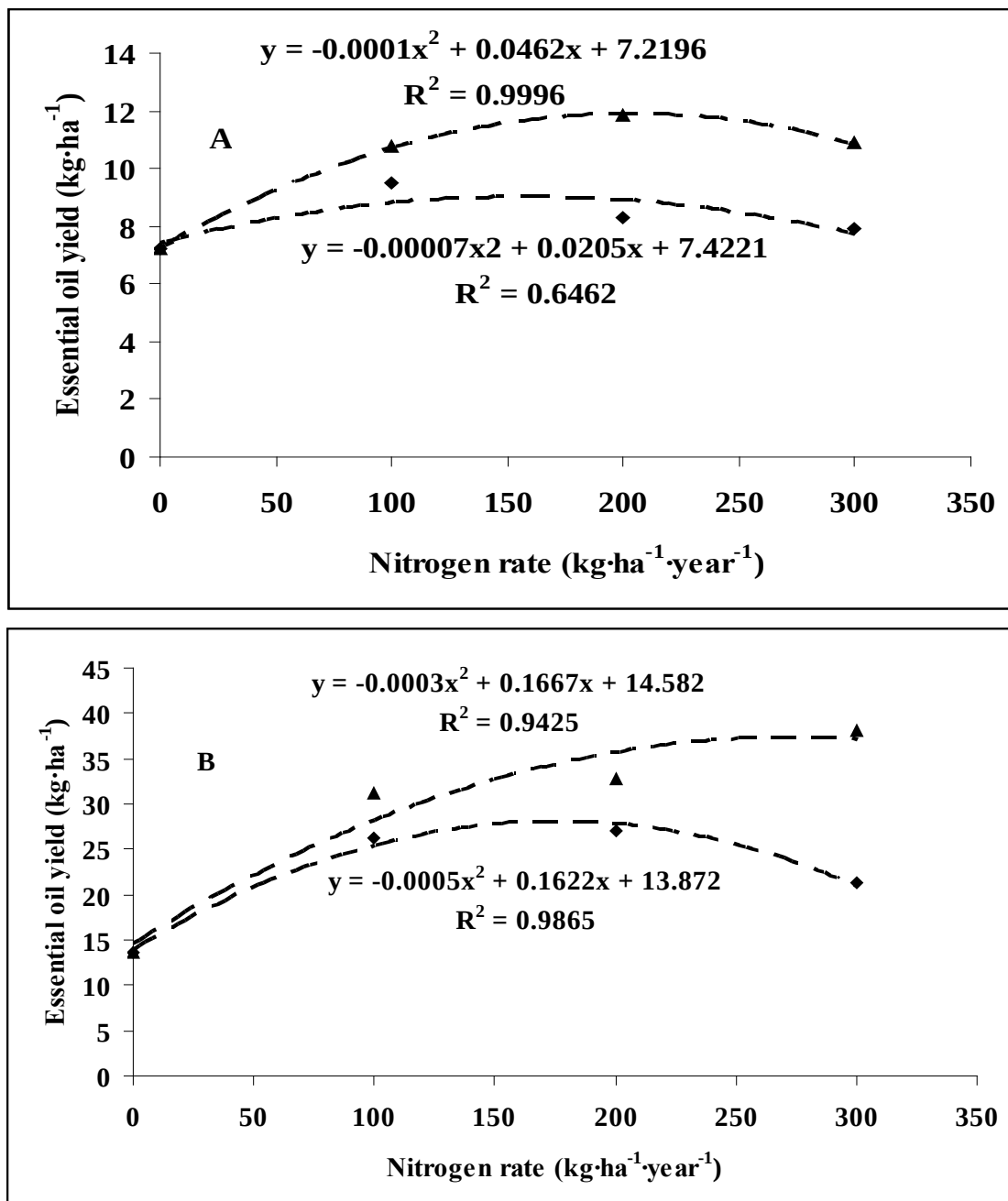


Figure 3.2: Relationship between rose-scented geranium essential oil yield (kg·ha⁻¹) to N applied using (♦) conventional or (▲) organic fertilizers (kg·ha⁻¹·year⁻¹) at (A) first (February to May 2005; summer/autumn) and (B) second (September to December 2005; spring/summer) harvests

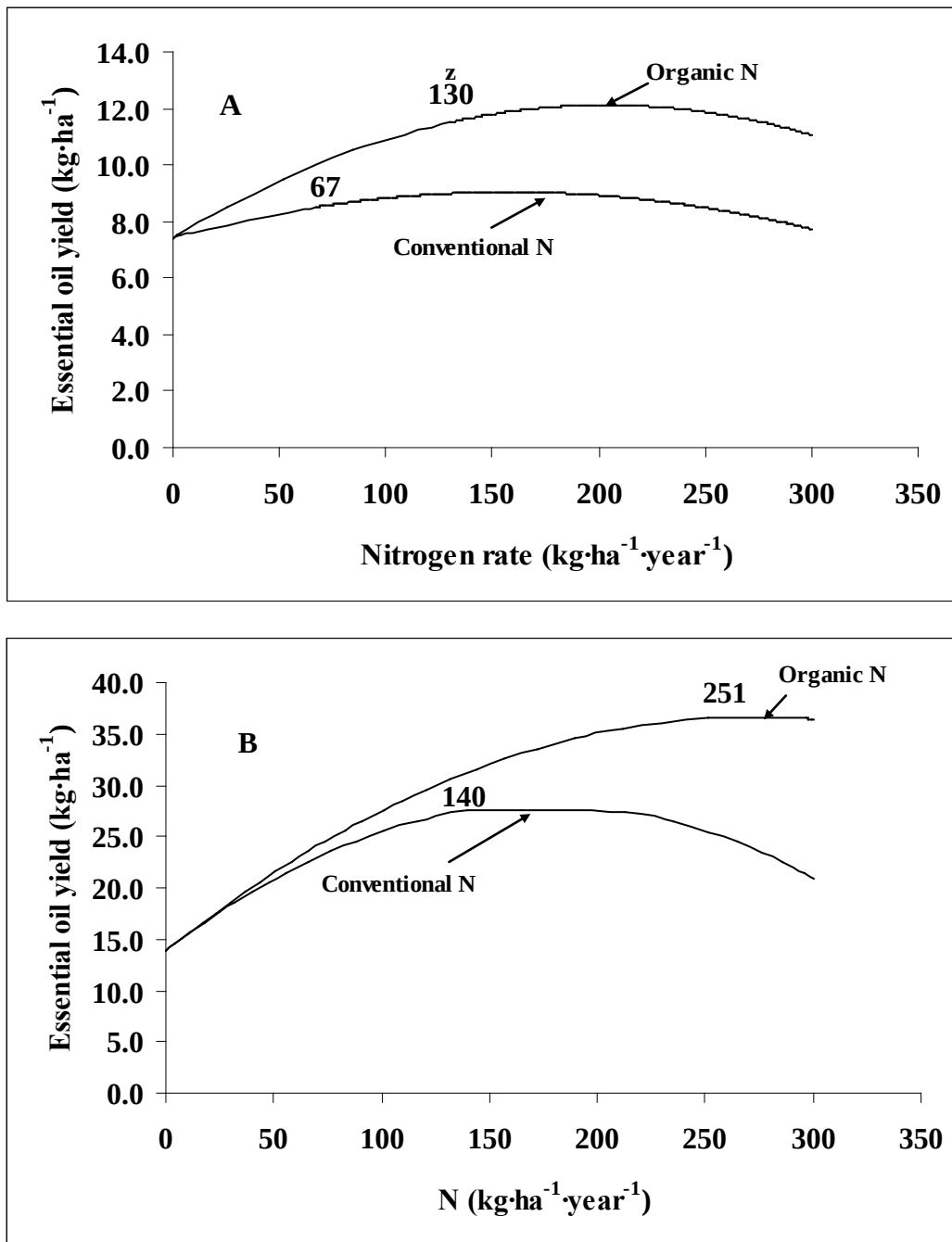


Figure 3.3: Estimation of optimum nitrogen for rose-scented geranium essential oil production at (A) first (February to May 2005; summer/autumn) and (B) second (September to December 2005; spring/summer) harvests with conventional or organic fertilizers

^z Values on the line graph represent optimum N (kg·ha⁻¹·year⁻¹)

The optimum estimated N values for the first harvest were 67 and 130 (kg·ha⁻¹·year⁻¹) for conventional and organic N, respectively (Fig. 3.3A). Application of organic N at a rate of 25 kg·ha⁻¹·year⁻¹ resulted in the same essential oil yield as conventional N at

the rate of $67 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$. This shows that N in the organic form was more efficient to support plant growth than conventional N. This might be due to slow releasing and less vulnerability to loss of organic N, compared to conventional N (Tisdale *et al.*, 1993; Rajeswara Rao, 2001).

In the second harvest (Fig. 3.3B), up to a certain N level, essential oil yield was about the same for both N sources, but with further increase in N level the response started to vary. Essential oil yield of $28 \text{ kg}\cdot\text{ha}^{-1}$ was achieved by application of 140 and 101 $\text{kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ of conventional and organic fertilizer, respectively. The difference in the optimum N values between the two harvests could be due to seasonal effects, since the first harvest was in summer/autumn (during rainy season), while the second harvest was in spring/summer. According to Weiss (1997) the plant requires clear sunny days for maximum leaf growth and high oil content, rather than cold temperatures or cloudy days.

Determining which source of N and at what rate it should be applied is important to consider in rose-scented geranium production. When N fertilizer is applied consideration has to be given for optimum plant growth, benefit to the farmer in comparison to no application and finally effect of N on the environment. Most plant species respond well to N and even excess application has no toxicity effect (Tisdale *et al.*, 1993). This usually encourages growers or farmers to apply more than what is required for optimum growth. As a result, benefits that were supposed to be obtained with optimum application are reduced and the environment is adversely affected through pollution. The net benefit to the farmer is realized after deducting the cost of fertilizer. That is:

$$\text{Net benefit} = [(\text{essential oil yield} \times \text{essential oil price}) - \text{cost of fertilizer}]$$

The net profit to be gained depends on different factors, such as the region and conditions where rose-scented geranium was grown, cost of fertilizer at the time of production and market value of the essential oil. In other words, it has agronomic as well as economic aspects. The agronomic aspect includes determining from each kg increase in N fertilizer how much essential oil can be produced in return. The response of the plant to the applied N could depend on soil characteristics (soil type

and conditions), variation in weather or season and crop management practices such as pest, disease and weed control (Tisdale *et al.*, 1993). The economic aspect considers the principle law of diminishing returns. That is, the response of the plant to the applied N decreases with each successive increase in N level. In the present study, this can be illustrated by Fig. 3.4.

According to Brik & Vitousek (1986), nitrogen use efficiency (NUE) is defined as the ratio between the amount of organic matter lost from a plant or permanently stored in wood and the amount of N lost or permanently stored by the plant. Generally NUE is interpreted as plant productivity per unit N uptake or loss (Vitousek, 1982). In the present study, NUE of the plant showed negative linear correlation to levels of N from the two sources in both growing cycles (Fig. 3.4). Nitrogen use efficiency decreased with an increase in nitrogen level. Rajeswara Rao (2001) and Rajeswara Rao *et al.* (1990c) also reported a decrease in NUE of rainfed palmarosa (*Cymbopogon martini* (Roxb.) with an increase in N level. The present investigation indicated that application of $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ resulted in higher essential oil production efficiency of the plant for both sources and in both harvesting seasons. For the first harvest (February to May 2005; summer/autumn), application of $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ of N had 90.9% and 64.7% higher efficiencies in essential oil production than application at $300 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$, for conventional and organic N, respectively (Fig. 3.4A). In this harvest, although the NUE pattern was the same for conventional and organic N, i.e. a decrease with an increase in N level, the response was greater with organic N. This reason might be due to the difference in characteristics of conventional (leaching or volatilisation) to organic N. As mentioned earlier, high rainfall that occurred (Appendix A) during this particular harvest might also have contributed to the variation. This might be the reason why organic N resulted in 53.7% greater in NUE than conventional N at a rate of $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ (Fig. 3.4A).

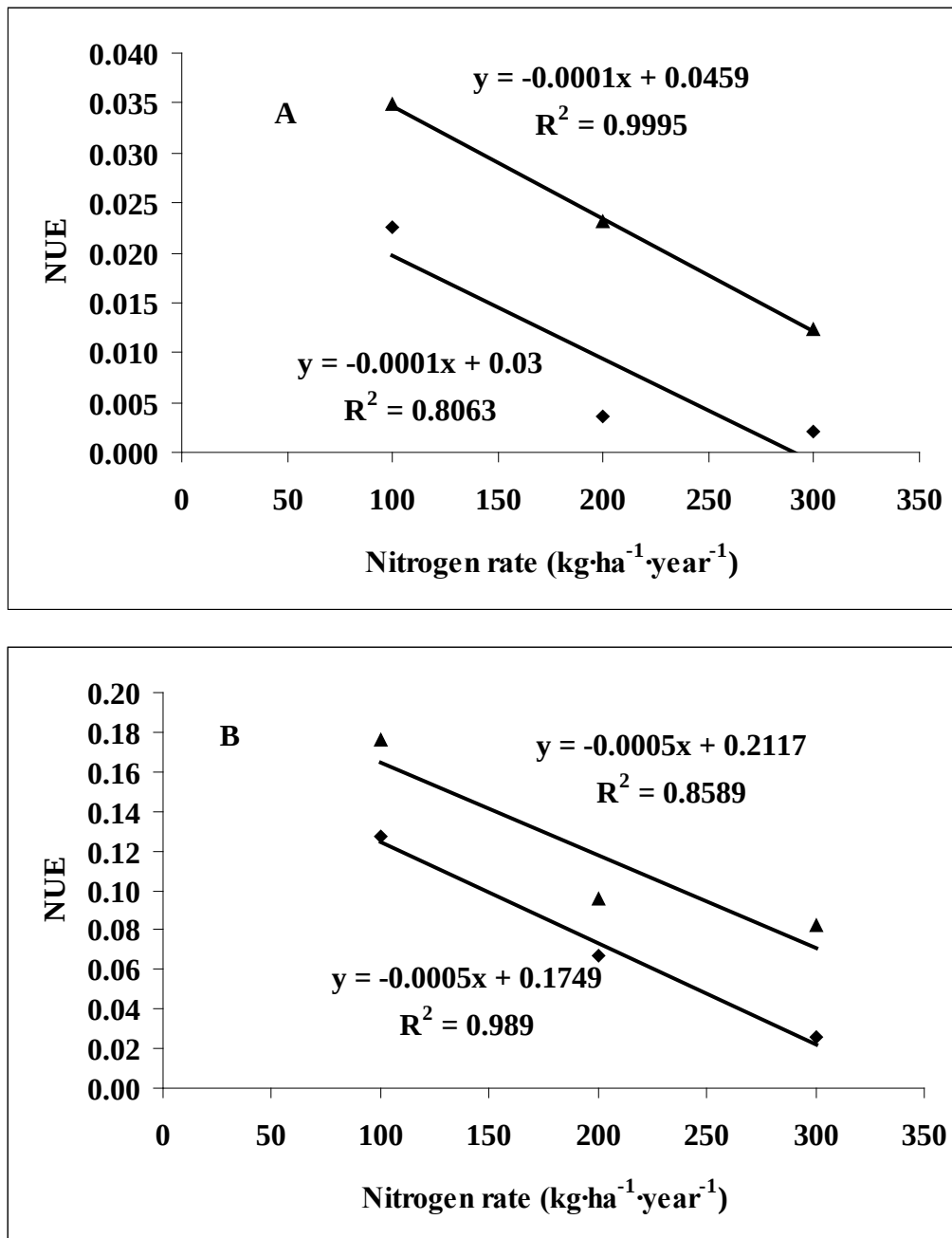


Figure 3.4: Nitrogen use efficiency (NUE) of rose-scented geranium at (A) first (February to May 2005; summer/autumn) and (B) second (September to December 2005; spring/summer) harvests using (♦) conventional or (▲) organic fertilizers. Values presented are means ($n = 16$)

In the second harvest, the trend was the same (Fig. 3.4B), namely NUE decreased with an increase in N level, and organic N gave highest in essential oil production efficiency. Application of 100 kg·ha⁻¹·year⁻¹ of N resulted in 79.9% and 53.4% higher efficiency in essential oil production than application of 300 kg·ha⁻¹·year⁻¹ N for

inorganic and organic N sources, respectively (Fig. 3.3B). The plant was more efficient in essential oil production with organic (38.2%) than conventional N at 100 kg·ha⁻¹ year⁻¹. Freney (1997) suggested that N loss can be reduced or NUE of a plant can be improved by either using slow release fertilizers or through fertigation. When essential oil from the two harvests were compared at 100 kg·ha⁻¹·year⁻¹ of N, high returns were obtained from the second harvest (82.2% and 80.2% more efficient; conventional and organic, respectively) than the first harvest. This shows that the economic return from the plant were influenced by the harvesting period or season. Generally, this investigation indicated that application of more N than what is required for optimum growth of the plant had no positive effect on essential oil production. In addition, the net benefit from N application is dependent on the growing period. In the present study a spring/summer season gave higher NUE than summer/autumn, and application of organic N resulted in higher NUE than conventional N.

Growth parameters

The influence of amount of N and source on growth parameters of rose-scented geranium are presented on Table 3.3. Application of N at different levels, in the conventional or organic form had no significant effect on plant height and leaf to stem ratio of rose-scented geranium as compared to the control, in both harvests. However, a fertigation study conducted in India indicated that an increase in N significantly affected rose-scented geranium height as compared to the control (Sabina Aiyanna *et al.*, 1998). In other essential oil producing plant species, such as basil (*Ocimum basilicum* L.), Sifola & Barbieri, 2006) reported that an increase in N level up 300 kg·ha⁻¹ had no significant effect on plant height and leaf to stem ratio, while Singh (2008) on the other hand, reported that an increase in N had a direct influence on plant height of palmarosa (*Cymbopogon martinii* [roxb.] Wats. var. Motia Burk). In most cases leaf to stem ratio of a plant is higher without than with N. This is because plants without N accumulate more carbon and this results in denser stems as compared to succulent stems with N (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). However, in the present study, this was not noticed due an increase in leaf mass with an increase in N level, which resulted into higher leaf to stem ratio values than the control.

Table 3.3: Effect of nitrogen level and source on growth parameters of rose-scented geranium i.e. first harvest from February to May 2005 (summer/autumn) and second harvest from September to December 2005 (spring/summer)

Source of N	Treatment	Total dry mass (g·plant ⁻¹) ^z		Plant height (cm)		Plant spread		Leaf:stem ratio		Branch Number	
	N (kg·ha ⁻¹ ·year ⁻¹)	Harvest									
		1	2	1	2	1	2	1	2	1	2
Control	0	168.9 ^a	505.1 ^a	38.3	43.8	183.0 ^a	224.1 ^a	1.77	1.67	44.3 ^a	24.6 ^a
Conventional	100	201.1 ^a	669.8 ^b	37.3	48.3	197.3 ^b	282.3 ^{bc}	1.50	1.71	53.6 ^a	31.5 ^{ab}
	200	177.0 ^a	555.5 ^a	40.8	48.3	186.7 ^a	260.6 ^a	1.55	1.71	50.9 ^a	28.8 ^a
	300	190.0 ^a	564.0 ^a	38.5	48.2	179.4 ^a	248.4 ^a	1.42	2.08	55.0 ^a	30.0 ^a
Organic	100	671.4 ^b	725.4 ^b	40.8	47.3	233.7 ^c	270.1 ^b	1.58	1.50	59.4 ^b	45.5 ^b
	200	590.3 ^b	624.9 ^a	40.5	49.3	208.6 ^b	272.1 ^b	1.40	1.52	51.4 ^a	32.4 ^{ab}
	300	710.1 ^b	649.6 ^a	44.8	47.8	226.7 ^c	300.3 ^c	1.62	1.97	50.4 ^a	41.1 ^b
LSD (α =0.05)		280.2	163.8	NS ^y	NS	12.6	41.4	NS	NS	11.5	10.9
Conventional (mean for all N levels)		189.4 ^a	580.1	38.8	46.8	187.8 ^a	267.1	1.49	1.83	53.2	30.1 ^a
Organic (mean for all N levels)		657.3 ^b	667.0	42.0	48.1	219.7 ^b	273.5	1.53	1.66	53.7	39.7 ^b
LSD (α = 0.05)		155.0	NS	NS	NS	10.8	NS	NS	NS	NS	7.2

^z statistical comparison is among values within the same column; different letters within a column indicate significant difference means at the 95% confidential level using Tukey's test; ^y NS = Nonsignificant; $P \geq 0.05$

Application of conventional N improved total dry matter production (Table 3.3) in the second harvest ($100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$) but the difference was not significant in the first harvest as compared to the control. Plant spread was also improved with conventional N at $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ (Table 3.3) in the first and second harvest, as compared to the control. Number of branches per plant was, however, not influenced by conventional N application in both harvests. Generally, increasing the levels of conventional N beyond $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ had no significant effect on any of the above stated growth parameters, in both harvests.

Nitrogen in organic form significantly increased total dry matter production, plant spread and branching of the plant as compared to the control, in both harvests (Table 3.3). In the first harvest, total dry matter had a direct relationship with an increase in organic N, but in the second harvest organic N at $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ was only significantly different than the control, while increasing N beyond this had no effect. Plant spread followed the same trend in both harvests; an increase with increasing organic N as compared to the control, but among organic N the differences were not significant. Organic N also increased the number of branches per plant with $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ in the first and second harvest and $300 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ in the second harvest resulted into more number of branches than the control. Comparison between over all levels of organic to conventional N (Table 3.3) indicates that organic N improved total dry matter production and plant spread in the first harvest and branching in the second harvest as compared to conventional N.

Leaf area index (LAI; $\text{m}^2\cdot\text{m}^{-2}$) was not significantly influenced by an increase in N applied from both sources during the first harvest (Fig. 3.5A). Although the difference was not significant a positive quadratic relationship was observed between LAI and N applied, organic or conventional N. This positive relationship between the N sources, especially with organic N and LAI might have contributed to the high biomass and essential oil production obtained in this particular harvest. Similarly in the second harvest (Fig. 3.5B), LAI increased in a quadratic trend with an increase in N in both conventional and organic N. The response was higher with organic than with conventional N. The increase in LAI with an increase in N (up to certain N level) regardless of source is

believed to be not only due to an increase in individual leaf expansion but also due to an increase in plant leaf number (Appendix A). Similar inferences were also made by Sifola & Barbieri (2006) in basil (*Ocimum basilicum* L.). In the present study, the decrease in LAI with an increase in N applied in both sources could be related to loss of leaves, especially the bottom leaves, due to shading, since this is reported to be one of the disadvantages of higher N rates application in rose-scented geranium (Weiss, 1997). On other plant species such as maize, the relationship between N rates and LAI was reported as linear by Amanullah *et al.* (2007).

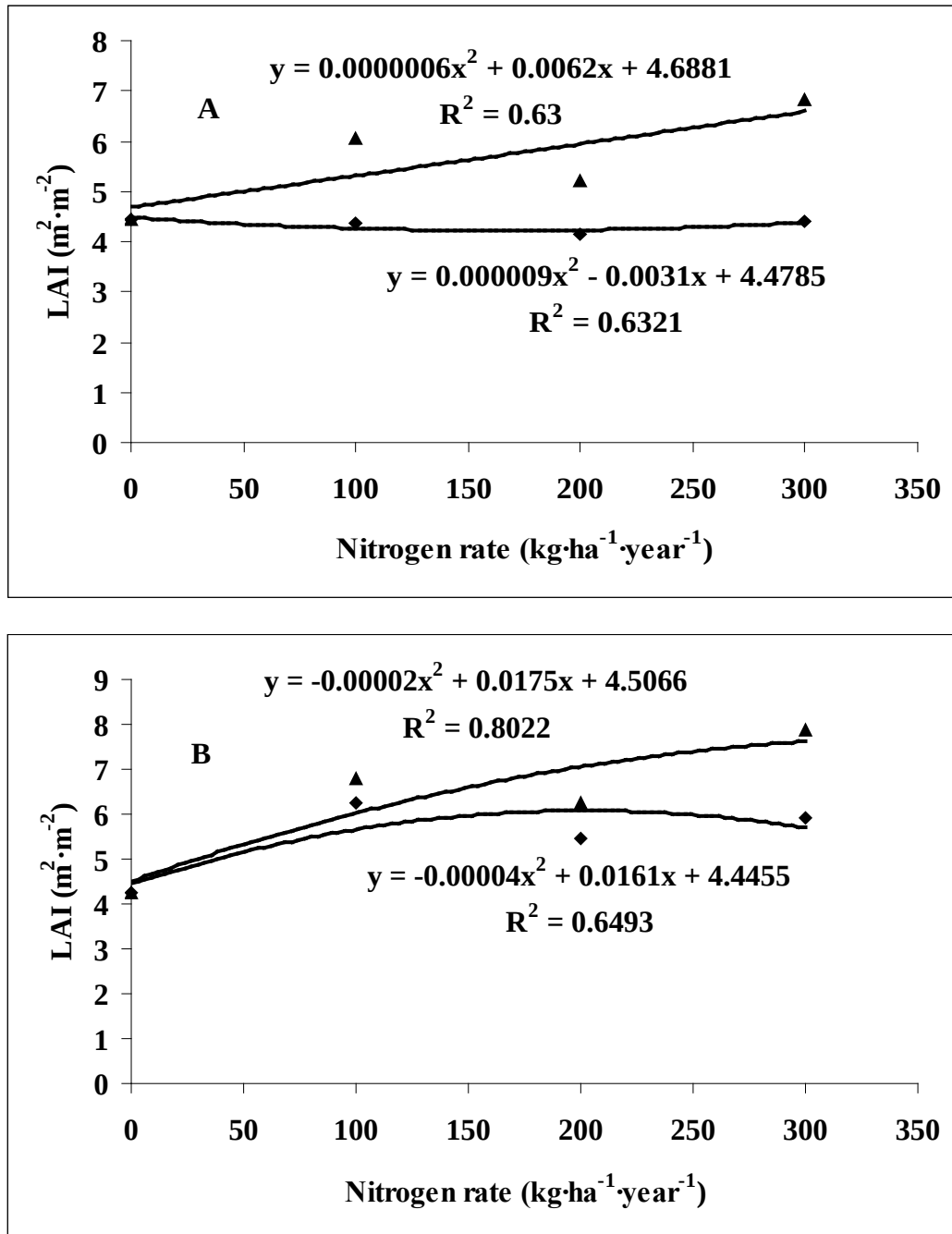


Figure 3.5: Leaf area index (LAI; $\text{m}^2 \cdot \text{m}^{-2}$) of rose-scented geranium at (A) first (February to May 2005; summer/autumn) and (B) second (September to December 2005; spring/summer) harvests using (♦) conventional or (▲) organic fertilizers. Values presented are means ($n = 16$)

According to Field & Mooney (1986), specific leaf area (SLA) is an expression used to determine the link between leaf mass and leaf area. Some researchers also express it as an inverse indicator of leaf thickness or density. Figure 3.6 shows the relationship between the leaf N content ($\text{mg}\cdot\text{g}^{-1}$) and specific leaf area ($\text{cm}^2\cdot\text{g}^{-1}$) of conventional and organic N. In the first harvest (Fig. 3.6A), the relationship was negatively correlated. That is, leaf N content decreased with an increase regardless of N source in SLA. This indicates that leaf N content increased with an increase in leaf thickness (decreasing SLA). As previously stated, this might be due to the adaptability process of the plant. In the second harvest, however, the slope of the regression line was positive (Fig. 3.6B). Leaf N content, therefore, increased with an increase in SLA. That is, leaf N content was higher in thinner leaves (higher SLA values) than in thicker leaves (lower SLA values). According to Salisbury & Ross (1992), leaves at control (zero N) and lower N levels resulted in greater accumulation of carbon than in succulent leaves with higher N levels. The effect of applied N on Leaf area ratio (LAR) of rose-scented geranium was not significant but with an increase in N applied the values of LAR decreased (Appendix A).

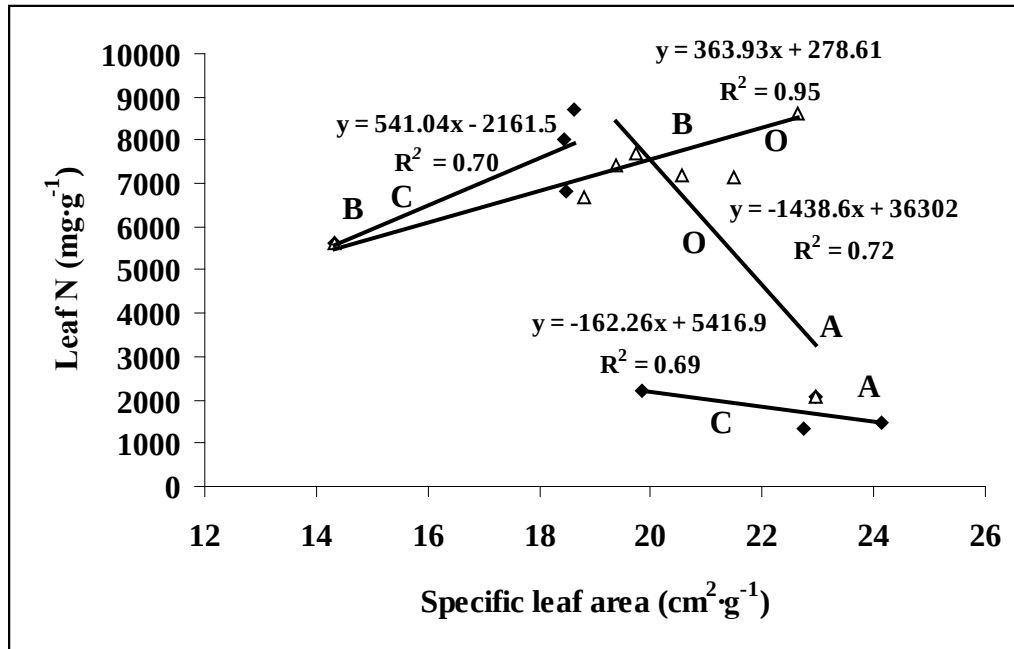


Figure 3.6: Relationship between specific leaf area (cm²·g⁻¹) and leaf N content (mg·g⁻¹) of rose-scented geranium at (A) first (February to May 2005; summer/autumn) (♦) and (B) second (September to December 2005; spring/summer) harvests (▲) using conventional (C) or organic (O) fertilizers. Values presented are means ($n = 16$)

Despite all the above mentioned advantages of N on biomass and growth parameters of the plant, a positive correlation was found between leaf senescence and plant spread (Fig. 3.7). That is, leaf senescence had no relationship with level of N from both sources and leaf nitrogen analysis (Appendix A), but instead it was affected by the size of the plant. This might affect the harvest biomass and consequently the essential oil yield, since more than 50% of rose-scented geranium essential oil is present on the leaves. Therefore, careful management of N application is highly recommended to control excessive plant spread.

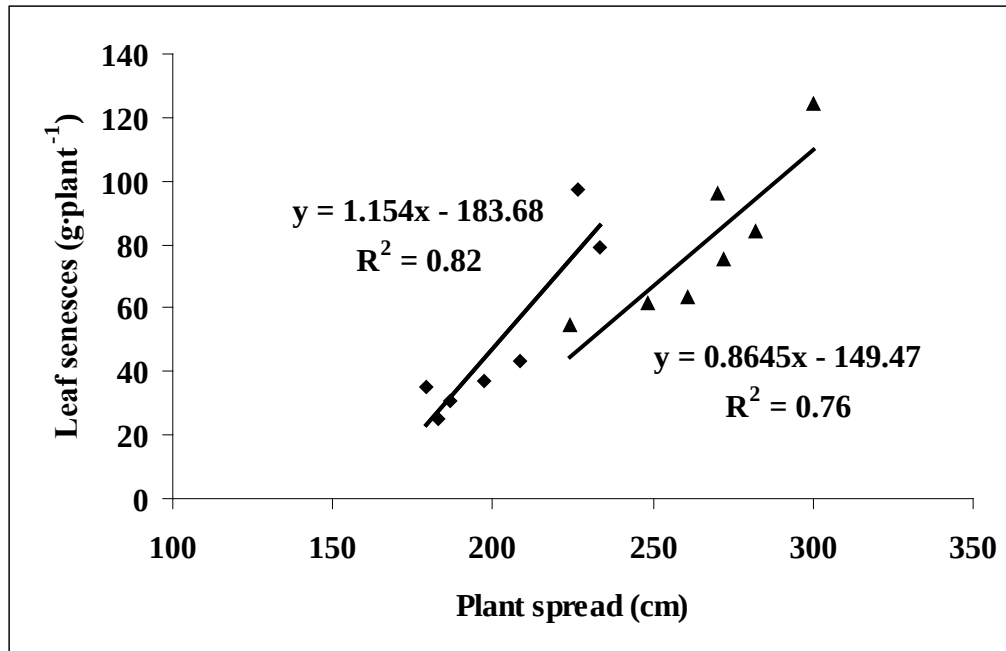


Figure 3.7: Relationship between plant spread (cm) and leaf senescence (g·plant⁻¹) of rose-scented geranium at first (February to May 2005; summer/autumn) (♦) and second (September to December 2005; spring/summer) harvests (▲) using conventional or organic fertilizers. Values presented are means ($n = 16$)

Essential oil content and composition as influenced by N levels and source

Essential oil content (% fresh mass basis)

Although no statistical analysis was performed (samples per source per treatment were pooled for essential oil extraction; Section 3.2), oil content (% fresh mass basis) of the plant was affected by N level or N source (Fig. 3.8). In the second harvest (September to December 2005; spring/summer), a positive quadratic relationship was observed between essential oil content (%) and N applied, organic ($R^2 = 0.94$) or conventional ($R^2 = 0.97$) N (Fig. 3.8B). This positive relationship, an increase in essential oil content with an increase in N applied (up to certain N level) regardless of source is believed to be due to an increase in plant leaf number (Appendix A). Similarly in the second harvest (Fig. 3.5B), LAI increased in a quadratic trend with an increase in N in both conventional and

organic. LAI response was higher with organic than with conventional N, similar response as the essential oil content of the plant with applied N. In addition, strong relationship was also observed between LAI ($\text{m}^2\cdot\text{m}^{-2}$) and essential oil content (%) (Appendix A), during this harvest. This might indicate that factors which have effect on LAI of the plant either by influencing an individual leaf expansion or plant leaf number (Appendix A) have an influence on the essential oil to be recovered during distillation. However, no relationship was found between essential oil content and N applied or source (Fig. 3.5A; conventional, $R^2 = 0.35$ and organic, $R^2 = 0.12$) during the first harvest (February to May 2005; summer/autumn).

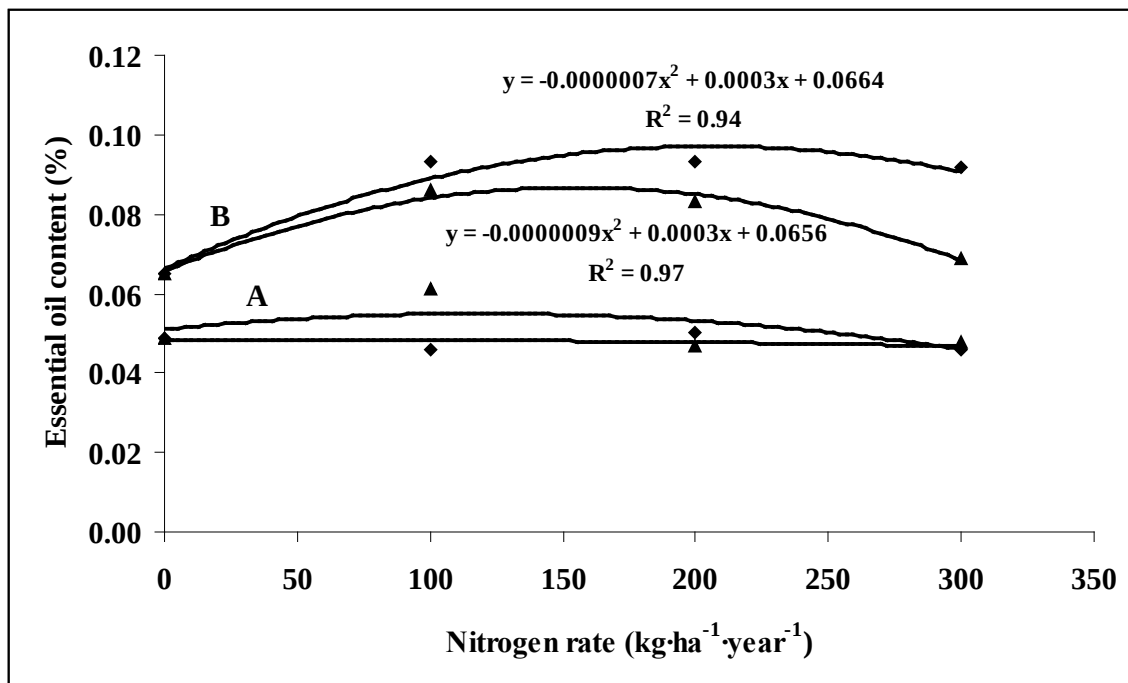


Figure 3.8: Rose-scented geranium essential oil content as influenced by nitrogen rate (0 to 300 $\text{kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$) at (A) first (February to May 2005; summer/autumn) (♦) and (B) second (September to December 2005; spring/summer) harvests (▲) using conventional or organic fertilizers. Individual values are means ($n = 8$; pooled sample of eight plants was used for essential oil distillation)

Essential oil content (% fresh mass basis) also varied between the harvests, greater in the second harvest (Fig. 3.8; September to December 2005; spring/summer) than the first harvest (February to May 2005; summer/autumn). This could be related to environmental variations that occurred between the harvesting periods. For the same cultivar, similar suggestions were also made by Motsa *et al.* (2006) where essential oil content was 0.096% for autumn harvest followed by 0.083% and 0.058% for summer and winter harvests, respectively. In the present study, essential oil content of the plant ranged between 0.05 to 0.06 and 0.07 to 0.09 for the first and second harvests, respectively (Figure 3.8). Generally, the essential oil contents (% fresh mass basis) achieved in the present study fell within the range (0.04 to 0.2%) of values reported for this crop by different authors (Weiss, 1997; Sabina Aiyanna *et al.*, 1998; Motsa *et al.*, 2006; Eiasu *et al.*, 2009).

Essential oil composition

Rose-scented geranium essential oil is characterized by concentration of the main constituents. These are citronellol, geraniol, iso-menthone, citronellyl formate, geraniol formate (Weiss, 1997; Miller, 2002; Peterson *et al.*, 2006) and guaia-6,9-diene (Rajeswara Rao, 2000). Oil composition data from this study is shown in Table 3.4. The values presented are compositions of pooled samples per treatment per harvest, and thus they were not statistically analysed. However, the characteristic of the essential oil was within the range of internationally accepted (Lis-Balchin, 1995) rose-scented geranium oil. Although no statistical analysis was performed, N level and source were found to have unnoticeable effect on essential oil composition of the plant (Table 3.4).

Citronellol percentage increased with an increase in N applied regardless of N source and was higher with organic than conventional N in the first harvest (February to May 2005; summer/autumn), for all levels of N. In the second harvest (September to December 2005; spring/summer), application of conventional N and 100 kg·ha⁻¹·year⁻¹ organic N had no influence compared to the control, while further application of organic N

increased citronellol percentage, with the highest at $300 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$. Application of conventional N in both harvests had no influenced geraniol percentage compared to the control. Except for the $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ level, organic N did not also influence geraniol percentage either. Ram *et al.* (2003) indicated that citronellol content of geranium increased with increasing conventional N up to $160 \text{ kg}\cdot\text{ha}^{-1}$, while geraniol content was decreased. In the first harvest, conventional N up to $200 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ had no noticeable effect on guaia-6,9-diene percentage, compared to the control. Further application ($300 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ N) reduced guaia-6,9-diene percentage. All levels of organic N also lowered guaia-6,9-diene percentage compared to the control. In the second harvest, guaia-6,9-diene percentage was not influenced by source or level of N applied. The reason for lack of consistency in the essential oil composition, regardless of the harvesting period (Table 3.4), was most probably due to seasonal effects as reported by Motsa *et al.* (2006). Scheffer (1993) also indicated that oil composition of the plant primarily depended on harvesting season.

Table 3.4: Change in concentration of major constituents of rose-scented geranium essential oil as influenced by amount and source of N in the first (February to May 2005; summer/autumn) and second (September to December 2005; spring/summer) harvests

Source of N	N (kg·ha ⁻¹ ·year ⁻¹)	First harvest (%)						
		Linalool	Iso-menthone	Citronellol	Geraniol	Citronellyl formate	Geranyl formate	Guaia-6,9-diene
Control	0	0.27	4.04	25.40	6.98	16.12	5.71	9.64
Conventional	100	0.27	4.46	25.83	7.74	16.36	6.01	9.43
	200	0.30	3.17	26.78	7.62	17.13	6.29	8.82
	300	0.54	3.68	29.23	8.66	17.88	6.79	6.53
Organic	100	0.74	3.19	31.13	8.81	20.68	9.99	5.30
	200	0.59	2.42	36.66	5.36	23.62	8.13	4.52
	300	0.89	2.97	35.89	7.98	22.99	9.40	4.39
Second harvest (%)								
Control	0	0.55	1.75	30.02	10.68	19.54	6.18	6.51
Conventional	100	0.57	4.28	30.16	11.83	18.88	6.33	6.61
	200	0.49	3.55	29.50	12.53	18.52	6.59	6.23
	300	0.82	3.27	28.90	10.44	20.02	6.51	6.89
Organic	100	0.84	4.05	30.52	12.90	18.15	6.92	5.95
	200	0.66	0.25	31.89	11.25	19.99	6.88	6.57
	300	0.76	3.37	32.18	11.20	19.05	6.55	6.31

Citronellol and geraniol are among the most important components of rose-scented geranium essential oil and the essential oil is characterized by their ratio (Doimo *et al.*, 1999; Motsa *et al.*, 2006). The desirable ratio between the components is 1.0 to 3.0 (Anistescu *et al.*, 1997; Bauer *et al.*, 1990). In this investigation, the ratio between citronellol and geraniol (C:G) is presented in Fig. 3.9. Citronellol to geraniol ratio (C:G) in the second harvest (September to December 2005; spring/summer) was stable and within the above stated range for all the treatments (mean value of 2.67). In the first harvest (February to May 2005; summer/autumn) citronellol concentration tended to vary with source of N and increased with organic N (Fig. 3.9) and this resulted in higher C:G values, especially with 200 kg·ha⁻¹·year⁻¹ organic N. Generally, the C:G values in the first harvest was higher compared to the second harvest and could have been related to weather effects. This was similar to a report by Motsa (2006) where C:G ratio in this growing region was high during autumn/winter (low minimum night temperature) growth period, which might be the case for the first harvest. Doimo *et al.* (1999) and Motsa *et al.* (2006) also reported C:G ratios were highest in winter and late winter/spring harvests. The same authors described that better quality of the oil was obtained when the C:G ratio was low, which was the case in the second harvest of the present study. According to Rajeswara Rao *et al.* (1996) cold weather conditions (mean maximum temperature of 27.3 °C and mean minimum temperature of 11.3 °C) during a regrowth period favour geraniol and geranyl formate concentration of the crop. In general, the characteristic of the essential oil of the present study was within the range of internationally accepted (Lis-Balchin, 1995) rose-scented geranium essential oil.

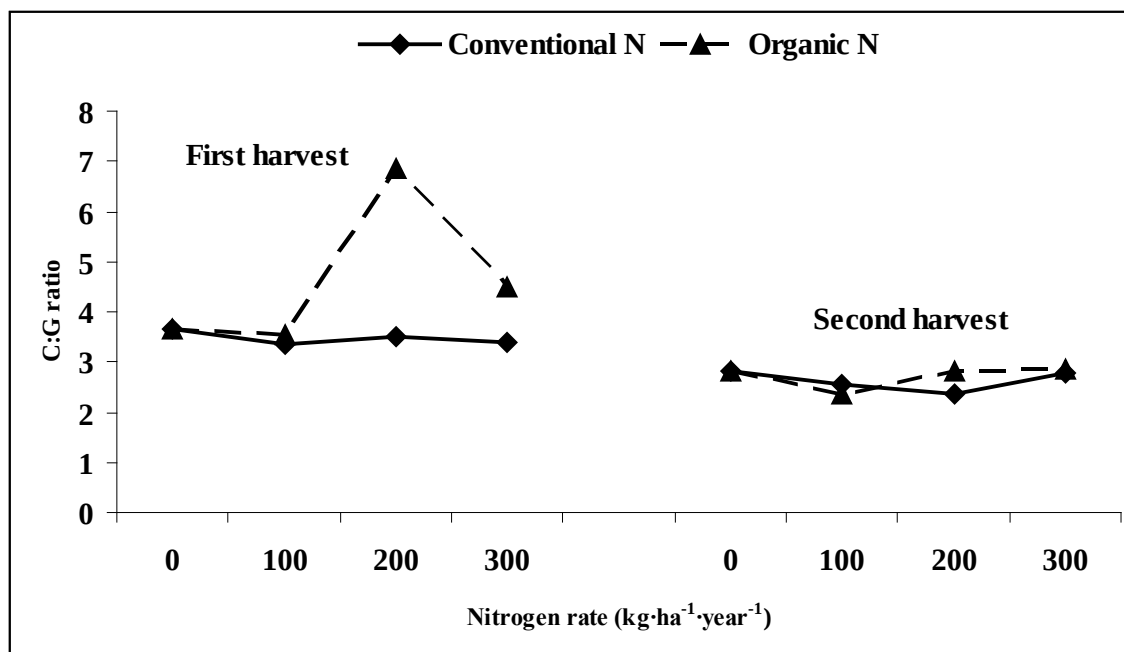


Figure 3.9: Citronellol to geraniol (C:G) ratio of rose-scented geranium essential oil at (A) first (February to May 2005; summer/autumn) and (B) second (September to December 2005; spring/summer) harvests using conventional or organic fertilizers. Individual values are means ($n = 8$; pooled sample of eight plants was used for essential oil distillation)

4.4 CONCLUSIONS AND RECOMMENDATIONS

Results of this study revealed a general trend that increased N levels increased herbage and oil yield. This effect was more pronounced for organic N than conventional N in both harvests, including cumulative yields. This shows that rose-scented geranium can be influenced by source of N (conventional or organic) in herbage and essential oil yield production. On the other hand, increased N showed a tendency to increase citronellol levels, particularly in the autumn months and to decrease guaia-6,9-diene. This trend may, for certain commercial applications, negate the benefit of increased yield with increased N application. The set hypotheses were both accepted, as biomass and oil yields responded positively to nitrogen top dressings after harvest. The crop also responded better to organic than conventional N sources.

CHAPTER FOUR

TIME OF NITROGEN APPLICATION ON REGENERATED ROSE-SCENTED GERANIUM BIOMASS YIELD, ESSENTIAL OIL YIELD AND OIL COMPOSITION

4.1 INTRODUCTION

Different studies confirmed that nutrient management is a very important task in crop production. The major goal of applied fertilizer is to improve plant growth and consequently yield. The applied fertilizer has to provide adequate nutrition and has to be available when the plant needs it (Tisdale *et al.*, 1993; Brady & Weil, 2002). Soil nutrient levels should not be in excess of the plant requirements. Excessive application might have a negative impact by either causing toxicity on the plant, reducing seed formation and flowering by encouraging vegetative growth, might increase the risk of wind damage through lodging or also cause environmental pollution through leaching. In addition, during fertilizer application not only the rate but also equally the time of application is important (Tisdale *et al.*, 1993).

Response of plants to nutrient application varies with time or plant growth stage. Mostly, plants respond well if fertilizer is applied at the vegetative stage of the plant (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). When fertilizer is applied at this stage, it must target to provide enough nutrition for optimum growth of the plant (Mengel, Kirkby, Kosegarten & Appel, 2001). However, if the applied nutrient does not meet the optimum required target, either in deficit or excessive amount then a negative impact will be noticed (Mengel *et al.*, 2001). For instance, with nitrogen nutrition under deficit conditions there is stunt growth, leaf chlorosis which reduces synthesis of proteins and chlorophyll and as the result poor CO₂ assimilation and carbohydrates synthesis which lead to premature flower and fruit developments (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). When it is excessive, leaves turn to dark green, with abundant vegetative growth, poor root growth and high shoot to root ratio, delay in flowering and seed formation, rapid growth and soft tissues are

reported. This favours the development of diseases and insects and increase the risk of lodging (Taiz & Zeiger, 2002).

Nitrogen is characterised by its mobility, that is under inadequate supply or severe conditions soluble nitrogen is transported from the older leaves to the young leaves (Tisdale *et al.*, 1993). According to Brady & Weil (2002), fertilization of mobile nutrients such as nitrogen (N) and partially potassium (K) should have to be during rapid growth of the plant. This is to ensure the optimum uptake and minimise losses. However, this information in rose-scented geranium was not available and understanding the re-growth trend of the crop and identifying the rapid re-growth stage of the plant was important in order to improve N management of the crop. For instance in maize, a small amount of nitrogen application is recommended during planting, while most of the required N should have to be applied as topdressing before rapid nutrient uptake stage of the plant that is four to six weeks from planting (Agronomic guide, 2009-2010). Deibert & Utter (2002) reported that maximum NPK uptake of two navy dry edible bean (*Phaseolus vulgaris*) cultivars varied between the cultivars and their growth stages. In spring wheat, N recovery increased when N application was delayed from tillering until second node growth stage (Petersen, 2004). Though, a study in China indicated that time of N application had no significant effect on wheat and maize N recovery (Shulan *et al.*, 2004). In Kinnow (*Citrus reticulata* Blanco) production, January application of urea, super phosphate and sulphate of potash was more efficient in improving fruit quality and quantity than April and July application (Salik, Muhammad & Shakir, 2000). However, information on the time of N application on biomass, oil yield, oil content and composition of rose-scented geranium is lacking. To achieve maximum use and efficiency of the applied, fertilizer application rate has to be matched with time and method of application (Ihsan, Mahmood, Mian & Cheema, 2007). A report from Uganda by ADC (1998) indicated that annual fertilization of geranium has to be 30: 35: 25 kg·ha⁻¹ of N: P: K, respectively. In addition, 25 kg·ha⁻¹ of N can be applied as topdressing in eight splits, one dose immediately after every harvest then followed by another dose in a two weeks interval. This practice was also adopted by South African producers (BioAfrica, 2007). According to Weiss (1997), the crop is believed to be labour intensive.

Nitrogen requirements of seedlings after emergence was reported to vary with growing phases and during the first growth phase only a small amount of N is required but in the second phase (15 to 31 days after emergence) the N requirement increases. The variation in N requirement with growth phases can be related to the differences in photosynthetic activity (Widders, 1989). That is, during early growth stages photosynthesis is very low because of limited leaf number and photosynthetically active leaf surface area of the plant. In addition, the root system at this stage is not fully developed and this also minimizes the uptake of N. However in the second growth stage, the plant starts increasing in growth and development as a result of root growth, leaf number and area expansion and this leads into an increase in N requirement of the plant (Salisbury & Ross, 1992). In such phases, if the management in N application coheres with its requirement an increase in dry mass production and partitioning can be achieved (Widders, 1989). Similar management techniques might be important in rose-scented geranium production, since during cutback most of the above-ground biomass is removed during harvest and as a result the plant is mostly left with only a few number of bottom leaves, which most of the time are not as photosynthetically active as the young and matured leaves (Rajaswara Rao *et al.*, 1990b; Wiess, 1997; Demarne, 2002; Motsa, 2006). In rose-geranium the harvestable plant part is the above-ground biomass and this misleads farmers especially with N application, since one might think biomass production of the crop is directly related to N levels, which was not the case according to the findings of Chapter 3, section 3.3.

In forage legumes, re-growth after biomass loss is more dependent on N reservoir than carbon concentration of the plants (Vance *et al.*, 1979; Groat & Vance, 1981; Kim *et al.*, 1993). A report by Avice, Lemaire, Ourry & Boucaud (1997) indicated the mobilization of C and N reservoirs after shoot removal in order to support fast re-growth of shoots. The same authors also found out that in alfalfa, shoot removal resulted in the depletion of C and N concentration of tap roots, lateral roots and crown stems.

Re-growth of a plant after biomass loss can further be affected by the type of plant species, environmental factors, soil nutrient availability, plant growth stage and the intensity of shoot removal (Kozłowski *et al.*, 1991; Richards, 1993; Leriche *et al.*,

2003; Hester *et al.*, 2004). Nitrogen fertilizer application to boost the consequent harvest is a common practice in rose geranium production. However, if N application does not coincide with the period when N is mostly required, efficiency to support the re-growth might be low. Therefore, in order to understand this the re-growth trend and the time of N application after every harvest, a study was conducted. It focused on identifying a single time N application per re-growth cycle, that is N application when it is mostly required by the crop. The objectives were to understand the re-growth trend of the plant in order to match it with time of nitrogen topdressing and its effect on rose-scented geranium biomass and oil yield, essential oil content and composition. The hypothesis tested was that if N top dressing coincides with the period of most rapid growth, yield response will be best.

4.2 MATERIALS AND METHODS

Experimental site, experimental design and treatment details

The study was conducted at the University of Pretoria's Hatfield Experimental Farm, Pretoria. Geographic location of the study site and equipments used for climatic data collection (see Appendix A) are described in Chapter 3, Section 3.2. The top 0 to 30 cm soil of the experimental site was sandy clay loam of the Hutton form (The Soil Classification Working Group, 1991) with a clay content of 22.3%, a pH of 6.1 (1:2.5, soil: water ratio), and a total carbon of 6.1 ($\text{g}\cdot\text{kg}^{-1}$). Total N, P, K, Ca, Mg, and Na contents were 0.04%, 12 $\text{kg}\cdot\text{ha}^{-1}$, 152 $\text{kg}\cdot\text{ha}^{-1}$, 352 $\text{mg}\cdot\text{kg}^{-1}$, 96 $\text{mg}\cdot\text{kg}^{-1}$, and 11 $\text{mg}\cdot\text{kg}^{-1}$, respectively.

Two trials were conducted: first trial was on re-growth trend of the plant, a trial which was set to identify the rapid growth period of the plant and then relate this finding with the time of N application. Therefore, the second trial was on time of N application through delaying N top dressing after cutback.

4.2.1 Re-growth trend

Understanding the re-growth trend of rose-scented geranium and identifying the rapid re-growth stage of the plant was important before setting up the timing of nitrogen topdressing trial. Thus, a trial was set with three nitrogen levels treatments (0, 100, and 400 kg·ha⁻¹ N·year⁻¹) using limestone ammonium nitrate (LAN; 28% N) as source of N. For the first re-growth cycle the growth period was from February to May 2005 (summer/autumn) and for the second re-growth cycle it was from June to September 2005 (winter/spring). The study was laid out in a randomised complete block design, with four blocks each of 10 m long and 20 m wide and blocking was against the slope.

Soil solution sampling: nitrate (NO₃⁻) concentration

Nitrate leaching or content was monitored by wetting front detectors (WFDs) (Stirzaker, Annandale, Stevens & Steyn, 2007) installed at 0-20, 20-40 and 40-60 cm depths located in the middle of the plots. The detectors were installed in all nitrogen level treatments (0, 100, and 400 kg·ha⁻¹ N·year⁻¹). Soil solution samples collected from the wetting front detectors were analyzed for nitrate (NO₃⁻) concentration using a C99 Multiparameter Bench Photometer (Hanna Instruments, Italy).

Data collection and analysis

Data collection was on a weekly basis and plant samples per treatment per replication were analyzed for fresh herbage biomass (kg), leaf area (m²·m⁻²) and dry matter accumulation (kg). Soil solution was collected after every irrigation or rainfall after activation of the WFDs (wetting front detectors) (Stirzaker *et al.*, 2007; Tesfamariam *et al.*, 2009). To determine the fresh mass accumulation trend of rose-scented geranium during a re-growth period, Richard's growth model (Eq. [4.1]) was fitted to the data, where: y = biomass accumulation of rose-scented geranium (t·ha⁻¹); a, b, c, d = regression parameters; e = exponential function; x = time (weeks after cut back of rose-scented geranium).

$$y = \frac{a}{(1 + e^{b-cx})^{1/d}} \quad [4.1]$$

During sampling time (on a weekly basis), two plants per treatment per replication were taken (destructive sampling) for growth parameter measurements and each plant was separated into leaf and stem, where after fresh and dry mass (dried in an oven for 72 hrs at 70 °C) was determined. Total leaf area per plant was determined using a belt driven leaf area meter LI 3100 (LiCor Inc., Lincoln, Nebraska, USA). Leaf area index (LAI) was calculated using the equation 4.2, where, LA was leaf area and HA was harvested area.

$$LAI = \frac{LA(m^2)}{HA(m^2)} \quad [4.2]$$

4.2.2 Time of nitrogen topdressing vs. re-growth cycle

In this study limestone ammonium nitrate (LAN) with 28% N was used as source of N at a rate of 100 kg·ha⁻¹ N·year⁻¹. This rate was selected based on findings from the previous study (Chapter 3). With regard to source, inorganic N as LAN was used with the assumption that it is more vulnerable to loss as compared to organic N. Therefore, this study was conducted to determine if the loss of conventional N could be adjusted by time of nitrogen application while meeting rose-scented geranium N requirements during the re-growth period. Superphosphate (P₂O₅; 11.5% P) and potassium chloride (KCl; 50% K) were used as sources of P and K at rates of 90 and 60 kg·ha⁻¹ year⁻¹ respectively, in all the treatments. The yearly N, P and K application was split into three, which gave a rate of 33.3, 30 and 20 kg·ha⁻¹ per harvest, respectively. There were sixteen treatments: control (0 kg·ha⁻¹ year⁻¹), immediately after harvest (IAH) and application after 1 to 14 weeks from cut back. Each treatment comprised a total area of 31.25 m², with five rows of each 6.25 m length. The middle three rows were used for herbage yield, essential oil yield and composition analysis, while the two rows on either side acted used as border rows.

Data collection and analysis

For this trial, harvesting was at four-monthly intervals. During each harvest plant samples were taken for essential oil content and composition determinations (Chapter

3, section 3.2). During the study period three harvests were made, namely first re-growth cycle (October 2005 to February 2006), second re-growth cycle (March to June 2006) and third re-growth cycle (July to October 2006).

At each harvesting, two plants per treatment per replication were taken (destructive sampling) for growth parameter measurements and each plant was separated into leaf and stem, where after fresh (kg) and dry mass (kg; dried in an oven for 72 hrs at 70°C) was determined. Total leaf area per plant ($\text{m}^2 \cdot \text{m}^{-2}$) was determined using a belt driven leaf area meter LI 3100 (LiCor Inc., Lincoln, Nebraska, USA). Leaf area index (LAI) was calculated using the equation 4.2.

In both trials, planting date, planting density, time of establishment and harvesting height information were presented in Chapter 3, Section 3.2. Similarly, irrigation water was applied when needed using a computerized pressure compensated drip irrigation system (NETAFIM, Cape Town, South Africa). The dripper flow rate was $2\ell \cdot \text{h}^{-1}$ in the pressure range of 120 to 200 kPa. To be comparable with the spacing between rows of the trial, the drip lines were installed 1 m apart and the in-line spacing between the emitters was 0.3 m. Before commencing irrigation, soil water content was monitored using a neutron probe (Neutron water meter, Model 503 DR, CPN Corporation, CA, USA) on a weekly basis. Rainfall was supplemented with irrigation. The profile was refilled to field capacity when the soil water content dropped below 60% of plant available water.

4.3 RESULTS AND DISCUSSION

4.3.1 *Fresh herbage of re-growth trend and plant growth parameters*

The collected data re-growth was fitted into a curve as shown in Fig. 4.1 (since the trend was the same with the second re-growth cycle, the presented data is only for the first re-growth cycle which was between February and June 2005). The curves represent the fit to Richards growth function and the model gave the best fit to the collected data ($r^2 = 0.96, 0.98$ and 0.99 ; $0, 100$ and $400 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$, respectively). Based on the original growth data, rose-scented geranium re-growth cycle followed a

sigmoid shape trend. The re-growth rate of the plant was slower for the first eight weeks and between twelve and fourteen weeks after cutback (Fig. 4.1). According to Taiz & Zeiger (2002), slow or lagging period of plants at initial stage of growth can be related to preparation of the plant for the rapid growth stage such as synthesis of the necessary enzymes which is then followed by a rapid growth stage and slowing phase where the increment becomes linear. In the present study, rapid re-growth was attained between the 9th and 12th weeks of the re-growth period while between 14 and 17 weeks the fresh biomass accumulation of the plant leveled off. Towards the end of the growth phases, plant growth becomes stable where the growth stays constant or even declines as the available plant nutrition becomes depleted (Taiz & Zeiger, 2002). This might imply for the present study where biomass accumulation of the crop was steady between 14th and 17th weeks of the growth period. This was true for all of the applied nitrogen treatments (Fig. 4.1). Maximum fresh biomass accumulation of the plant was attained in week 17 of the re-growth cycle. This is in agreement with findings of Motsa *et al.* (2006) and Eiasu (2009) for the particular growing region and cultivar in spring/summer and summer/autumn growing conditions.

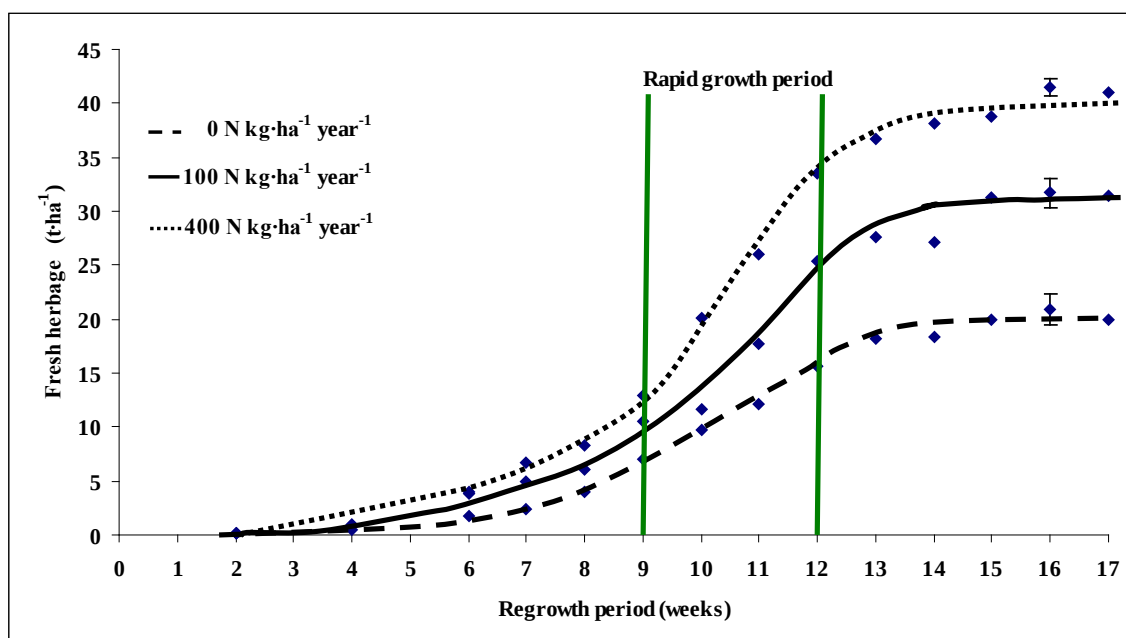


Figure 4.1: Fresh herbage accumulation trend of rose-scented geranium at 0, 100 and 400 kg·ha⁻¹ N year⁻¹ fitted to Richard's growth model for the first re-growth cycle, February to June 2005. Recorded values are means ($n = 8$)

Response of plants to nutrient application varies with plant physiological growth stage. Mostly, plants responded well if fertilizer is applied at the vegetative plant growth stage (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). According to Demarne (2002) rose-scented geranium has no clear physiological stages since the plant propagates vegetatively through stem cuttings.

During the course of the experimental period, the three nitrogen treatments resulted in significant differences between treatments. Differences in fresh herbage ($\text{t}\cdot\text{ha}^{-1}$) accumulation among the treatments became more evident after week 9 of the re-growth cycle. As stated earlier, this could be related to rapid growth stage of the plant that corresponded with the nitrogen nutrition and rate which supplemented the growth to emphasize the necessity of nitrogen nutrition on re-growth of the plant, as compared to the control. The accumulated fresh biomass was highest for $400 \text{ kg}\cdot\text{ha}^{-1} \text{ N year}^{-1}$ as compared to 0 and $100 \text{ kg}\cdot\text{ha}^{-1} \text{ N year}^{-1}$.

Leaf area index (LAI) and dry matter accumulation

Leaf area index for the re-growth cycles is shown in Fig. 4.2. The presented data shows that LAI of rose-scented geranium varied due to applied nitrogen. In both the re-growth cycles (1 and 2), application of nitrogen significantly increased LAI. The increase was more prominent during the first re-growth cycle, than in the second re-growth cycle. LAI increased gradually for the first 8 weeks of the first re-growth cycle but between weeks 9 and 12 the increment was more rapid. The slow re-growth trend (LAI) in the first 8 weeks could be related to slow metabolic activities at the early stage of the re-growth period to contribute to leaf growth. In many plant species such as alfalfa, continued root carbohydrate depletion was reported until the top re-growth was 15 to 20 cm tall, which was related to a point where enough leaf area met the growth and respiratory requirements of the plant (Wiersma, Bertam, Wiederholt & Schneider, 2007). After cut back or pruning, plants undergo a recovery stage for a short period of time in a re-growth cycle (Varnam & Sutherland, 1999). Recovery stage from pruning in tea plants (*Camellia sinensis*) was reported to have a low starch concentration on the stem as compared to other growth stages of the plant (Selvendran & Selvendran, 1972). In the present study, it could also be related to the growth and

developmental characteristics of the plant, where after every cut back it underwent, there is an initial lag period to synthesize essential enzymes for the following rapid growth period (between the 9th and 12th weeks after cutback). From week 13 onwards, the increase in LAI levelled off. Slow LAI might be due to the slow maturity of the plant as a result of limited growth to achieve higher LAI values. Steady LAI values can be due to maximum leaf expansion or number of leaves in which the plant bears during a re-growth period. The decline in LAI values can be in association with leaf senescence due to nutrition depletion (especially nitrogen nutrition) towards the end of the re-growth period.

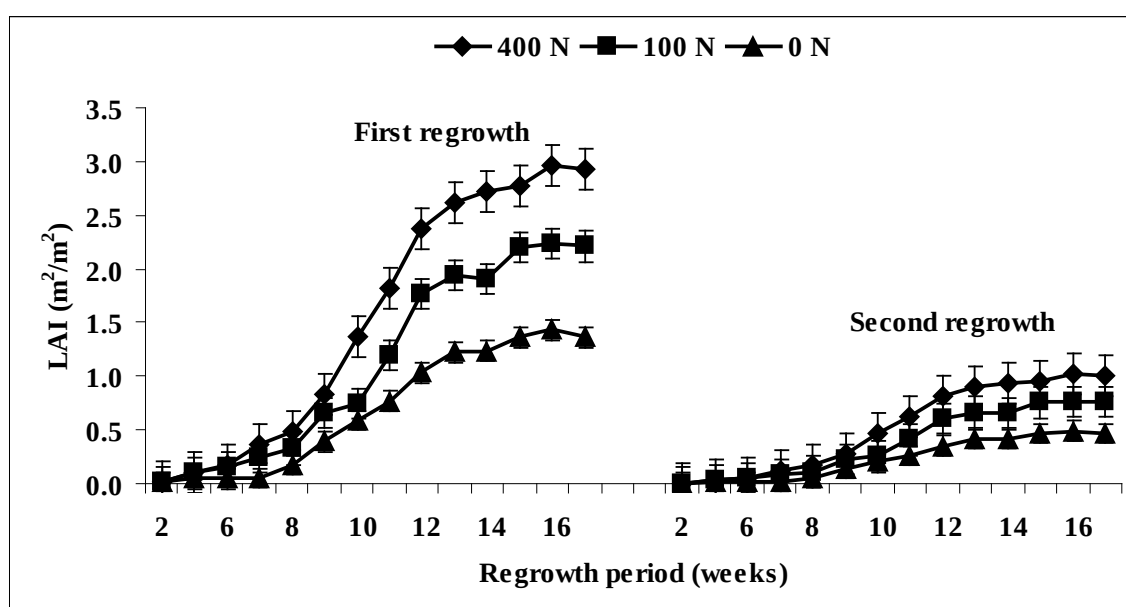


Figure 4.2: Leaf area index (LAI) trend of rose-scented geranium for 0 (▲), 100 (■) and 400 (♦) kg·ha⁻¹ N year⁻¹ treatments during February 2005 to May 2005 (first re-growth cycle) and June 2005 to September 2005 (second re-growth cycle)

Regarding nitrogen level, application of N at either the 100 or 400 kg·ha⁻¹ N year⁻¹ rates significantly increased LAI throughout the re-growth cycle, compared to the control (Fig. 4.2). The highest LAI values were achieved with N application at a rate of 400 kg·ha⁻¹ year⁻¹ followed by 100 and 0 kg·ha⁻¹ N year⁻¹, respectively (Fig. 4.2). The increase in LAI in response to the increased N rates is a well documented for many crop species, for example: in sunflower an increase in leaf expansion was

reported in relation to N rates (Radin & Boyer, 1982), in barley cultivars an increase in leaf number was reported (Dale & Wilson, 1978) and in pearl millet an increase in tillering was reported (in the present study which might be in relation to branching) (Coaldrake & Pearson, 1985). The suggestion made by Coaldrake & Pearson (1985), partially might be applicable to the present study, as stated in Chapter 3, section 3, an increase in branch number was noticed with N application ($100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$) compared to the control but farther increase had no significant effect. Generally, the LAI values of the present study were lower than what was reported in Chapter 3. This might be related to soil pH effect where in the present study the soil pH ranged between 4.9 and 5.4 while for the study in Chapter 3 it was between 6.2 and 6.6. According Weiss (1997), the crop requires slightly acidic conditions (5.5 to 7) for optimum biomass accumulation. Soil pH is also widely accepted as a dominant factor that regulates soil nutrient bio-availability (Robson, 1989). In comparing re-growth cycles (Fig. 4.2), show that LAI in the first re-growth cycle (February to May 2005) was higher as compared to the second cycle (June to September 2005). This might be related to environmental conditions during the trial periods, since the first re-growth cycle occurred during a warmer period (Appendix B) and rose-scented geranium is believed to perform best under sunny and warm weather conditions to attain optimum growth (Weiss, 1997). The plant is also reported to be sensitive to low temperature (less than 6°C) (Weiss, 1997), which often occurred during the second re-growth period. Low LAI of the plant to nitrogen levels during this period could also be due to low metabolic activities of the plant to utilize the applied nitrogen due to weather conditions of the re-growth period.

Total dry matter accumulation of the plant followed a similar trend to LAI (Fig. 4.3) throughout the re-growth periods (data combined for all N treatments of re-growth cycle 1 and 2). A correlation between the total dry matter accumulation and LAI (re-growth cycles 1 and 2) showed strong positive linear relationship ($r^2 = 0.97$). That is, the plant accumulated dry matter (kg) with every increase in LAI ($\text{m}^2\cdot\text{m}^{-2}$). Motsa *et al.* (2006) and Eiasu *et al.* (2009) reported similar findings for the cultivar grown in this region.

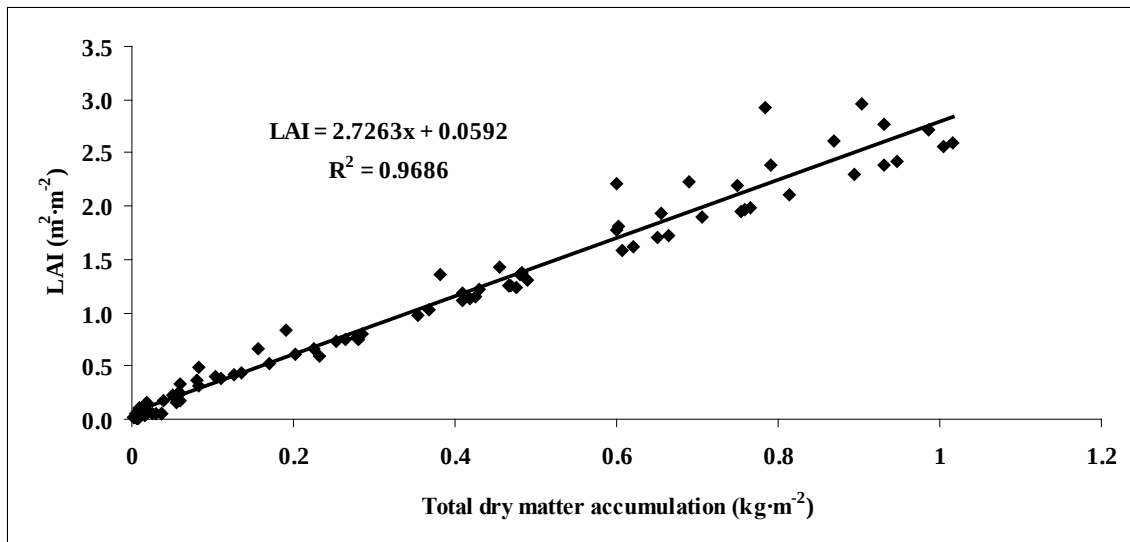


Figure 4.3: Relationship between total dry matter accumulation (kg·m⁻²) and LAI (m²·m⁻²) of rose-scented geranium (data combined for all N treatments of the re-growth cycles during February to May 2005 and June to September 2005)

Soil solution nitrate level (NO₃⁻)

Figure 4.4, shows soil solution nitrate (NO₃⁻) concentration for re-growth cycle 1 (February to May 2005). Nitrate (NO₃⁻) concentration of the collected soil solution samples generally increased with an increase in nitrogen level applied. Nitrate values (mg·L⁻¹) were highest when nitrogen was applied at a rate of 400 kg·ha⁻¹ N year⁻¹ (1/3 split application, 133.3 kg·ha⁻¹ N per re-growth cycle) followed by 100 N kg·ha⁻¹ year⁻¹ (1/3 split application, 33.3 kg·ha⁻¹ N per re-growth cycle). The soil solution NO₃⁻ concentration was also higher in the top soil layers (0-20 and 20-40 cm), compared to the deeper layers (40-60 cm). Differences were more evident in the beginning of the re-growth cycle. This might be related to low above-ground biomass (leaf area) to create a sink for uptake of the N, which resulted in abundance of nitrogen (NO₃⁻) right after fertilizer application (LAN; 28% N). The fertilizer used in the study is characterized by approximately half of the nitrogen in NO₃⁻ form and the second half in NH₄ form (Hadfield, 1967). As stated in the materials and methods section, the plants were cut back to 15 to 20 cm above ground height during harvesting, which leave the plant with limited above ground biomass to utilize the applied nutrients. In

addition, re-growth trend of the plant at an early stage of cut back was lagging (Fig. 4.1), which might also have contributed to less uptake of the NO_3^- solution. Nitrogen fertilization in this study was based on the recommendation by ADC (1998) where N top dressing was applied immediately after harvest. This might also have contributed to the high nitrate values at the beginning of the re-growth cycle but as the plants started to produce more biomass the values noticeably dropped. That is, the demand of the plant might have been met by mineralization of the applied fertilizer (LAN). According to this finding, therefore, application of N nutrition at an early stage of the crop's re-growth stage might not be advisable, particularly at the described low harvesting height or at higher N level. At this harvesting height, the crop is mostly left with limited above ground biomass and/or with few numbers of old leaves which most of the time are reported to be photosynthetically limited or inactive (Rajaswara Rao *et al.*, 1990b; Wiess, 1997; Demarne, 2002; Motsa *et al.*, 2006); as a result the re-growth trend of the subsequent harvest was negatively influenced.

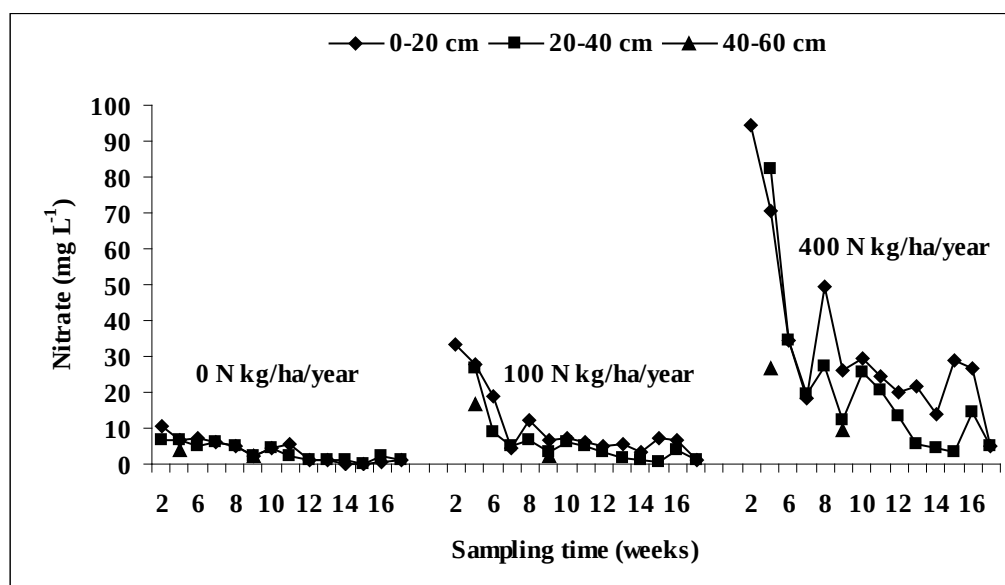


Figure 4.4: Soil solution nitrate levels collected using wetting front detectors at depths of 0-20, 20-40, and 40-60 cm of rose-scented geranium re-growth period during February 2005 to May 2005 for 0, 100 and 400 kg·ha⁻¹ N year⁻¹

Generally, nitrate concentration of the collected soil solutions (re-growth cycle from February to May 2005; Fig. 4.4) was within the range of 0-250 mg·L⁻¹ as reported by Biró *et al.* (2005) and Tesfamariam *et al.* (2009) for horticultural lands. However, the values were higher for the first four weeks and at eight weeks of the re-growth cycle for 400 kg·ha⁻¹·year⁻¹ nitrogen treatments, as compared to the recommended nitrate concentration (44 mg·L⁻¹) for drinking water in South Africa (Korentajer, 1991; Tesfamariam *et al.*, 2009). WFDs at 40-60 cm soil layer collected solution only twice during the re-growth period (Fig. 4.4). This leachate might be related to irrigation and rainfall which occurred at the early re-growth cycle (Appendix B), which resulted into washing down of the nitrate below the efficient root zone of the plant. This might show the disadvantage of too much or early nitrogen application after cutting back of the crop, since the biomass at this stage was very low to utilize the applied nitrogen. Therefore, the amount of N applied at early re-growth stage of the crop must be controlled. That is, time of N top dressing is important either at or before rapid re-growth period of the plant.

4.3.2 Time of nitrogen topdressing vs. re-growth cycle

Herbage and essential oil yield

Delaying nitrogen topdressing (conventional N in the form of LAN, N 28%) after harvest, to certain periods in a re-growth cycle, was found to have a significant positive effect on biomass production of the plant (Fig. 4.5). This effect was more pronounced in the first and third re-growth cycles. Biomass production peaked when nitrogen was applied between the 7 and 9 weeks after cut back. Compared to the control (0 kg·ha⁻¹·year⁻¹ N), early and late nitrogen applications were found to have a negative effect on biomass production of the plant. This might be related to the previous findings (Fig. 4.4) where early application could favour loss of N through leaching, since the biomass during this particular period was very low to utilize the applied N. This is in agreement with the previous findings (Section 4.3.1) where high level of nitrate concentration was recorded at the early re-growth stage, especially at higher N rate (400 kg·ha⁻¹·year⁻¹). Similarly, the negative effect of late application might be related to a delay in peak N requirement of the plant. That is, specific N

requirement period for optimum re-growth of the plant, which in the present study was between the 7th and 9th weeks after the previous harvest (cut back). In canola production, N application at either pre-sowing, fourth leaf stage or split application at fourth leaf and late cabbage stage was reported to advantage dry matter accumulation than no N (control) and late cabbage stage application (Moody, 2007). Drake, Raese & Smith (2002) described the influence of time of nitrogen application on apple fruit yield and quality. As illustrated in Fig. 4.5, biomass production of rose-scented geranium in the third re-growth cycle followed a similar trend as the first re-growth cycle, where there was low biomass accumulation in the control (0 kg·ha⁻¹·year⁻¹ N) and during late N application. Though the trend was similar, the peak due to N application time in the second re-growth cycle (Fig. 4.5) was not pronounced as in the first and third re-growth cycles. This might be due to environmental factors (Appendix B) since most of the second re-growth cycle period was in winter season and in this season the re-growth of the plant is very limited (Motsa *et al.*, 2006).

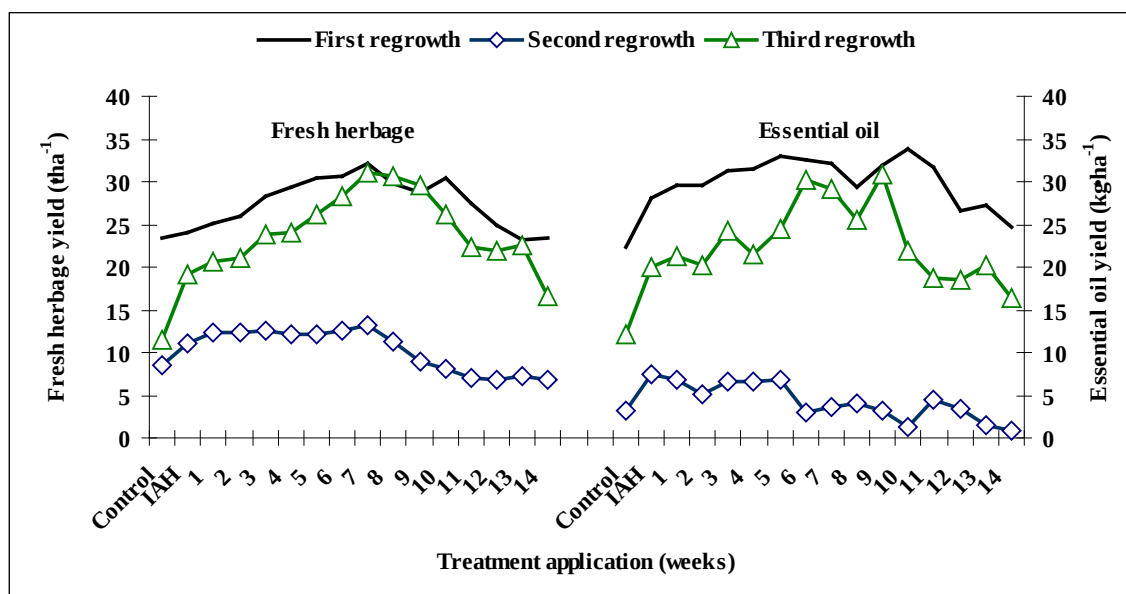


Figure 4.5: Response of rose-scented geranium fresh herbage and essential oil yield to nitrogen topdressing time (weeks) in three different re-growth cycles (first re-growth cycle, October 2005 to February 2006; second re-growth cycle, March to June 2006; and third re-growth cycle, July to October 2006) at a rate of 100 kg·ha⁻¹·year⁻¹ N. Values presented are means ($n = 8$)

Generally, the finding of this trial was comparable with the previous re-growth trend trial (Section 4.3.1). Nitrogen topdressing immediately after harvest (IAH), at early (1 to 6 weeks after cut back) or late (10 to 14 weeks after cut back) period of the re-growth cycle had no significant effect in boosting the biomass or essential oil production of the plant (Fig. 4.5). This means that nitrogen supply did not match the demand of the crop to meet the highest nitrogen requirement period. In rice, the appropriate time for nitrogen application was at panicle initiation (Bacon, 1980) and in spring wheat delayed N application was reported to increase grain yield than application at planting (Ortiz-Monasterio, 2008). Therefore, maximum re-growth biomass production of rose-scented geranium, in the particular growing region, can be achieved with nitrogen topdressing between the 7th and 9th weeks of the re-growth period. Essential oil production also followed similar trend with the exclusion of a slight drop during the 8th week (third re-growth cycle). This was due to unexplained (human error that could have occurred during distillation of the particular specimen) low essential oil content (%) that was recorded at this particular period but according to the statistical analysis the difference was not significant as compared to the peak essential oil production periods (7th and 9th weeks; Fig. 4.5). According to Kumar *et al.* (2001) and Motsa *et al.* (2006) rose-scented geranium essential oil yield was more dependent on fresh herbage yield than essential oil content.

Essential oil characteristics

Essential oil content (% fresh mass basis)

Essential oil content did not show clear variation due to the effect of delaying nitrogen topdressing (Fig. 4.6). The only noticeable variation in oil content was among the re-growth cycles. Oil content was high in the first (October 2005 to February 2006) and third (July to October 2006) re-growth cycles as compared to the second re-growth cycle (March to June 2006) (Fig. 4.6). This could be related to environmental variations that occurred amongst the re-growth periods. Using the same cultivar, similar results were obtained by Motsa *et al.* (2006) where essential oil content was 0.08% for spring/summer harvest and 0.064% for autumn/winter harvest. In the present study, essential oil content of the plant ranged between 0.10 to 0.12, 0.01 to 0.06 and 0.08 to 0.11 for the first, second and third harvests, respectively.

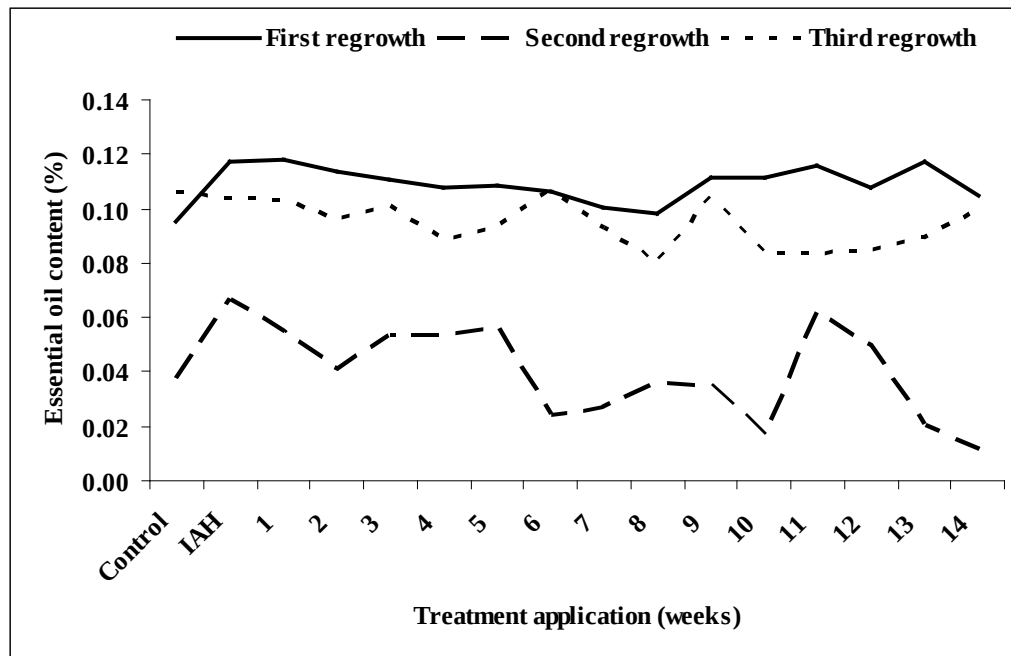


Figure 4.6: Response of rose-scented geranium essential oil content to delayed nitrogen topdressing at a rate of $100 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ N during the first re-growth (October 2005 to February 2006), second re-growth (March to June 2006) and third re-growth cycles (July to October 2006)

As illustrated in Fig. 4.7, a positive relationship ($r^2 = 0.87$; Weibull's model) was found between essential oil content and LAI of rose-scented geranium. This could be related to an increase in leaf expansion and number through maturity of the plant as a function of weather conditions presented during the re-growth cycles, which resulted in an increase in LAI and then levelled off (Fig. 4.7). Leaf number of the plant was also reported to have a direct relationship with essential oil content (Weiss, 1997). In rose-scented geranium essential oil production, leaves are the most important part of the plant since more than 50% of the essential oil is stored in the leaves (Kumar *et al.*, 2001). In the present study, lowest LAI values and essential oil contents were collected at the second re-growth cycle (March to June 2006), which corresponded to low LAI (Fig. 4.7), as compared to the first (October 2005 to February 2006) and third re-growth cycles (July to October 2006). Findings of the present study partially agree with Croteau *et al.* (1981) who reported that camphor content of camphor plants increased with an increase in leaf area, where the increase or decrease in essential oil

content (rose-scented geranium) was re-growth cycle dependent than LAI. According to Rajeswara Rao *et al.* (1990b), in rose-scented geranium, high leaf proportion during harvesting period was reported to have a significant effect in essential oil recovery through distillation. However, according to Rajeswara Rao *et al.* (1993) and Motsa (2006), no relationship was found between rose-scented geranium essential oil content and LAI. It was also reported that oil content of the plant decreased towards maturity due to leaf ageing. Generally, the plant is believed to require warm and sunny weather conditions for maximum growth (Weiss, 1997) and these conditions are also reported to be favourable for higher essential oil content of the plant (Araya *et al.*, 2011).

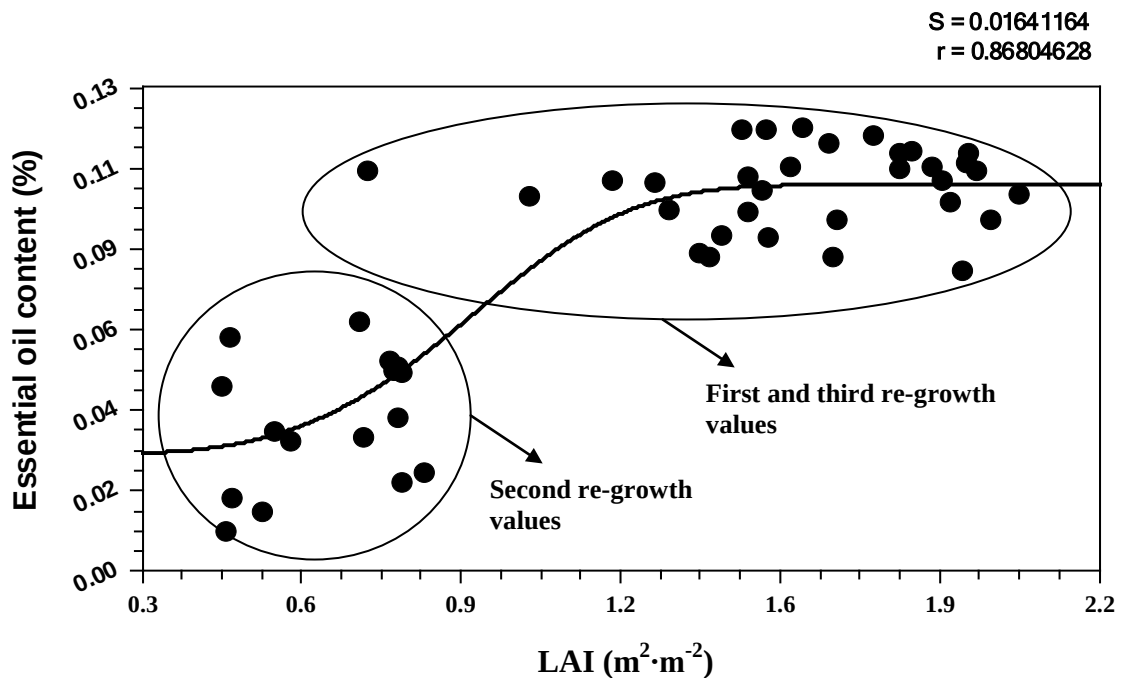


Figure 4.7: Relationship between rose-scented geranium essential oil content (%) and LAI ($\text{m}^2 \cdot \text{m}^{-2}$) with nitrogen topdressing time at a rate of $100 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ N using Weibull's model². Data combined for the first (October 2005 to February 2006), second (March to June 2006) and third (July to October 2006) re-growth cycles. ² $\text{Essential oil content} = 1.03 - 7.09e^{-9.72 \text{ LAI}^{3.95}}$, where e = exponential function

Essential oil composition

Rose-scented geranium essential oil composition is highly dependent on weather conditions (Parakasa Rao *et al.*, 1995; Diomo *et al.*, 1999a) and oil extraction methods (Gomes *et al.*, 2004). In the first re-growth cycle (October 2005 to February 2006), a gradual increase in geraniol component of the essential oil was observed with a delay in nitrogen application but this trend was not evident in the second re-growth cycle (March to June 2006) (Fig. 4.8). In the second re-growth cycle, geraniol concentration peaked at 'immediately after harvest' (IAH) nitrogen application time then this was followed by a steady gradient throughout the re-growth period. This shows that the response was not consistent and it might be incorrect to conclude that peak geraniol concentration (%) of the essential oil (especially in the first harvest) was related to the applied treatments. As stated by different authors from this growing region (Araya *et al.*, 2006; Motsa *et al.*, 2006; Eiasu *et al.*, 2009), the variation in the essential oil constituents during re-growth periods could be related to environmental factors that occur during a re-growth period than the applied treatments effect. Citronellol, another important component which is responsible for sweet, soapy rose-like odour (Weiss, 1997) followed a gradual concentration (%) in both the re-growth cycles. The highest values were recorded in the first re-growth cycle than the second re-growth cycle (Fig. 4.8). Similarly, this could be due to environmental factors such as in Java citronella (*Cymbopogon winterianus*) where essential oil composition of the plant was influenced by season than harvesting time (Blank, Costa, Arrigoni-Blank, Cavalcanti, Alves, Innecco, Ehlert & de Sousa, 2007). Other components such as linalool, citronellyl formate, geranyl formate and guaia-6,9-diene followed steady trends in both re-growth cycles (Fig. 4.8). Fernando & Roberts (1984) also described that linalool composition of black tea varied due to seasonal changes. In the present study, iso-menthone concentration was steady during the second re-growth but in the first re-growth the trend was not clear (Fig. 4.8). Composition of the third re-growth cycle (July to October 2006) was not subjected to gas chromatograph analysis (GC).

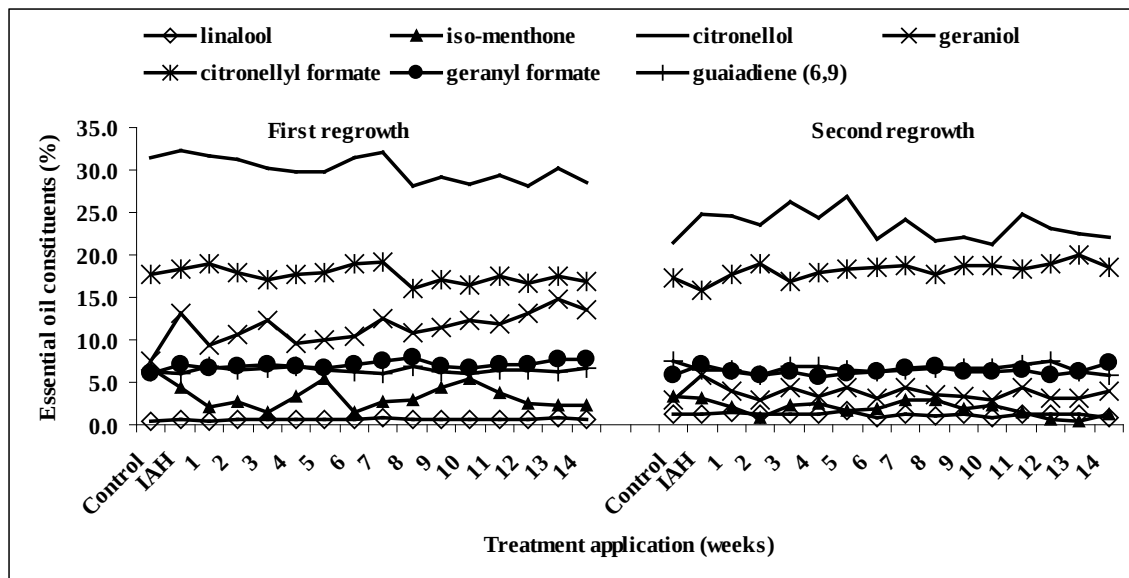


Figure 4.8: Response of rose-scented geranium essential oil composition to time of nitrogen topdressing at a rate of $100 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ N during the first (October 2005 to February 2006) and second (March to June 2006) re-growth cycles

Citronellol and geraniol are among the most important components of rose-scented geranium essential oil and the essential oil is characterized by their ratio (Doimo *et al.*, 1999b; Motsa *et al.*, 2006). The desirable ratio between the components is 1.0 to 3.0 (Anistescu *et al.*, 1997; Bauer *et al.*, 1990). In this investigation, the ratio between citronellol and geraniol (C:G) is presented in Fig. 4.9. Citronellol to geraniol ratio (C:G) in the second harvest (re-growth cycle) of the trial was high in all the treatments (mean value of 6.47) and in the control treatment for the first harvest (4.8). As stated earlier, in the first harvest geraniol concentration tended to increase with delaying nitrogen topdressing and this might have resulted in low C:G values. High C:G values in the second harvest could have been related to weather effects. This was similar to a report by Motsa (2006) where C:G ratio in this growing region was high during autumn/winter (low minimum night temperature) growth period, which might be the case for the second harvest. Doimo *et al.* (1999a) and Motsa *et al.* (2006) also reported C:G ratios were highest in winter and late winter/spring harvests. The same authors described that better quality of the oil was obtained when the C:G ratio was low. According to Rajeswara Rao *et al.* (1996) cold weather conditions (mean maximum temperature of 27.3°C and mean minimum temperature of 11.3°C) during a

re-growth period favour geraniol and geranyl formate concentration of the crop. In the present study, mean maximum temperature was 29.2 and 23.3°C and mean minimum temperature was 15.6 and 9°C for the first and second re-growth cycles, respectively. But in general, the characteristic of the essential oil of the present study was within the range of internationally accepted (Lis-Balchin, 1995) rose-scented geranium essential oil.

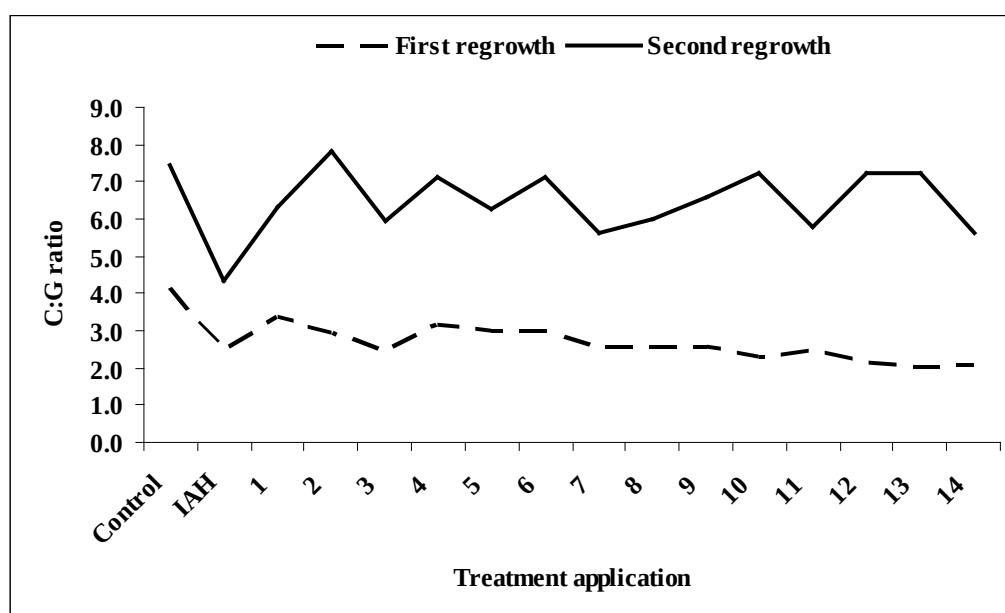


Figure 4.9: Citronellol to geraniol (C:G) ratio of rose-scented geranium essential oil with delayed nitrogen topdressing at a rate of 100 kg·ha⁻¹·year⁻¹ N during the first (October 2005 to February 2006) and second (March to June 2006) harvests

4.4 CONCLUSIONS AND RECOMMENDATIONS

Fresh herbage yield, total dry matter accumulation, and LAI of rose-scented geranium were found to follow a lag period (slow re-growth period) which was from the 1st to the 8th week from cut back, a rapid growth period (9 to 12 week from cut back) and a stationary and/or decline period (13 to 17 week from cut back). Fresh herbage yield ($\text{kg}\cdot\text{ha}^{-1}$), dry matter accumulation ($\text{kg}\cdot\text{ha}^{-1}$) and LAI ($\text{m}^2\cdot\text{m}^{-2}$) were highest at the rate of $400 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ N followed by 100 and then $0 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ N, respectively. In addition, soil solution nitrate (NO_3^-) concentration increased with an increase in nitrogen level. Nitrate values ($\text{mg}\cdot\text{L}^{-1}$) were high when nitrogen was applied at the rate of $400 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ (1/3 split application, $133.3 \text{ kg}\cdot\text{ha}^{-1}$ N per re-growth cycle) followed by $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ N (1/3 split application, $33.3 \text{ kg}\cdot\text{ha}^{-1}$ N per re-growth cycle) in the soil layer. Nitrate loss to deeper soil layers (40 to 60 cm) was also found when nitrogen was applied at the early re-growth period and leachate concentration was more pronounced at $400 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ N. Therefore, nitrogen management in terms of rate and time of application is important in rose-scented geranium production.

Delaying nitrogen topdressing (conventional N in the form of LAN; N 28%) after harvest to between the 7th and 9th week after cut back, was found to have a significant positive effect on biomass and essential oil production. This effect was more distinct in the first and third re-growth cycles. Essential oil content of the plant did not show any response to a delay in nitrogen topdressing. Though the applied treatments did not show clear effects on oil content, high essential oil content was recorded in the first and third re-growth cycles. A positive relationship was also found between essential oil content and LAI. Rose-scented geranium essential oil composition is highly influenced by weather parameters though geraniol concentration increased with a delay in nitrogen topdressing, in the first re-growth cycle and this also resulted into a lower citronellol to geraniol (C:G) ratio, which favour essential oil quality of the crop. Generally, the characteristic of the essential oil of the present study was within the range of internationally accepted rose-scented geranium essential oil. Therefore, it can be concluded that proper time for nitrogen topdressing in rose-scented geranium production has to be during rapid growth stages of the plant, which was between the 9th and 12th weeks after cut back. That is, nitrogen topdressing has to be applied either

between the 7th and 8th week or at the 9th week of the re-growth period, to attain optimum biomass and essential oil yield production. The set hypothesis could therefore be accepted, as best yield response was observed when N top dressing coincided with the period of most rapid growth. In addition, production of rose-scented geranium during cooler periods is not advisable due to limited biomass production which might encourage leaching of nitrogen.

CHAPTER FIVE

INFLUENCE OF SEASON ON NITROGEN FERTILIZATION OF ROSE-SCENTED GERANIUM AND THE ESTIMATION OF FOLIAR NITROGEN STATUS USING SPAD-502 CHLOROPHYLL METER

5.1 INTRODUCTION

Essential oil production of crops is reported to be influenced by several factors, including nitrogen rate, source and season of production (Rajeswara Rao *et al.*, 1990a; Araya *et al.*, 2006). For instance, rose-scented geranium (*P. capitatum* x *P. radens*) essential oil production and oil composition was reported to be affected by change in temperature, humidity, rainfall (Weiss 1997; Kumar *et al.*, 2001), fertilization (Araya *et al.*, 2006), soil moisture availability (Eiasu, 2009), harvesting season and plant shoot age (Rajeswara Rao *et al.*, 1996; Motsa 2006), soil type and pH (Araya *et al.*, 2011; Weiss, 1997), photoperiod (Adams & Langton, 2006) and distillation method and techniques (Hey & Waterman, 1993; Coleman, 2003). Weiss (1997) also suggested that climatic factors which favour high herbage yield in rose-scented geranium, have the contrary effect on oil yield, and vice versa; and further suggested that if one of these products is reduced it can be balanced by increasing the other factor.

Like any other crop, season plays an important role in production of rose-scented geranium. For instance, in India rainy/monsoon and autumn seasons were found to be conducive for good crop growth, high biomass yields ($\text{t}\cdot\text{ha}^{-1}$), better essential oil concentration (%) and essential oil yield ($\text{l}\cdot\text{ha}^{-1}$), whereas in summer seasons low biomass and essential oil yield as well as low oil quality were recorded. Winter and spring were also found to support good growth when the crop was irrigated (Rajeswara Rao *et al.*, 1996). On the other hand, crops harvested in the spring (semi-dry period) had greater oil content than those cut after monsoon rains (Mani & Sampath, 1981; Prakasa Rao *et al.*, 1995), whereas oil from plants grown in semi-arid conditions showed similar variation (Bhattacharya *et al.*, 1993). When the plant is

grown in areas where there are no distinct seasons, highest herbage production mostly occurred during the flowering season (spring) (Demarne, 2002). Fleisher & Fleisher (1985) reported high herbage yield in May and high oil content in August, under Israeli environmental conditions. On the other hand in South Africa, Motsa (2006) reported higher fresh herbage and essential oil yield production in summer, followed by autumn and winter seasons when harvesting was done at a three month interval. However, the yield was lower than when the harvest frequency was every four months. Information on the effect of nitrogen rate and source applied in different growing seasons (at three month harvesting frequency) has not been documented before.

Rose-scented geranium responds well to application of fertilizers (Rajeswara Rao *et al.*, 1990a; Araya *et al.*, 2006). According to different studies (Rajeswara Rao *et al.*, 1990a; Weiss, 1997; Araya *et al.*, 2006), herbal plants (such as rose-scented geranium) in general are reported to require larger quantities of the essential, plant nutrients, such as nitrogen, phosphorus and potassium, for optimum growth. Among these essential elements, nitrogen plays an important role in yield and growth of rose-scented geranium. Since most of the above ground biomass is removed at harvest, fertilization of the crop after every harvest becomes essential. Traditionally, fertilization of the crop includes application of chemical fertilizers, addition of organic matter and liming (Weiss, 1997). However, understanding the advantages and disadvantages of the source of nutrients (conventional or organic fertilizers) is important. According to a study by Araya *et al.* (2006), production of the crop was found to be affected by source and rate of nitrogen nutrition used. Commonly, farmers use chemical fertilizers (conventional fertilizers) sparsely because of their comparatively high costs (Chabalier, 1992).

When there is fertilization balance or when other factors, especially phosphorous, are not limiting, geranium is found to respond well to nitrogen fertilizer application (Chabalier, 1992). When nitrogen is deficient, while phosphorous, potassium and moisture are sufficiently available it was found that only nitrogen affect the biomass yield of the crop, while it may have little or no effect on the quality of the oil (Rajeswara Rao *et al.*, 1990a,b). To get faster growth, it is advisable to apply nitrogen fertilizer to cuttings before planting and after harvesting of the crop. This encourages

new growth but it is also important to know the exact amount and application level in order to minimize losses and cost of fertilizer. For instance, application of a total of $170 \text{ kg}\cdot\text{ha}^{-1}$ ammonium sulphate ($36 \text{ kg}\cdot\text{N}$) was found not to increase yield in Kenya, but on the other hand $100 \text{ kg}\cdot\text{ha}^{-1}$ ammonium sulphate was found to increase biomass yield in India (Rajeswara Rao *et al.*, 1990a,b). In Israel, a high level of ammonium sulphate ($500 \text{ kg}\cdot\text{ha}^{-1}$ prior to planting and $300 \text{ kg}\cdot\text{ha}^{-1}$ as top dressing, before and after first harvest) yielded $70 \text{ t}\cdot\text{ha}^{-1}$ biomass and $185 \text{ kg}\cdot\text{ha}^{-1}$ oil from three harvests per year (Fleisher & Fleisher, 1985). A high amount of nitrogen may yield high biomass. However, it may also result in rust development caused by *Puccinia pelargonii-zonalis* due to shading. According to Weiss (1997), little or no difference in biomass production was observed with high levels of different nitrogen fertilizers, therefore it was suggested to use the recommended amount, which is in the study region it was reported to be $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ either in the form of limestone ammonium nitrate or LAN, 28% N) or blend of organic fertilizers (Araya *et al.*, 2006).

In rose-scented geranium production, determining which source of N and at what rate to be applied per harvesting season is important to consider, for the following reasons. When N fertilizer is applied, consideration should be given to provide enough nutrition for optimum growth of the plant, benefit the farmer (in comparison to no application) and the effect it has on the environment. Most plant species respond well to N and even at excess levels, it has no toxicity effect on the plant (Tisdale *et al.*, 1993). This usually encourages one to apply more than what is required for optimum growth and as a result it reduces the benefits that are supposed to be obtained with optimum application and also affects the environment through pollution. Techniques for proper N management are thus become important.

The most common practices used to determine plant nutrition management are soil tests, plant tissue analysis and long-term N fertilization trials but these techniques are believed to be expensive and time consuming (Tisdale *et al.*, 1993). Therefore, a new instrument was developed recently by Minolta Camera Co. Ltd. (Osaka, Japan) called the SPAD-502 chlorophyll meter (Minolta Camera Co. Ltd., 1989). The SPAD-502 chlorophyll meter (Minolta Camera Co. Ltd., Osaka, Japan) is an instrument

(handheld meter) used to give a quick and non destructive estimation of leaf greenness in plants (Kariya *et al.*, 1982; Yadava, 1986; Minolta Camera Co. Ltd., 1989). Leaf greenness is directly related to relative chlorophyll content and positively related to foliar N concentration of plants (Chapman & Barreto, 1997; Schröder *et al.*, 2000; Sexton & Carrol, 2002). This instrument measures the SPAD values (Soil Plant Analysis Development; units developed by Minolta Camera Co. Ltd.) which are directly related to chlorophyll content (Wood, Reeves & Himelrick, 1993; Markwell, Osterman & Mitchell, 1995). This is achieved by transmitting red light at 650 nm (a near peak wavelength where chlorophyll absorbs light) and infrared light at 940 nm (a near peak where almost no absorption occurs). These wavelengths are frequently transmitted by the instrument through the leaf within the chamber (2 mm x 3 mm, where a leaf sample is being held) calculates the SPAD values 'units' from the optical density differential between the two wavelengths (Minolta Camera Co. Ltd., 1989). However, at this stage no information is available on the use of the instrument on rose-scented geranium for estimation of foliar N concentration.

The effect of nitrogen fertilization on rose-scented geranium production was studied extensively (Rajeswara Rao *et al.*, 1990a; Araya *et al.*, 2006). However, there is no information on the effect of nitrogen source (conventional or organic nitrogen) and levels in different production seasons on rose-scented geranium foliage and essential oil production. These needed to be studied to understand the response of the crop to source of nitrogen in different harvesting seasons and also to determine the best production season of the crop. Therefore, this study was conducted to identify the influence of timing of N application in relation to harvesting season, source and rate on rose-scented geranium production; and to estimate N uptake of the crop using a chlorophyll meter (SPAD 502). In this study the following hypotheses were tested:

- (i) Biomass and oil yield will respond best to N top dressings in spring and summer, when highest yields are expected.
- (ii) The SPAD 502 chlorophyll meter can be successfully used to estimate leaf N status of rose-scented geranium.

5.2 MATERIALS AND METHODS

Experimental site, experimental design and treatment details

The experiment was conducted under field conditions at the University of Pretoria's Hatfield Experimental Farm, Pretoria, South Africa (25° 45'S, 28° 16'E) as a randomised complete block design, over four harvesting seasons, at three-monthly intervals, (winter, June – August 2006; spring, September – November 2006; summer, December 2006 – February 2007; and autumn, March – May 2007). Soil characteristics and weather conditions of the growing area are as described in Chapter 3, Section 3.2.

Healthy and rooted cuttings (45 days old) of rose geranium were planted in the experimental plots on 29 January 2004, at a spacing of 0.625 m between plants and 1 m between rows, which gave a total of 16 000 plants·ha⁻¹ (suggested by the industry). Before commencing treatment application the plants were allowed to establish and deplete previously applied N fertilizer, during the establishment period. In early June 2006, plants were cut back to a height of 20 to 25 cm above ground level and at the same time treatments applications were started. Two sources of N, namely conventional (limestone ammonium nitrate or LAN, 28% N) and organic fertilizers (blend of four organic fertilizers) (Table 5.1) were tested at three levels (100, 200 and 300 kg·ha⁻¹ N·year⁻¹). A control (no N) was also included and treatments were replicated four times. Phosphorous (P) and potassium (K) fertilizers were applied at 90 and 102.1 kg·ha⁻¹ year⁻¹ respectively, in all the plots (including the control treatment). For conventional N treatments, super phosphate (10.5% P) and potassium chloride (50% K) were used as sources of P and K, respectively. The yearly P and K were split into four applications, with one quarter of the yearly amount applied in equal splits after each harvest. Similarly, the yearly N either in conventional or organic form was also applied in four equal splits (one portion of equal splits after every cutting back, harvest, of the plants) and harrowed in manually. Application of organic N treatments was designed to achieve comparable levels to conventional N and at the same time ensuring adequate P and K levels. As shown in Table 5.1, achieving P and K to rates of 90 and 102.1 kg·ha⁻¹·year⁻¹ while ensuring the desirable N kg·ha⁻¹·year⁻¹ rates was impossible, since the organic fertilizers used were in complete fertilizer blends. That is, increasing the rate of one nutrient affected the

other's rate (Table 5.1). Therefore, the P and K levels were normalized to the desired rates (same as conventional treatments) by addition of super phosphate (10.5% P) and potassium chloride (50% K), respectively.

Table 5.1: Nitrogen (N), phosphorous (P) and potassium (K) content of the organic fertilizers used for the trial (winter, spring, summer and autumn 2006/07)

Fertilizer type	Nutrient content (kg·100 kg ⁻¹)			Treatment (N kg·ha ⁻¹ ·year ⁻¹)	Total Required (kg·ha ⁻¹)	Computed nutrient content per ha			Applied per area (kg·22.5 m ⁻²)
	N	P	K			N	P	K	
2:3:2(16)	4.6	6.9	4.6	100.0	796.0	36.6	54.9	36.6	1.8
3:1:5(18)	6.0	2.0	10.0		550.0	33.0	11.0	55.0	1.2
4:1:1(15)	10.0	2.5	2.5		304.0	30.4	7.6	7.6	0.7
KCl	0.0	0.0	50.0		5.9			2.9	0.0
P ₂ O ₅	0.0	10.5	0.0		157.5		16.5		0.4
					1813.4^z	100.0	90.0	102.1	4.1
2:3:2(16)	4.6	6.9	4.6	200.0	400.0	18.3	27.4	18.3	1.8
3:1:5(18)	6.0	2.0	10.0		400.0	24.0	8.0	40.0	1.8
4:1:1(15)	10.0	2.5	2.5		352.5	35.3	8.8	8.8	1.6
7:2:2(11)	7.0	2.0	2.0		1750.0	122.5	35.0	35.0	7.8
KCl	0.0	0.0	50.0		0.0			0.0	0.0
P ₂ O ₅	0.0	10.5	0.0		102.5		10.8		0.2
					3005.0	200.0	90.0	102.1	13.2
3:1:5(18)	6.0	2.0	10.0	300.0	210.0	12.6	4.2	21.0	1.4
4:1:1(15)	10.0	2.5	2.5		1000.0	100.0	25.0	25.0	6.7
7:2:2(11)	7.0	2.0	2.0		2677.0	187.4	53.5	53.5	18.0
KCl	0.0	0.0	50.0		5.1			2.6	0.0
P ₂ O ₅	0.0	10.5	0.0		69.5		7.3		0.2
					3961.6	300.0	90.0	102.1	26.3

^z Bold values represent cumulative of the required fertilizer (kg·ha⁻¹·year⁻¹) or nutrient content that can be achieved with the particular quantity.

The experimental site had four blocks, with four rows per block assigned to each of the treatments. The four rows of each experimental plot were 5.6 m long, with a 1 m path between plots, which gave a total area of $22.5 \text{ m}^2 \cdot \text{plot}^{-1}$. From the four rows per treatment, the two centre rows were used for measurement of fresh herbage and essential oil yield and oil composition analysis, while the two rows on either side were used as borders to reduce treatment interference. Rainfall was supplemented with drip irrigation (flow rate of $2 \ell \cdot \text{h}^{-1}$ at a pressure range of 120 to 200 kPa) and prior to irrigation, soil water content was measured to a depth of 60 cm using a neutron probe (Neutron water meter, Model 503 DR, CPN Corporation, CA, USA) on a weekly basis. This depth was taken based on the assumption that most of the efficient roots of this crop occur within this depth). Irrigation commenced when the soil water content dropped below 60% of plant available water and the profile was refilled to field capacity.

Data collection and analysis

At each harvest, fresh herbage yield, oil yield and oil composition were determined. The crop was harvested (20 to 25 cm above ground level) at three-monthly intervals and herbage yield per hectare was computed. Foliage essential oil extraction (steam distillation) and essential oil composition (gas chromatography, GC) was determined using the same methodology as described in Chapter 3, Section 3.2. Essential oil yield ($\text{kg} \cdot \text{ha}^{-1}$) per treatment (per replication) was computed based on the essential oil content (%) and fresh herbage yield ($\text{t} \cdot \text{ha}^{-1}$) of the same treatment per season.

At each harvest, two plants per treatment per replication were taken for growth analysis measurements and each plant was separated into leaf and stem, where after fresh and dry mass (dried in an oven for 72 hrs at 70°C) were determined. Total leaf area per plant was determined using a LI 3100 belt driven leaf area meter (LiCor Inc., Lincoln, Nebraska, USA). Standard cultivation and plant protection practices were followed to control weeds and insects during the experimental period.

To estimate the leaf N content of the crop, a Minolta SPAD 502 chlorophyll meter was used to take SPAD readings (Figure 5.1). Chlorophyll meter readings were taken

on fully expanded functional leaves (4th from the apex) at various growth periods on monthly intervals. Readings were taken by putting the chlorophyll meter's sensor slot parallel to the main vein of the rose-scented geranium leaves (Figure 5.1). Four plants were randomly selected per treatment per replication and used for SPAD value monitoring, throughout the trial period. From the selected plants, 30 readings were taken and averaged. After the SPAD measurements, the 20 measured leaves were sampled for leaf N content determination. Total N content was analyzed by semi-micro Kjeldahl digestion and distillation according to the method of Bremner & Mulvaney (1982). Leaf N concentration was expressed on a dry mass basis. To interpret the chlorophyll meter readings, reference rows (well fertilized plots; about 80% higher than the optimum N level) were developed, based on the recommendation of Peterson *et al.* (1993) and Shapiro *et al.* (2006).



Figure 5.1: Illustration of Minolta SPAD 502 chlorophyll meter reading technique in rose-scented geranium

Nitrogen use efficiency (NUE) was calculated as described by Rajeswara Rao *et al.* (1990c). The equation used to compute the NUE of the plant is described below (Eq. [5.1]), where, Y_n is total biomass ($\text{t}\cdot\text{ha}^{-1}$) or essential oil ($\text{kg}\cdot\text{ha}^{-1}$) yield for a specific N

fertilized treatment, Y_o is total biomass ($\text{t}\cdot\text{ha}^{-1}$) or essential oil ($\text{kg}\cdot\text{ha}^{-1}$) yield in the zero N plot (control) and N is the amount of N fertilizer ($\text{kg}\cdot\text{ha}^{-1}$) applied.

$$\text{NUE} = \frac{Y_n - Y_o}{N} \quad [5.1]$$

Statistical analysis and interpretations were based on comparison of treatment means as well as comparison between cumulative harvests. The collected data were subjected to analysis of variance (ANOVA) and analysis was carried out using the SAS package (Statistical Analysis System Institute Inc., 1999-2001). To estimate the foliar nitrogen status of rose-scented geranium using the SPAD-502 chlorophyll meter, SPAD readings (units) and leaf N content (%) over the three growing seasons (spring, summer and autumn) per source of N (conventional or organic) were correlated. Microsoft Excel (Windows Microsoft 2003) and Curve fitting software for windows (Hyams, 1997) were used to fit quadratic functions to the data.

5.3 RESULTS AND DISCUSSION

Rose-scented geranium herbage yield, essential oil yield and oil content were found to vary with nitrogen rate, source and season. The statistically analyzed data showed that application of conventional or organic N had no significant effect on fresh herbage yield of rose-scented geranium, compared to the control, in winter (June – August 2006) and autumn (March – May 2007) growing seasons (Fig. 5.2 and 5.3, respectively). Essential oil yields ($\text{kg}\cdot\text{ha}^{-1}$) in both seasons also followed a similar trend, except for the $300 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ organic N treatment, which resulted in a significant difference as compared to the other treatments in winter (97% and 73% oil yield higher than the control and conventional N at $300 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$, respectively) . Similarly in autumn, essential oil yield of the $200 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ organic N treatment was significantly lower compared to the rest of the treatments. This could be related to slightly higher essential oil content (0.075%) when organic N was applied at $300 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ in winter and a slight drop in essential oil content (0.094%) at $200 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ organic N in autumn. Essential oil yield ($\text{kg}\cdot\text{ha}^{-1}$) is a multiplicative

result of fresh herbage yield ($\text{t}\cdot\text{ha}^{-1}$) and essential oil content (%) of the plant. Generally, fresh herbage yield ($\text{t}\cdot\text{ha}^{-1}$) and essential oil yield ($\text{kg}\cdot\text{ha}^{-1}$) during these seasons (winter and autumn) were significantly ($P < 0.05$) lower as compared to spring and summer season harvests. Similarly, Motsa *et al.* (2006) also reported low fresh herbage ($\text{t}\cdot\text{ha}^{-1}$) and essential oil yield ($\text{kg}\cdot\text{ha}^{-1}$) for the same cultivar during autumn and winter growing seasons.

Warm weather conditions are reported to be favourable for optimum production of this plant (Weiss, 1997; Kumar *et al.*, 2001). On the contrary, Rasejwara Rao *et al.* (1996) suggested that summer conditions are not favourable for geranium production, since the plants are subjected to high temperatures (atmospheric and soil) and moisture stress, which impact negatively on the photosynthetic activity of the plant. In the present study, the lack of response of the plant to the applied N could be related to environmental factors such as temperature, since the weather conditions in winter growing period was cooler (mean maximum of 21.6°C and mean minimum of 5.3°C) and in autumn a drop in temperature was noticed towards the end of the season (May with mean maximum of 23.9°C and mean minimum of 6.2°C) (Appendix A), which might have contributed to low biomass as well as low essential oil yield. Amongst weather factors, temperature is known to play an important role in plant production (Salisbury & Ross, 1992; Taiz & Zeiger, 2002). In addition, rose-scented geranium is also believed to require warm and sunny weather conditions for maximum growth (Weiss, 1997)

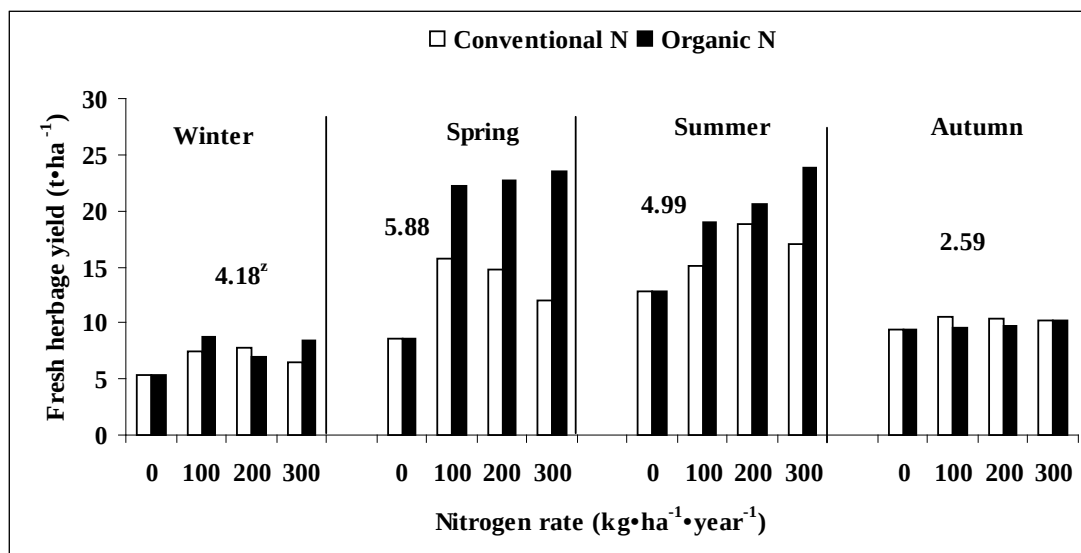


Figure 5.2: Fresh herbage yield of rose-scented geranium in response to conventional or organic nitrogen fertilization at a rate of 0 to 300 kg·ha⁻¹·year⁻¹ N, during 2006/07 growing seasons (winter, spring, summer and autumn). Individual values presented per growing season are means ($n = 16$). ^z LSD ($\alpha = 0.05$)

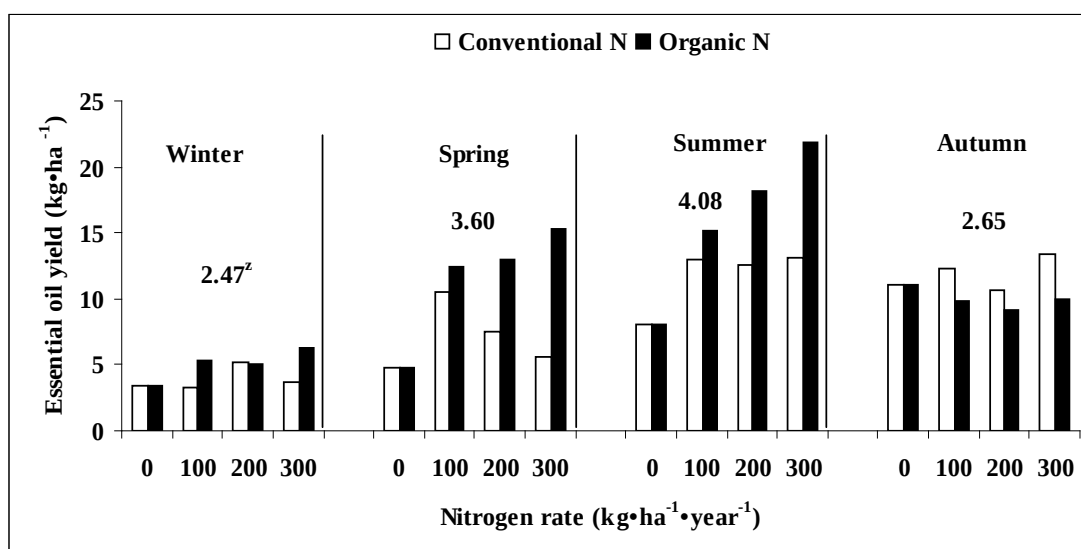


Figure 5.3: Rose-scented geranium essential oil yield in response to conventional or organic nitrogen fertilization at a rate of 0 to 300 kg·ha⁻¹·year⁻¹ N, during 2006/07 growing seasons (winter, spring, summer and autumn). Individual values presented per growing season are means ($n = 16$). ^z LSD ($\alpha = 0.05$)

In spring and summer growing seasons, application of N from both sources (conventional and organic) increased fresh herbage as well as essential oil yield significantly (Fig. 5.2 and 5.3). The increases in fresh herbage yield over the control due to conventional and organic N at $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ were 81.9% and 157.6% (spring) and 18.9% and 49.2% (summer), respectively. The $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ level of N also increased essential oil yield by 120.4% and 161.3% in spring and 62.1% and 90.3% in summer season, compared to the control for conventional and organic N, respectively.

Increasing N level beyond $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ with both N sources (conventional or organic) did not contribute to further significant increases in fresh herbage yields for either season (spring or summer) (Fig. 5.2) or the cumulative yields over the four seasons (Fig. 5.5). For essential oil yield (Fig. 5.3), further N increments provided no advantage in the autumn and winter season but for spring and summer harvest seasons, further positive responses to increased N levels above $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ were observed, with significantly higher oil yield at $300 \text{ kg}\cdot\text{ha}^{-1}$ organic N (Fig. 5.3). Similar results were also true for the cumulative essential oil yield over the four seasons (Fig. 5.5). Rajeswara Rao *et al.* (1990b) also reported an increase of 19.1% in fresh herbage yield and 24.1% in essential oil yield at $100 \text{ kg}\cdot\text{ha}^{-1}$ N, but no further responses at higher N levels. However, Prakasa Rao *et al.* (1985) reported an increment in fresh herbage and essential oil yield of geranium with each increment of applied N. Similarly, in India, application of $200 \text{ kg}\cdot\text{ha}^{-1}$ N increased fresh herbage and essential oil yield over no N and $100 \text{ kg}\cdot\text{ha}^{-1}$ N (Singh *et al.*, 1996; Singh, 1999).

When environmental factors (other than temperature) are optimal conditions, developmental rate of many plant species depends primarily on mean daily temperature. During a growing season, a plant's growth rate depends on accumulation of specific quantity of heat for its maturity, which is known as growing degree days (GDD) or growing degree units (GDUs). Mathematically, GDD is expressed as the average of daily maximum and minimum temperatures minus the base temperature (a temperature below which a plant growth is zero (CRS report for congress, 2005); in the present study 6°C was used according to Weiss (1997) and Table 5.2 shows thermal time recorded. As stated earlier, low temperature ($^{\circ}\text{C}$) of winter and autumn seasons, during the study period, might have contributed to the low biomass

accumulation of the plant (Table 5.2) at harvest (three month harvesting intervals after cutback).

Table 5.2: Rose-scented geranium growing-degree days (GDD) during four growing seasons (winter, spring, summer and autumn 2006/07)

Growing season	Thermal time (GDD)		
	One month	Two months	Three months
Winter	206.17	447.68	684.99
Spring	343.30	842.95	1301.81
Summer	547.66	1086.57	1622.58
Autumn	514.44	930.74	1210.66

To understand the effect of thermal time (GDD) on rose-scented geranium growth, long term weather data (16 years per season) was computed based on the above stated formula and then averaged per season (Fig. 5.4). Based on the simulation, the plant requires more days under cold weather conditions (winter or autumn seasons) to reach equivalent maturity (begin flowering) than in a summer season. The plant is believed to require warm and sunny weather conditions for maximum growth (Weiss, 1997), which was the case in spring (September – November 2006; mean maximum 27.7°C and minimum 12.8°C temperature) and summer (December 2006 – February 2007; mean maximum 31.4°C and minimum 16.3°C temperature) seasons in the present study. These weather conditions might have contributed to optimum growth of the plant and concurrently the plant efficiently utilized the applied N during the growing period (Table 5.2; Fig. 5.4). However in winter or autumn, applying the same nitrogen amount and harvesting the plant at the same interval (three months from cut back) becomes questionable, since growth rate of the plant is slow (Fig. 5.4); therefore the required biomass might not be achieved for optimum essential oil yield (kg·ha⁻¹). In addition, a strong positive linear relationship ($R^2 = 0.97$) was found between fresh biomass accumulation (t·ha⁻¹) and thermal time accumulated (GDD) during the study period (Appendix C).

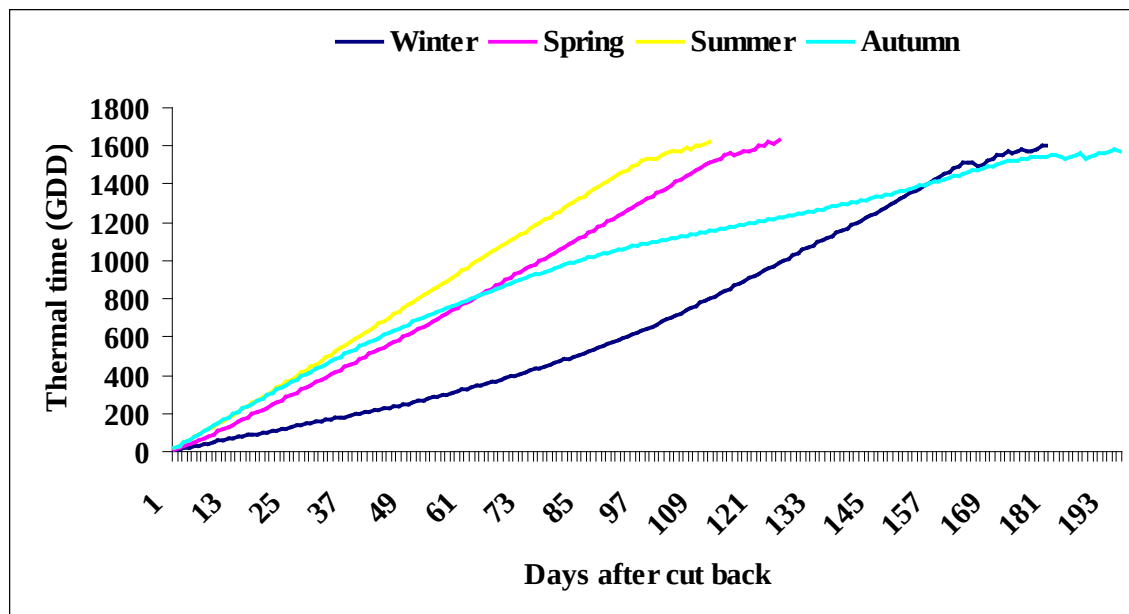


Figure 5.4: Long-term (16 years) simulation of thermal time (GDD) accumulation in rose-scented geranium for four growing seasons (winter, spring, summer and autumn). Individual values presented are means ($n = 16$)

The average effect of all levels of organic N was compared to the average effect of all levels of conventional N. In all instances, organic N produced higher herbage and oil yields ($15.20 \text{ t}\cdot\text{ha}^{-1}$ and $11.67 \text{ kg}\cdot\text{ha}^{-1}$, respectively) as compared to conventional N ($12.20 \text{ t}\cdot\text{ha}^{-1}$ and $8.97 \text{ kg}\cdot\text{ha}^{-1}$, respectively). As described by different authors (Weiss, 1997; Demarne, 2002), this species prefers to grow on well drained and high organic matter content soils for optimum herbage and essential oil yield. Application of organic matter in the form of either organic fertilizer (plant or animal residues) or manure are well known for their beneficial effect on the beneficiary microbial activities and in improving the soil structure (Tisdale *et al.*, 1993; Brady & Weil, 2002). Better root development and a two-fold increase in essential oil yield were reported when 5 to 10 tons of distillation residues were applied to geranium intercropped with legumes, compared to the geranium crop grown as a single crop (Chabalier, 1992). Improvement in water holding capacity of the soil was also reported with the addition of organic matter to the soil (Tisdale *et al.*, 1993; Brady & Weil, 2002). Scheffer *et al.* (1993) also reported that application of organic fertilizer (composed of cattle manure + straw) significantly increased essential oil yield of

Achillea millefolium L. The positive response of geranium to organic N could be due to the nature of organic fertilizers in improving soil structure, water holding capacity and soil microbial activity (Tisdale *et al.*, 1993; Rajeswara Rao, 2001). Furthermore, the possibility of lower N loss through leaching and the slow nutrient releasing nature compared to conventional fertilizers may be another reason for increased herbage and essential oil yield (Tisdale *et al.*, 1993).

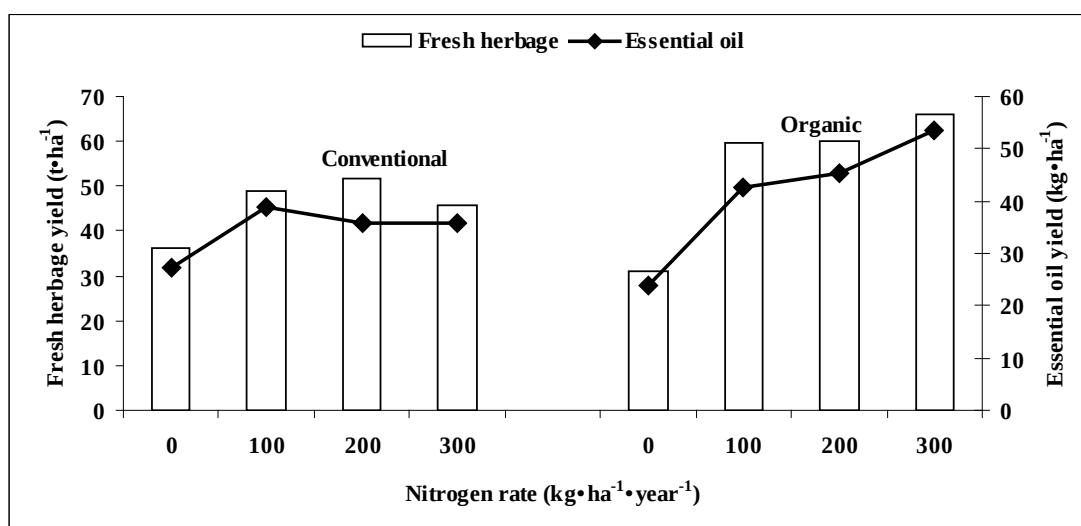


Figure 5.5: Cumulative rose-scented geranium fresh herbage (t·ha⁻¹) and essential oil yield (kg·ha⁻¹) production in response to conventional or organic nitrogen fertilization at a rate of 0 to 300 kg·ha⁻¹·year⁻¹ during 2006/2007. Individual values presented are means ($n = 32$). ^z LSD ($\alpha = 0.05$) values were 8.90 and 6.26 for fresh herbage (t·ha⁻¹) and essential oil (kg·ha⁻¹) yield, respectively

For essential oil yield (kg·ha⁻¹), there was a significant ($p < 0.05$) interaction between amount and source of N (Figure 5.6a) and, in addition, between source of N and application season (Fig. 5.6b). This implies that essential oil yield responded differently to an increase in N levels and season of application for the two sources of N. As organic N increased, essential oil yield increased, while it did not alter significantly when conventional N increased. Application of 300 kg·ha⁻¹·year⁻¹ in the form of organic N increased the essential oil yield by 21.6% compared to the control and by 25.2% compared to 100 kg·ha⁻¹·year⁻¹ organic N (Fig. 5.6a). Season of N application was also found to have a great influence on essential oil yield (Fig. 5.6b).

Application of organic N significantly increased essential oil yield in winter, spring and summer growing seasons by 38.4%, 65.7% and 43.4% compared to conventional N, respectively (Fig. 5.6b). Organic N gave the highest essential oil yield ($18.4 \text{ kg} \cdot \text{ha}^{-1}$) in summer compared to spring and winter growing seasons. However, in autumn the trend was different with conventional N. Essential oil yield increased by 1.2% and 15.4% compared to the control and organic N when conventional N was used (Fig. 5.6b). Source of N in this season resulted in variable response with regard to essential oil content.

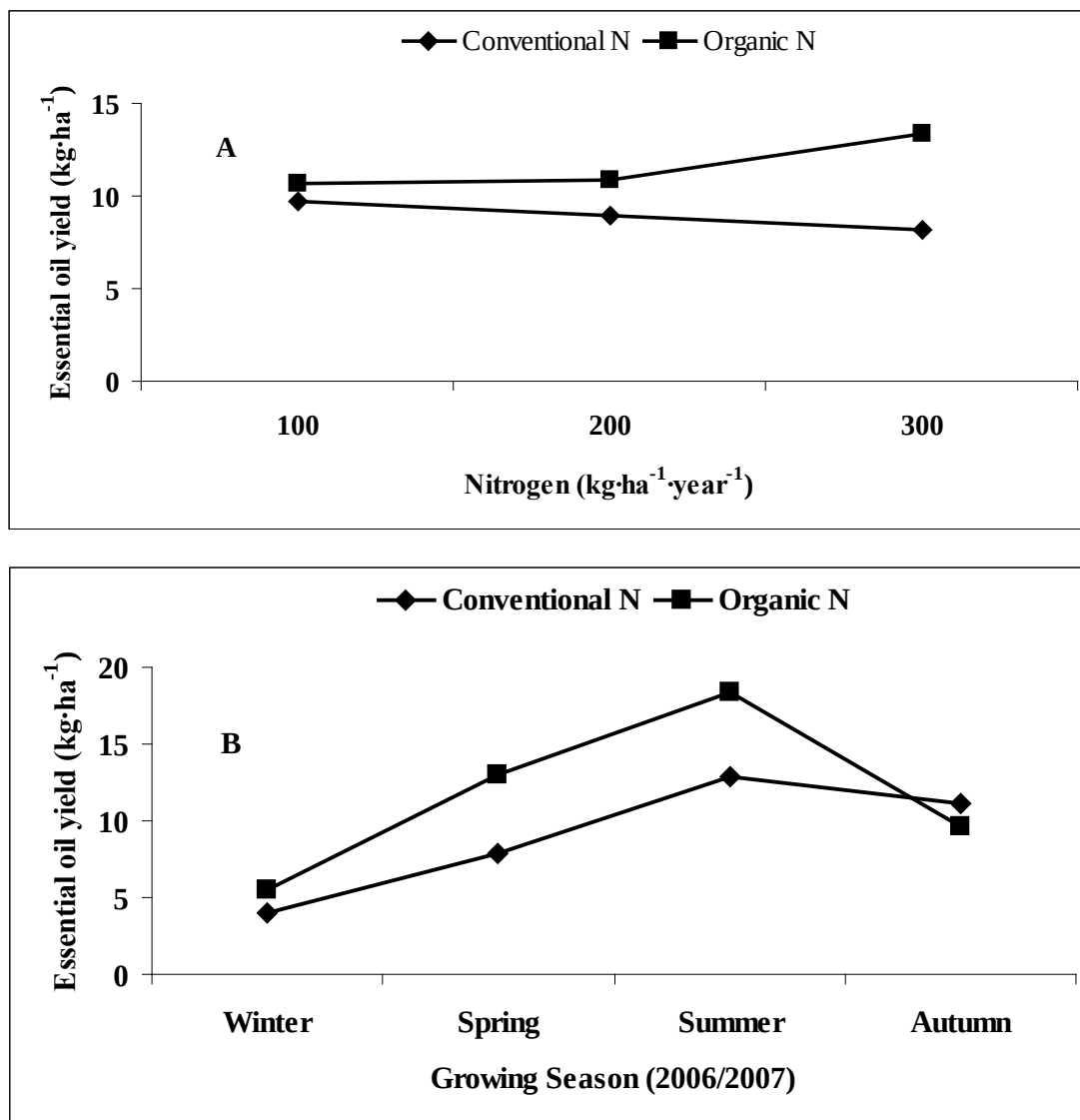


Figure 5.6: Interactions of (a) amount and source of N and (b) season and source of N on essential oil yield of rose-scented geranium. Values presented are means ($n = 16$ for amount $\times$ source of N; $n = 12$ for season $\times$ source of N)

Nitrogen use efficiency (NUE) vs season of production

Nitrogen use efficiency (NUE) of rose-scented geranium with regard to growing season is illustrated in Fig. 5.7 the discussion is focused on the spring (Fig. 5.7a) and summer (Fig. 5.7b) growing seasons when growth was optimal. Similar to the findings of a previous study (Chapter 3, Section 3.3), NUE of the plant had a negative linear correlation with levels of N for both N sources and growing seasons (Fig. 5.7). Nitrogen use efficiency decreased with an increase in nitrogen level. Rajeswara Rao (2001) and Rajeswara Rao *et al.* (1990c) also reported a decrease in NUE of rainfed

palmarosa (*Cymbopogon martini* Roxb.) with an increase in N level. The present investigation indicated that $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ N resulted in highest NUE (based on essential oil yield) for both N sources and in both harvesting seasons (spring and summer). That is, application of $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ N resulted in a 95.3% and 54.1%, (spring, Fig. 5.7a) and 66.1% and 35.8% (summer, Fig. 5.7b) higher NUE (essential oil yield) than $300 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ N of either forms (conventional or organic N, respectively) (Fig. 5.7).

During these growing seasons, although the NUE trend was the same for conventional and organic N, the response was best with organic N (Fig. 5.7) at any given rate of N, the NUE was highest for the organic N fertilizer. Comparing conventional to organic N, the plant was more efficient in essential oil production with organic (25.4% in spring and 31.2% in summer) than conventional N at $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$. This might be due to the difference in characteristics of the N sources, as described in the previous section.

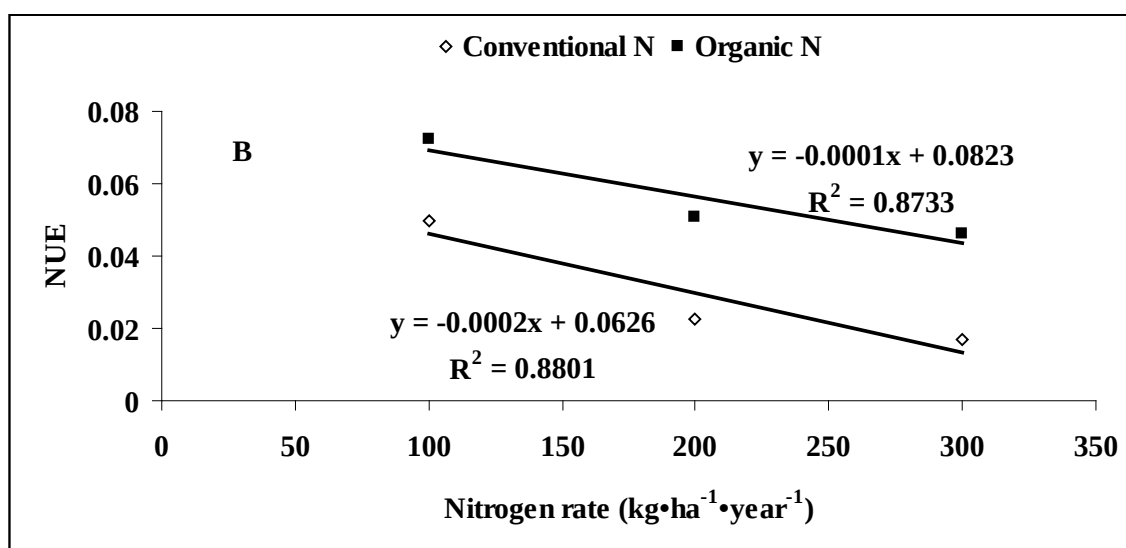
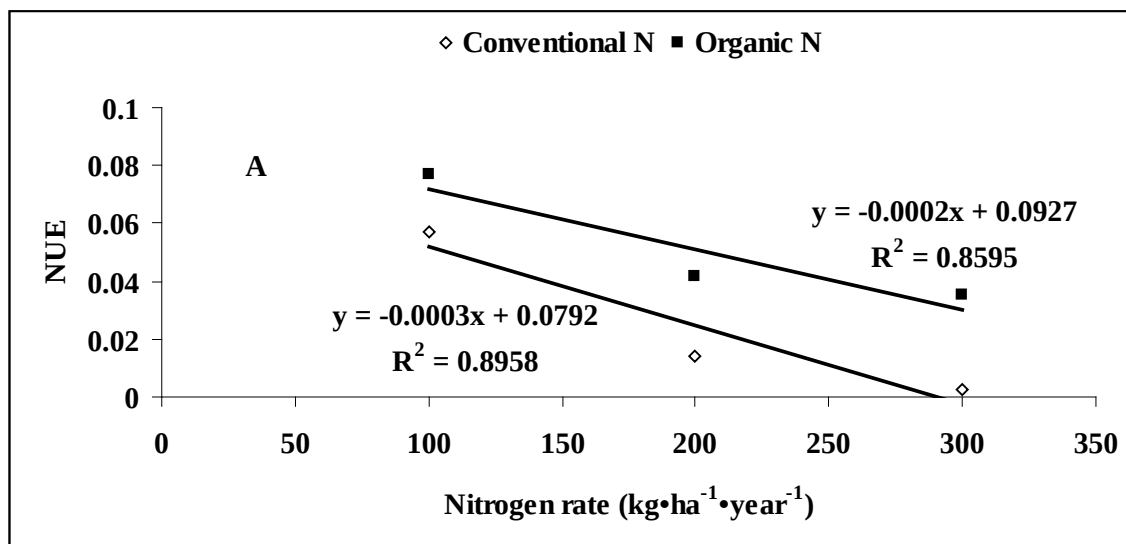


Figure 5.7: Nitrogen use efficiency (NUE) of rose-scented geranium essential oil yield ($\text{kg} \cdot \text{ha}^{-1}$) as influenced by nitrogen rate and season of production during (A) spring 2006 and (B) summer 2006/2007. Individual values presented per growing season are means ($n = 16$)

As suggested by Freney (1997) N loss can be reduced or NUE can be improved by either using slow releasing fertilizers or through fertigation process (through reduction in fertilizer amount used and leaching). When the produced essential oil from the two seasons were compared at $100 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ of N, high returns were obtained during the spring harvest (5.9% and 13.3% more efficient for conventional and organic, respectively) than the summer harvest (Fig. 5.7). This shows that the economic return

was influenced by the harvesting period or season. Generally, this investigation indicates that application of more N than what is required for optimum plant growth has no positive effect on essential oil production. In addition, the net benefits from N application are dependent on the growing period and in the present study spring and summer were more productive than winter and autumn. Application of organic N also resulted in higher N use efficiency than conventional N.

Essential oil content and composition as influenced by season of production

Essential oil content (%)

Although no statistical analysis was performed (samples per season per treatment were pooled for essential oil extraction), oil content (% fresh mass basis) was apparently not affected by either N level or N source (Fig. 5.8). A potted trial on geranium indicated that application of another macronutrient (magnesium) increased herbage yield without any effect on oil content of the plant (Weiss, 1997). In the present study, the only variation noticed was among the harvesting periods (seasons) (Fig. 5.8). Oil content was highest in autumn (March – May 2007) followed by summer (December 2006 – February 2007) and winter (June – August 2006) growing seasons. Spring (September – November 2006) harvest recorded the lowest essential oil content as compared to the other harvests (Fig. 5.8). This could be related to environmental variation that occurred among the harvesting periods. Motsa *et al.* (2006) recorded an essential oil content of 0.096% for autumn harvest followed by 0.083% and 0.058% for summer and winter harvests, respectively for the same cultivar. In the present study, essential oil content of the plant ranged between 0.04 to 0.08, 0.05 to 0.07, 0.06 to 0.09 and 0.09 to 0.13 for the winter, spring, summer and autumn harvests, respectively (Fig. 5.8). Generally, the essential oil contents (% fresh mass basis) achieved in the present study fell within the range (0.04 to 0.2%) of values reported for this crop by different authors (Weiss, 1997; Sabina Aiyanna *et al.*, 1998; Motsa *et al.*, 2006; Eiasu *et al.*, 2009). According to Weiss (1997), economic sustainability or profitability of the crop is more dependent on fresh herbage yield per hectare and oil quality than essential oil content. Different authors (Kumar *et al.*, 2001; Eiasu *et al.*, 2009) also suggested that under most conditions high essential oil content cannot compensate for low biomass production per hectare. Findings of the

present study concur with the above statement, i.e., higher essential oil content (Fig. 5.8) with lower fresh herbage yield ($\text{t}\cdot\text{ha}^{-1}$) in autumn did not result in higher essential oil yield ($\text{kg}\cdot\text{ha}^{-1}$) (Fig. 5.2 and 5.3). On the other hand, spring and summer harvests gave lower essential oil content but higher fresh herbage yield ($\text{t}\cdot\text{ha}^{-1}$), which resulted in higher essential oil yield ($\text{kg}\cdot\text{ha}^{-1}$) (Fig. 5.2 and 5.3).

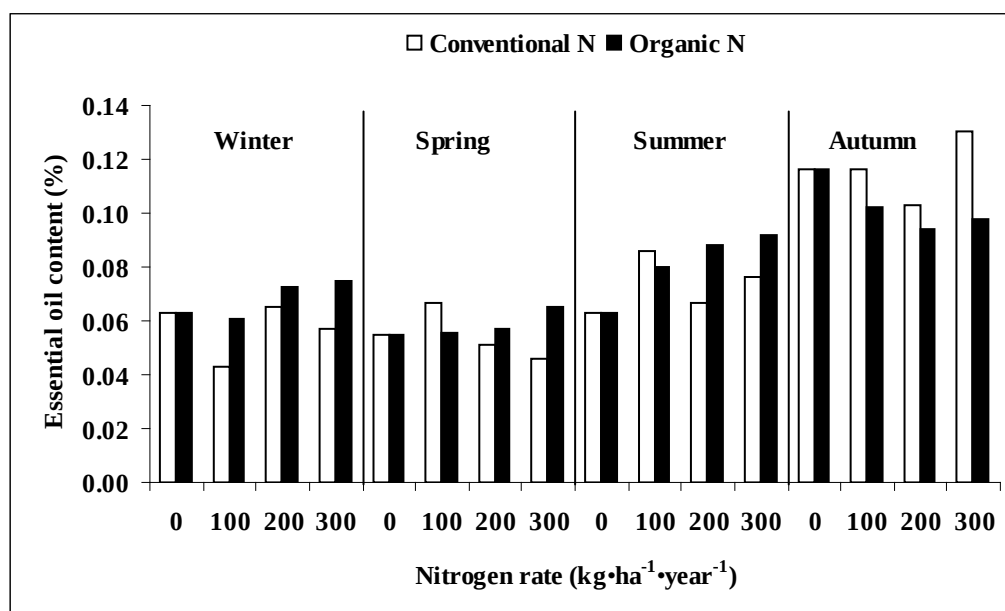


Figure 5.8: Rose-scented geranium essential oil content (% fresh mass) as influenced by nitrogen rate (0 to $300 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$) and season of production (winter, spring, summer and autumn) in 2006/2007. Individual values presented per treatment per growing season are means ($n = 8$; pooled sample of eight plants was used for essential oil distillation)

Essential oil composition

Rose-scented geranium essential oil is characterized by the concentration of the main constituents. These are citronellol, geraniol, iso-menthone, citronellyl formate, geraniol formate (Swamy *et al.*, 1960; Weiss, 1997; Miller, 2002; Peterson *et al.*, 2006) and guaia-6,9-diene (Rajeswara Rao, 2000) and of these the most important ones are citronellol, geraniol and guaia-6,9-diene. Oil composition data from this

study is shown in Figure 5.9. The composition values presented are for pooled samples (over all N treatments) per season, and thus they were not statistically analysed. Essential oil composition was in general within the range of internationally accepted rose-scented geranium oil (Lis-Balchin, 1995). Nitrogen level and source were found not to affect essential oil composition of the plant. The reason for lack of consistency in the essential oil composition, regardless of the harvesting season (Fig. 5.9), was most probably due to seasonal effects, as was reported by Motsa *et al.* (2006). Scheffer (1993) also indicated that oil composition of this species primarily depends on harvesting season.

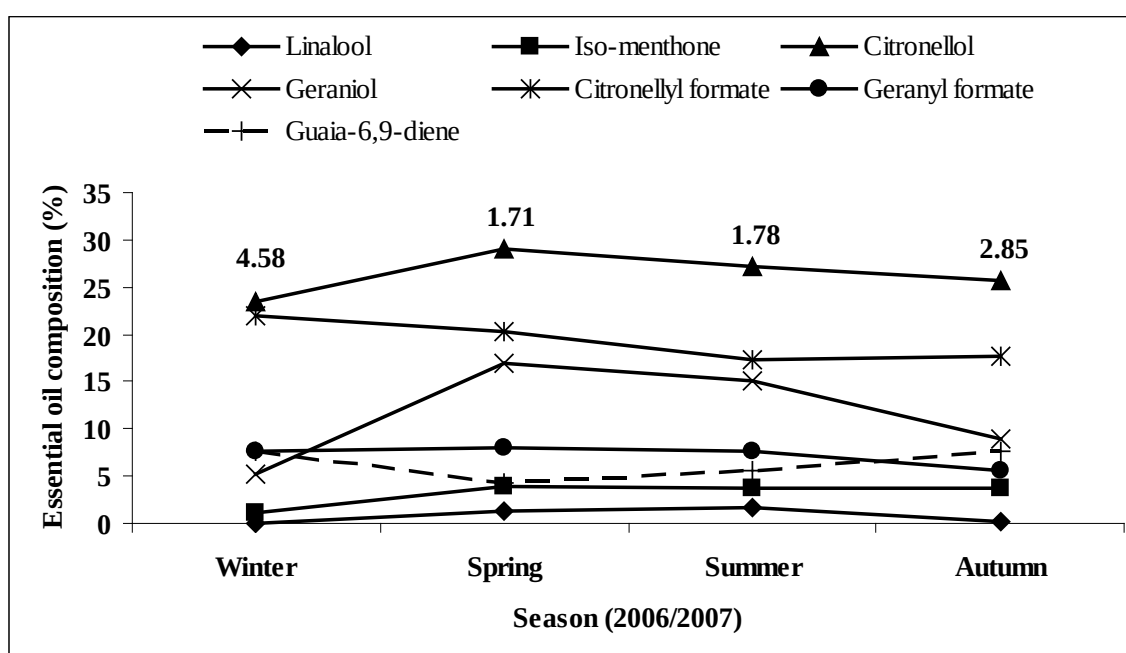


Figure 5.9: Rose-scented geranium essential oil composition as influenced by season of production (winter, spring, summer and autumn) in 2006/2007. Values above the lines represent citronellol: geraniol (C: G) ratio per harvesting season

Although the applied treatments (N level and source) had no noticeable effect on the essential oil composition of the plant, a major effect of harvesting period or season was noticed (Figure 5.9). Geraniol concentration (%), the compound responsible for the rosy like odour of the geranium oil (Weiss, 1997; Peterson *et al.*, 2006), was at its

peak in spring season, followed by summer and autumn (Fig. 5.9). These findings concurred with Doimo *et al.* (1999b), who reported an increase in geraniol concentration in spring and summer seasons and lower concentration of the compound in winter season. Motsa *et al.* (2006) also reported low geraniol concentration in a winter harvest. On the contrary, Prakasa Rao & Ganesha Rao (1995) as well as Rajeswara Rao (2002) reported high geraniol concentration in cooler weather conditions.

Citronellol concentration (%) trends in the present study (Fig. 5.9) partially agrees with the findings of Rajeswara Rao *et al.* (1996) who indicated that concentration of the component increased in summer as an adjustment to thermal stress but disagrees with findings of Motsa *et al.* (2006), who reported that citronellol concentration increased in the winter season. In this experiment, citronellol concentration followed a similar pattern as geraniol, with highest concentration in spring followed by summer and autumn and lowest concentration in winter (Fig. 5.9).

Citronellol and geraniol are among the most important components of rose-scented geranium essential oil and the essential oil quality is characterized by their ratio (C:G ratio) (Doimo *et al.*, 1999b; Motsa *et al.*, 2006). The desirable C:G ratio is 1.0 to 3.0 (Bauer *et al.*, 1990; Anistescu *et al.*, 1997). In this investigation, the C:G ratio (Figure 5.9) was more influenced by harvesting season differences than the applied treatments (N level and source). The C:G ratio was lowest, and therefore, most desirable (Doimo *et al.*, 1999b) in spring (1.71) and summer (1.78) season harvests, followed by autumn harvest (2.85) while it was highest (least desirable) in the winter harvest (4.58) (Fig. 5.9). This is in agreement with the findings of Doimo *et al.*, (1999b) and Motsa *et al.* (2006) who reported that C:G ratios were highest in winter and late winter/ spring harvests.

The conditions in winter and autumn seasons favoured guaia-6,9-diene concentration (Fig. 5.9), while in spring and summer, concentration of the component was comparatively within the internationally accepted range (1 to 7%) for geranium oil (Lis-Balchin, 1995). Iso-menthone concentration, however, was lowest in winter (1.04%) and high (3.67 to 3.87%) in spring, summer and autumn harvesting seasons.

Similarly, in summer a high concentration of the component was reported by Motsa *et al.* (2006).

Plants from winter harvesting season produced an essential oil without linalool (0%) and lower concentration of the component in autumn (0.26%) followed by spring (1.29%) and summer (1.70%) harvesting seasons (Fig. 5.9). Fernando & Roberts (1984) also described that linalool composition of black tea varied due to seasonal changes. Geranyl formate, another important component in geranium oil, also varied due to season and a substantial amount of the component was noticed in winter, spring and summer harvesting seasons (7.57 to 8.00%) but in autumn a slight drop (5.53%) of the component was noticed (Fig. 5.9). Harvesting season also influenced citronellyl formate concentration (Fig. 5.9), which followed a decreasing gradient from winter to summer and remained constant from summer to autumn (Fig. 5.9).

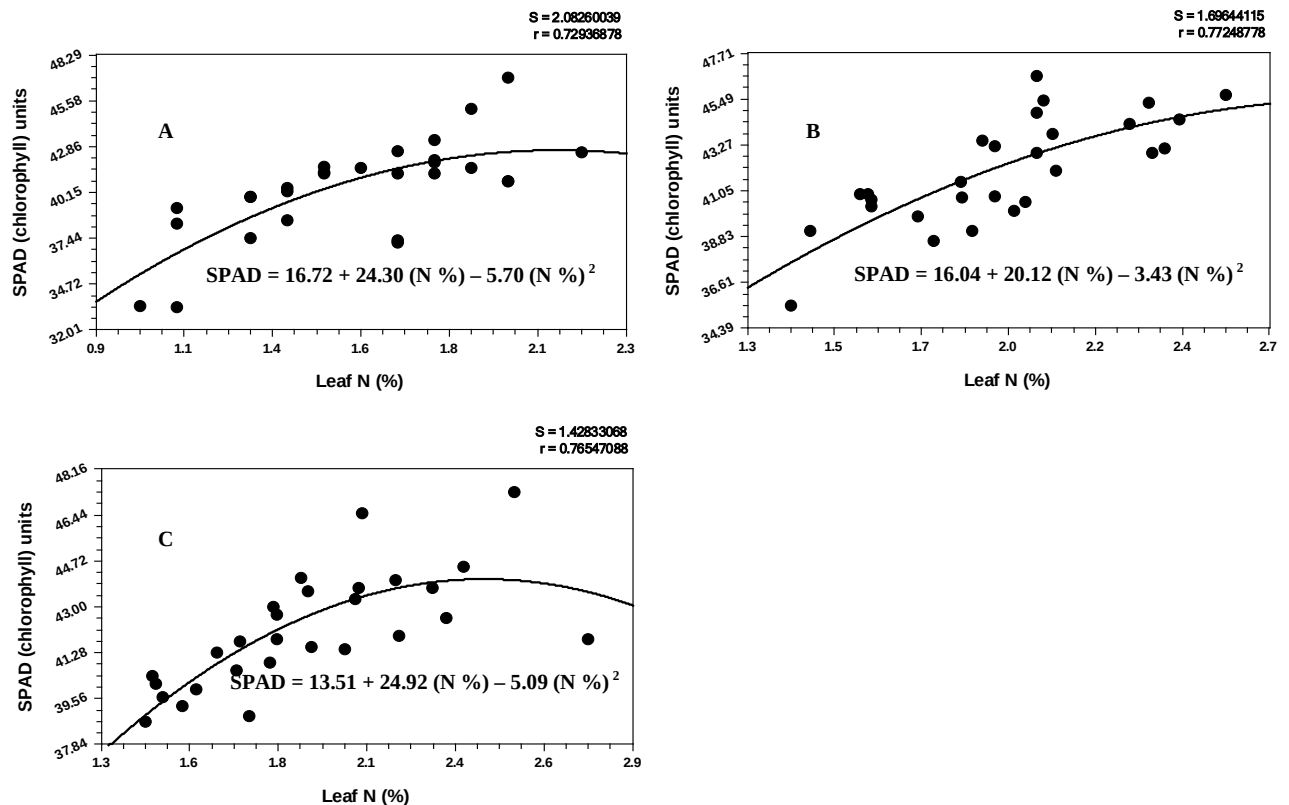
Generally, essential oil composition of the plant was influenced more by the effect of season or environmental conditions that occurred per growing period than the applied treatments (N level or source). In addition, the essential oil compositions recorded for the present study were also within the internationally accepted range of geranium oil, as stated by Lis-Balchin (1995).

Estimation of foliar nitrogen status using the SPAD-502 chlorophyll meter

The SPAD-502 chlorophyll meter (Minolta Camera Co. Ltd., Osaka, Japan) is an instrument (handheld meter) used to give a quick and non destructive estimation of leaf greenness in plants (Kariya *et al.*, 1982; Yadava, 1986; Minolta Camera Co. Ltd., 1989). Leaf greenness is directly related to relative chlorophyll content and positively related to foliar N concentration of plants (Chapman & Barreto, 1997; Schröder *et al.*, 2000; Sexton & Carrol, 2002). This instrument measures the SPAD values (Soil Plant Analysis Development; units developed by Minolta Camera Co. Ltd.) which are directly related to chlorophyll content (Wood, Reeves & Himelrick, 1993; Markwell, Osterman & Mitchell, 1995). This is achieved by transmitting red light at 650 nm (a near peak wavelength where chlorophyll absorbs light) and infrared light at 940 nm (a near peak where almost no absorption occurs). These wavelengths are frequently transmitted by the instrument through the leaf within the chamber (2 mm x 3 mm,

where a leaf sample is being held) calculates the SPAD values ‘units’ from the optical density differential between the two wavelengths (Minolta Camera Co. Ltd., 1989).

Different researchers have used this instrument to estimate the foliar N content of different crops, such as corn (Schepers *et al.*, 1992; Wood *et al.*, 1992; Piekielek *et al.*, 1995; Bullock & Anderson, 1998), rice (Peng *et al.*, 1996; Hussain *et al.*, 2000), coffee (Netto *et al.*, 2005), apple and grapevine (Porro *et al.*, 2001), potato (Gianquinto, Sambo & Bona, 2003), cotton (Wood *et al.*, 1992), tomatoes (Sandoval-Villa *et al.*, 2002), pepper (Hartz *et al.*, 1993) and pumpkins (Swiader & Moore, 2002). In the present study leaf SPAD readings (units) were correlated with leaf N concentration of the plant (Figure 5.10) and as illustrated below, the relationship between the two parameters was quadratic in function for the three harvesting seasons (spring, $R^2 = 0.73$; summer, $R^2 = 0.77$; and autumn, $R^2 = 0.77$). SPAD values (units) and leaf N content (%) significantly ($P < 0.05$) varied per harvesting season (Fig. 5.11). Nitrogen uptake and SPAD reading were significantly lower in spring compared to summer and autumn harvesting seasons but the difference between summer and autumn in both parameters was not significant. Generally, in spring (Fig. 5.10a), maximum SPAD value was 42.1 at 2.1% of leaf N concentration; in summer (Fig. 5.10b), maximum SPAD value was 45.5 at 2.9% of leaf N followed by autumn (Fig. 5.10c) with maximum SPAD value of 44.0 at 2.4% of leaf N concentration. This showed that harvesting season has a great influence on SPAD value readings as well as on nitrogen uptake of the plant (Fig. 5.11). Lower N leaf content and SPAD values of spring season (Fig. 5.11) could be related to a carryover influence of the winter season where growth of the plant was limited due to cold weather conditions. Perennial plants are also believed to enter into a period of winter dormancy; that is, a slow to a stop growth cycle as the temperature drops (Perry, 1971). As stated earlier winter season was found to be unfavourable for optimum growth of rose-scented geranium (Appendix C) and most perennial plants also do not start growth until the weather conditions are conducive for optimum growth (such as warm temperature and sufficient to sunlight) (Perry, 1971). In the present study rose-scented geranium growth was slow in the beginning of spring season and then started picking up as temperature warmed up, which might have affected the N uptake of the plant (Fig. 5.10a).



s^2 = standard error

r = correlation coefficient

Figure 5.10: Relationship between leaf N content (%) and leaf chlorophyll meter SPAD values of rose-scented geranium in (A) spring, (B) summer and (C) autumn season of production in 2006/2007. Individual values presented per growing season are means ($n = 120$)

In autumn, when the daylength gets shorter and temperatures start dropping, perennial plants then identify the changes and respond to it by storing more nutrients in their structure, in order to sustain growth during the winter dormancy period (Perry, 1971). Findings of the present study cohere with the above statement, i.e. leaf N concentration (%) as well as SPAD values in autumn were as high as in the summer season, when growth of the plant is known to be optimum and concurrently high N concentration was expected (Fig. 5.11). In a study by Woldetsadik *et al.* (2002), an increase in leaf N content (%) was also reported at peak growth of shallots (*Allium cepa* var. *ascalonicum* Backer). According to Weiss (1997), when the nutrient content of harvested geranium plants was analyzed, 0.98% of nitrogen was found in the leaves

and 1.13% and 0.80% phosphate and potassium content in the petioles, respectively. The leaf N content (%) of the present study was generally higher than what was reported by Weiss (1997), which might be related to variation in growing conditions of the plant. Based on the plant species, leaf N concentration of plants was generally reported to vary between 2-5% under optimum conditions (Parvizi *et al.*, 2004). Generally, SPAD-502 chlorophyll meter readings (SPAD units) matched well with that of leaf N concentration data (%) of rose-scented geranium and based on this the instrument can be used as an indication of leaf N status of the plant.

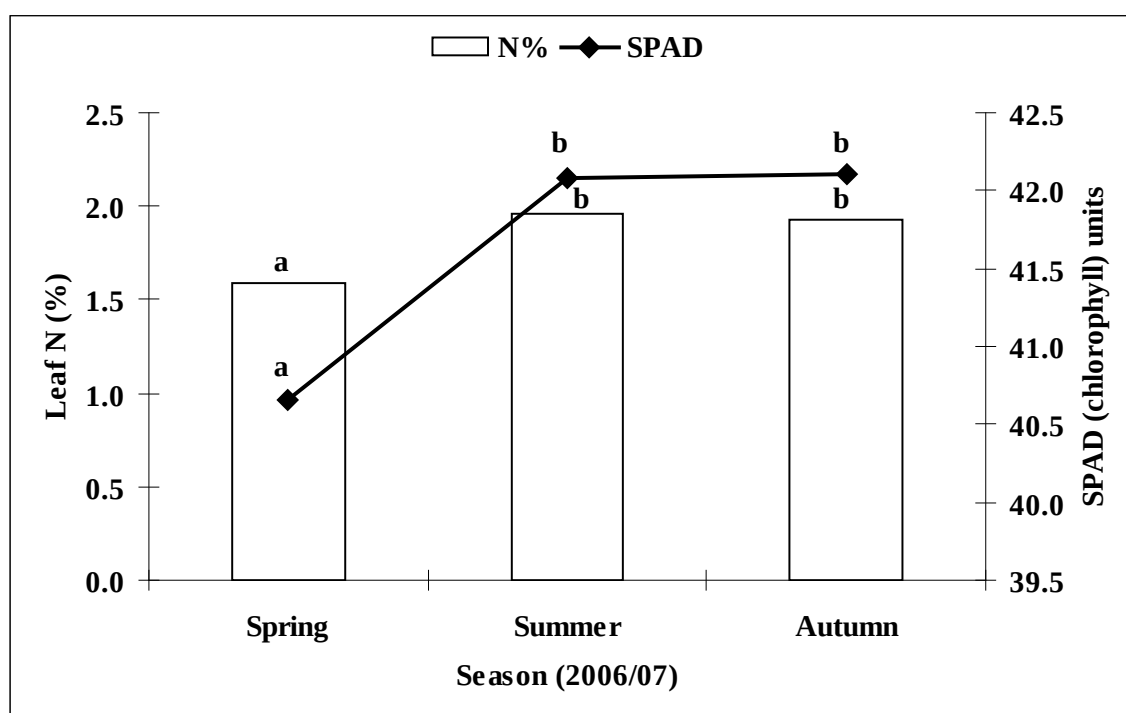


Figure 5.11: Leaf N content (%) and leaf chlorophyll meter SPAD values of rose-scented geranium in spring, summer and autumn harvesting seasons in 2006/2007. Individual values per growing season are means over all N levels and sources ($n = 28$). Statistical comparison is among values in the same graph type

The relationship between chlorophyll meter readings (SPAD units) and leaf N concentration is reported to be linear by different researches such as in rice (Takebe & Yoneyama, 1989), apple (Campbell *et al.*, 1990), maize (Schaper & Chacko, 1991),

winter wheat (Barraclough & Kyte, 2001), sugar maple (Cate & Perkins, 2003), coffee (Netto *et al.*, 2005) and in four hardwood plant species (sycamore, sweetgum, green ash and swamp cottonwood) that was reported by Chang & Robison (2003). The linear relationship between N (on dry mass basis) and SPAD readings varies and the relationship might be affected by factors such as the plant species used during calibration (Wood *et al.*, 1993), plant growth stages and cultivar (Takebe & Yoneyama, 1989), environmental conditions as well as stress factors (Campbell *et al.*, 1990; Wood *et al.*, 1993; Smeal & Zhang, 1994), and morphological condition of the leaf such as leaf thickness (Peng *et al.*, 1993). In addition, different studies also parameterized the relationship between SPAD units and leaf chlorophyll content (directly related to leaf N concentration) as linear (Marquard & Tipton, 1987; Dwyer *et al.*, 1991; Fanizza *et al.*, 1991; Schaper & Chacko, 1991; Gratani, 1992; Yamamoto *et al.*, 2002; Esposti *et al.*, 2003; Wang *et al.*, 2004), which cohered with Beer's Law (Eisenberg & Crothers, 1979) where pigment concentration has a direct relationship with radiation absorbance. However, other studies also reported a curvilinear relationship between the two parameters (Monje & Bugbee, 1992; Markwell *et al.*, 1995; Castelli *et al.*, 1996; Richardson *et al.*, 2002) and their interpretation for this relationship was due to a non-uniform distribution of the chlorophyll as well as radiation reflection (at 650 and 940 nm) on the leaf surface.

In the present study the relationship was a quadratic function (Fig. 5.10) which might suggest that the instrument (SPAD-502 chlorophyll meter) had underestimated leaf N (%) of the plant at higher concentrations. Similarly, a curvilinear relationship between SPAD values and foliar N concentration in *Eucalyptus nitens* and *E. globulus* (Pinkard, Patel & Mohammed, 2006) was also reported. The same authors further suggested that collecting samples with extreme values during calibration curve development are important. In addition, the instrument is also reported to have limitations, such as a higher foliar N concentration or luxurious N consumption is not recognized (Schepers *et al.*, 1992; Blackmer & Schepers, 1995). Wood *et al.* (1993) also suggested that at higher N levels, the relationship between leaf chlorophyll content and foliar N concentration might not be linear, since there is a possibility of the foliar N to be stored in the form of $\text{NO}_3\text{-N}$, which is a non-chlorophyll N form.

Foliar N concentration (%) of rose-scented geranium was significantly ($P < 0.05$) influenced by source of N (Fig. 5. 12). Conventional N at all levels and organic N at $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ had no significant effect on foliar N concentration of the plant as compared to the unfertilized treatment (control; zero $\text{kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$). However, organic N at 200 and $300 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ significantly increased foliar N concentration as compared to all levels of conventional N and the control. In addition, the SPAD meter readings followed a similar trend as the above findings, when computed with levels of N from both sources and treatment, where organic N at 200 and $300 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ were found to have higher SPAD values. This shows that, readings of the SPAD chlorophyll meter can be influenced by the source of N used and the instrument can also be used as complementary tool to determine the leaf N concentration of rose-scented geranium, in addition to other techniques (such as soil and plant analysis methods). According to Loh *et al.* (2002), SPAD chlorophyll meter readings were reported to correlate well with leaf N contents of non-horticultural crops but with horticultural crops great variability of the relationship was reported, which was not the case in the preset study. Findings of the present study also partially agrees with Westcott & Wraith (2003) who reported positive relationship between leaf SPAD chlorophyll meter readings and stem nitrate concentration of peppermint (*Mentha piperita* L.).

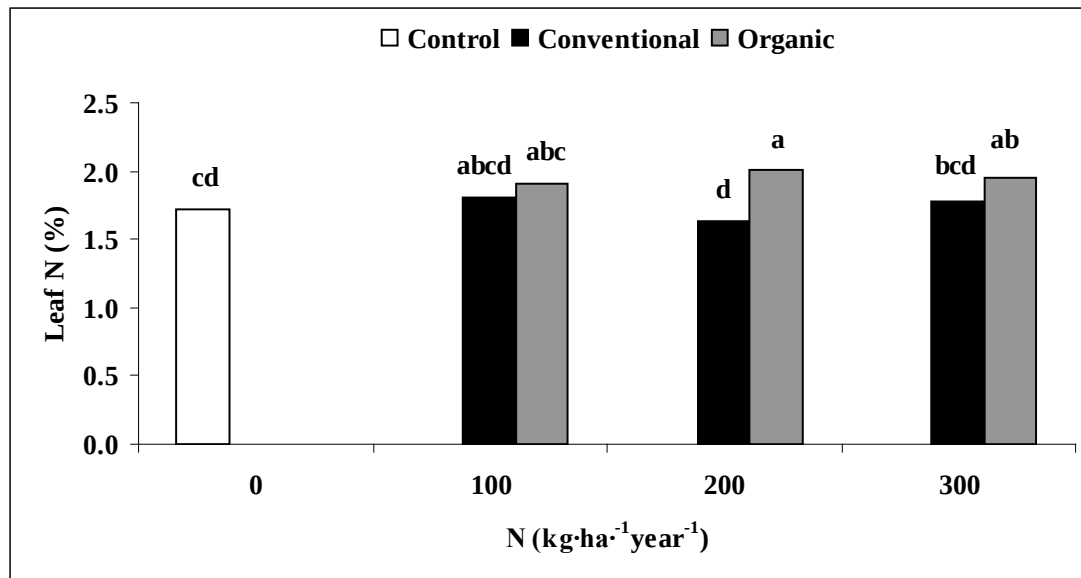
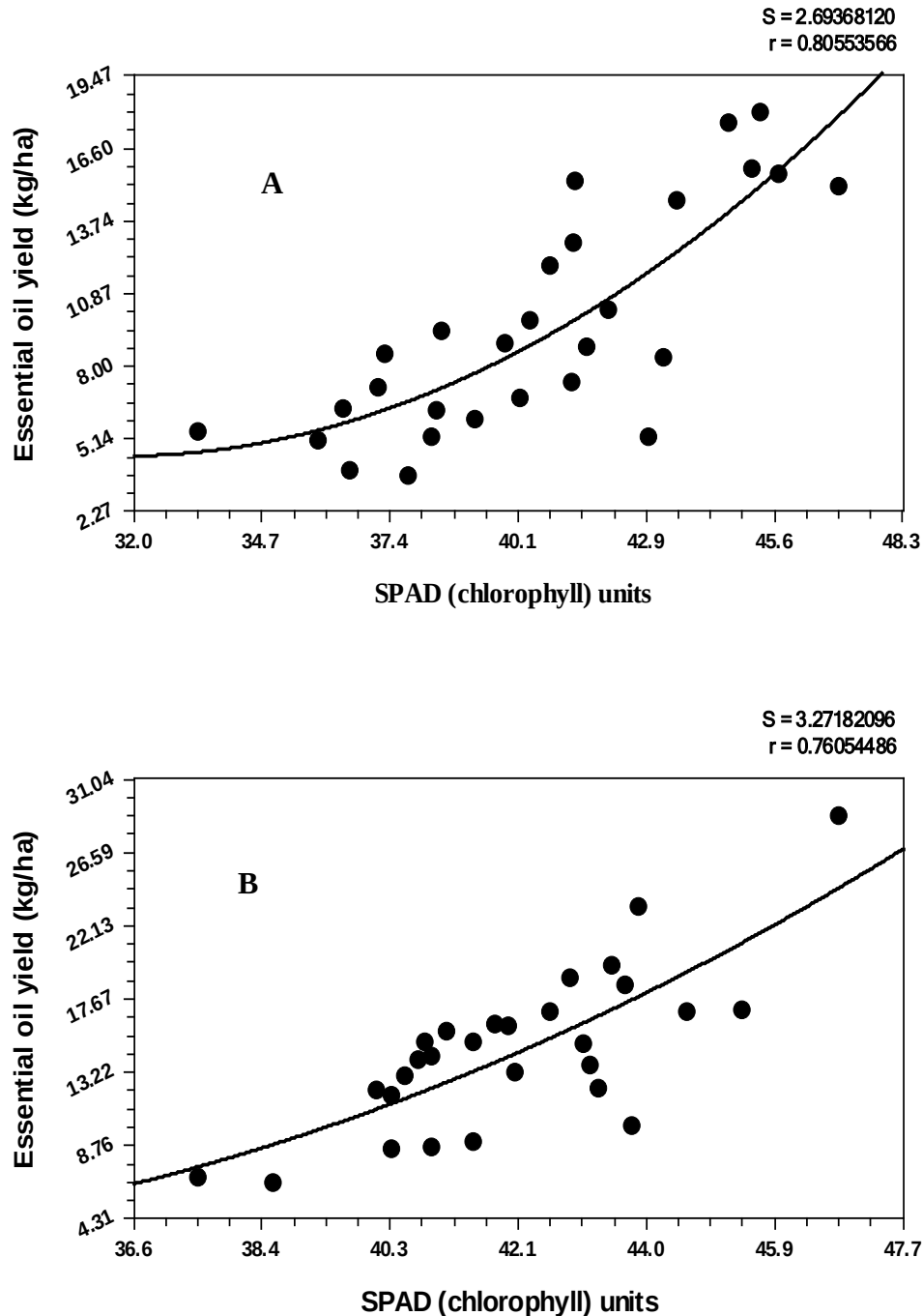


Figure 5.12: Leaf N content (%) of rose-scented geranium as influenced by nitrogen rate (0 to 300 kg·ha⁻¹·year⁻¹) and source (control, conventional or organic) during 2006/2007. Individual values presented are means ($n = 32$)

Figure 5.13 indicates the quadratic relationship between SPAD chlorophyll meter readings (units) and essential oil yield (kg·ha⁻¹·year⁻¹) in spring (Fig. 5.13a; $r^2=0.81$) and summer (Fig. 5.13b; $r^2=0.76$) harvesting seasons (Fig. 5.13). Rostami *et al.* (2008) and Blackmer & Schepers (1995) also reported a strong positive relationship between SPAD readings and grain yield (t·ha⁻¹) in corn or maize. Findings of the present study (Fig. 5.13) illustrates that SPAD chlorophyll meter can be a strong indicator of essential oil yield reduction (kg·ha⁻¹) in rose-scented geranium due to an induced N deficiency, especially during spring and summer growing seasons (Fig. 5.13). However, in autumn there was no relationship ($r^2 = 0.38$, Appendix C) between SPAD readings and essential oil yield. Similarly, N level and source did not influence essential oil yield (Fig. 5.3) in this season, which may also have contributed to the poor relationship. Rostami *et al.* (2008) also reported a strong quadratic relationship ($r^2 = 0.92$) between SPAD readings and N levels in corn or maize.



S^2 = standard error

r = correlation coefficient

Figure 5.13: Relationship between leaf chlorophyll meter SPAD values (units) and essential oil yield ($\text{kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$) of rose-scented geranium in (A) spring and (B) summer season of production in 2006/2007. Individual values presented are means ($n = 16$)

As stated earlier, SPAD chlorophyll meter readings are influenced by factors other than N status of the leaf and this includes factors such as, differences in leaf thickness, cultivar, hybrid, growth stage and so on (Shapiro *et al.*, 2006). In order to normalize this variation, different researchers developed a technique called SPAD index or sufficiency index (SI) (Blackmer & Schepers, 1995). SPAD index or sufficiency index is calculated by dividing bulk SPAD readings by maximum SPAD readings from the reference strips (well fertilized) which are then multiplied by 100 (Peterson *et al.*, 1993; Shapiro *et al.*, 2006). The values range between 50 and 100% (Peterson *et al.*, 1993; Shapiro *et al.*, 2006). Based on this, a sufficiency index was developed for the present study and values were correlated to levels of nitrogen (Fig. 5.14), in order to determine the need for N application. This was done only for spring, summer and autumn harvesting seasons (values computed together), since in winter the relationship between SPAD readings and N content was not significant. Practically, previous studies on N requirement of rose-scented geranium in this region indicated that $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ of N was sufficient for optimal production of rose-scented geranium in the region, either in organic or conventional form (Araya *et al.*, 2006). Therefore, this level was used as an optimum N level required for optimum essential oil production.

Over all sufficiency index values (SI) from the three seasons (spring, summer and autumn) per source of N (conventional or organic) were computed against applied N levels using a quadratic model (Fig. 5.14). Values from these seasons were combined together in order to develop a representative quadratic model which could be used per source of N. As illustrated below, conventional N at $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ (N level for optimum essential oil production) corresponded with an average chlorophyll meter based SI value of 85% and essential oil yield of $10 \text{ kg}\cdot\text{ha}^{-1}$. The mean conventional N level (spring, summer and autumn) required for maximum essential oil production $203 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$, which correspond to an average chlorophyll meter based SI value of 87% and essential oil yield of $12 \text{ kg}\cdot\text{ha}^{-1}$. Essential oil yield produced in the three seasons at $100 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ ranged between 9 and $12 \text{ kg}\cdot\text{ha}^{-1}$ and at $200 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ between 9 and $14 \text{ kg}\cdot\text{ha}^{-1}$. This shows that the recommended N levels based on the overall developed quadratic equation (Fig. 5.14a) is representative for the three individual seasons. Similar suggestions were also made by Varel *et al.* (2007).

Similarly for organic N (Fig. 5.14b), $100 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ (N level for optimum essential oil production) corresponded with an average chlorophyll meter based SI value of 86% and an essential oil yield of $12 \text{ kg} \cdot \text{ha}^{-1}$. The mean organic N level (spring, summer and autumn) required for maximum essential oil production was $395 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$, which correspond to an average chlorophyll meter based SI value of 94% and essential oil yield of $15 \text{ kg} \cdot \text{ha}^{-1}$. Essential oil yield produced in the three seasons at $100 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ organic N ranged between 11 and $14 \text{ kg} \cdot \text{ha}^{-1}$ and at $300 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ between 5 and $21 \text{ kg} \cdot \text{ha}^{-1}$, which shows that the recommended N levels based on the overall developed quadratic equation (Fig. 5.14a) falls within range.

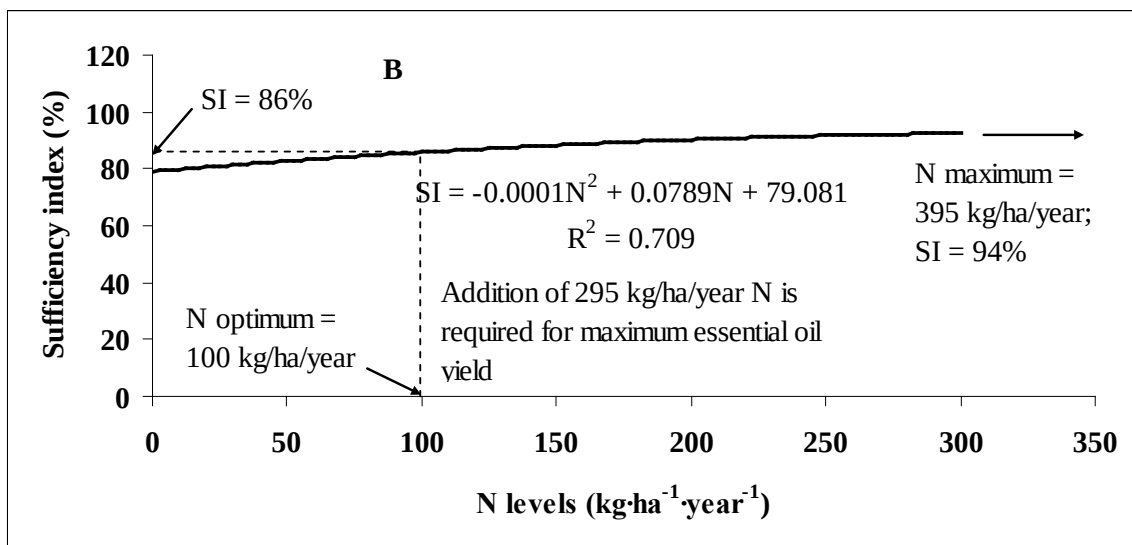
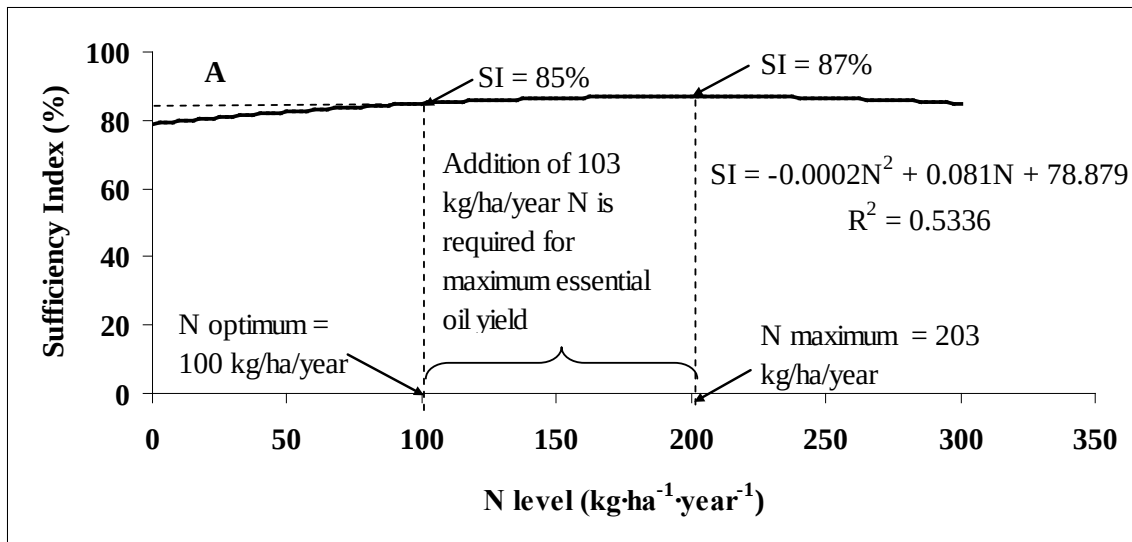


Figure 5.14: Quadratic response models from regression analysis of relative chlorophyll meter readings (SI) and N fertilizer rates at three harvesting seasons (spring summer and autumn 2006/2007) for conventional (A) and organic (B) N fertilizers

4.4 CONCLUSIONS AND RECOMMENDATIONS

Organic N at $300 \text{ kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ increased herbage and essential oil yield of rose-scented geranium in spring and summer but further increases in conventional N levels had no significant effect. N application either in winter or autumn did not improve production. In addition, this investigation indicates that application of more N than what is required for optimum growth had no positive effect on essential oil production, especially with conventional N. The net benefits from N application is dependent on the growing period and in the present study spring and summer applications were more beneficial than winter and autumn. Application of organic N also resulted in higher N use efficiency than conventional N.

Although no statistical analysis was performed, essential oil content (% fresh mass basis) of the plant was apparently not affected by either N level or N source. Essential oil content ranged between 0.04 to 0.08, 0.05 to 0.07, 0.06 to 0.09 and 0.09 to 0.13 for the winter, spring, summer and autumn harvests, respectively. The essential oil contents (% fresh mass basis) achieved in the present study generally fall within the range (0.04 to 0.2%) of values reported for this crop by different authors. Furthermore, Nitrogen level and source were found to have no noticeable effect on essential oil composition of the plant. However, essential oil composition was influenced more by the effect of season or environmental conditions that occurred per growing period. For instance, citronellol and geraniol concentration (%), were at peak levels in spring season, followed by summer and autumn and lowest concentration was found in winter. The ratio between these two components (C:G ratio) is also used as an indicator of rose-scented geranium essential oil quality and most desirable (low C:G ratio) essential oil was attained in spring, summer and autumn harvesting seasons and less desirable was recorded in winter.

The relationship between SPAD-502 chlorophyll meter readings (SPAD units) and leaf N content (% dry weight basis) was a quadratic function. SPAD-502 chlorophyll meter readings (SPAD units) matched well with leaf N concentration data of rose-scented geranium. Regardless of the factors that affect the readings, this instrument can be used as an indication of leaf N status of rose-scented geranium. Therefore, it

can be concluded that N rate, source and season of production should be considered to ensure optimal rose-scented geranium production. Both hypotheses set for this study could be accepted, as biomass and oil yield responded best to N top dressings in spring and summer, when yields were highest. The SPAD 502 chlorophyll meter gave good estimates of the leaf N status of rose-scented geranium.

CHAPTER SIX

HERBAGE YIELD, ESSENTIAL OIL YIELD AND OIL COMPOSITION OF ROSE-SCENTED GERANIUM AS INFLUENCED BY LIMING

6.1 INTRODUCTION

Rose-scented geranium (*Pelargonium capitatum* x *P. radens*, family Geraniaceae) produces one of the most expensive essential oils in the world. The essential oil from this crop is extracted by steam distillation and is for example used in aromatherapy, scenting of soaps and in perfumery in many parts of the world. Countries such as Réunion Island, China, Egypt, Morocco and India are known for their high production of rose-scented geranium essential oil (Lawrence, 1985; Prakasa Rao *et al.*, 1985; Scheffer *et al.*, 1993; Weiss, 1997). South Africa is now producing significant quantities of geranium oil. Studies as to how herbage yield, essential oil yield and oil composition respond to sources of N, plant shoot age and temperature and irrigation under South African conditions, were reported recently (Araya *et al.*, 2006; Motsa *et al.*, 2006; Eiasu *et al.*, 2008). How the plant responds to acidic soil conditions (and liming) has, however, not previously been studied. In addition, over fertilization (especially N) and inadequate liming are limiting production factors. For instance, in addition to the naturally existing acidic soils of South Africa (16 million ha) more than 5 million ha of high potential land have been acidified due to inappropriate agronomic practices (Laker, 2005).

Previous experience by this research group showed that seedlings often take long to establish, resulting in high death rates and sometimes poor growth after establishment. Stunted growth and yellowing of leaves was also observed in some cases. Poor vegetative growth causes low herbage yield and, consequently, low total essential oil production per hectare. Symptoms of poor growth were observed at the Hatfield Experimental Farm, where the soils are known to be acidic in nature.

Various authors have emphasized soil acidity (low soil pH) as a major constraint in agricultural crop production (Tisdale *et al.*, 1993; Brady & Weil 2002; Bolan *et al.*, 2003). Acidity of a soil can be caused by the acidic nature of the parent material (low in basic cations such as Ca^{2+} , Mg^{2+} , K^{+} and Na^{+}) or the release of H^{+} ions into the soil solution under extensive agricultural crop production when most base forming cations are taken up from the soil solution by the root system of the plant in exchange of H^{+} ions. Another cause could be the application of commercial N fertilisers. When N fertiliser is applied to the soil in either organic or inorganic form, it has to be converted into nitrate (NO_3^{-}) to be taken up by plants. During this process H^{+} ions are released into the soil solution, which consequently result in soil acidity (Tisdale *et al.*, 1993; Bolan *et al.*, 2003).

Acidic soil, with a soil pH lower than 5, is believed to result in root damage, with shallow and poor plant root systems. This leads to susceptibility to drought and poor nutrient uptake, and consequently stunted plant growth (Karlen *et al.*, 1977; Tisdale *et al.*, 1993), since soil pH is widely accepted as a dominant factor that regulates soil nutrient bio-availability (Robson, 1989). The common practice of shifting soil pH into a level which is favourable for optimum crop production is liming (Karlen *et al.*, 1977; Tisdale *et al.*, 1993). The main purpose of applying lime is to increase soil pH, soil Ca and Mg concentration, plant-available P, nutrient uptake by the plant, and to reduce the concentration of acidifying elements such as Al, Fe and Mn (Tisdale *et al.*, 1993).

In rose-scented geranium production, Weiss (1997) stated that the plant performs well when it is grown on slightly acidic soils (5.5 to 7 pH). Similarly, Demarne (2002) suggested application of lime for reconditioning the growth of rose geranium. However, no research data is available on the effect of soil pH and liming on rose-scented geranium grown under South African conditions. The study was therefore initiated with the hypothesis of soil amelioration is important when the crop is grown under acidic conditions. The objective of this study was, therefore, to evaluate the fresh herbage and essential oil yield response of rose-scented geranium grown on acidic soils to different liming rates and consequent changes in base saturation and

soil pH. The hypothesis for this study was that liming to ameliorate acidic soils will improve herbage and essential oil yield of rose-scented geranium.

6.2 MATERIALS AND METHODS

Experimental site, soil and leaf nutrient analysis

The experiment was conducted under field conditions at the University of Pretoria's Hatfield Experimental Farm, Pretoria, South Africa, located at 25° 45'S, 28° 16'E and at an altitude of 1372 m. The soil type is classified as a sandy clay loam Hutton (Soil Classification Working Group 1991). Before setting up the experiment, 10 soil samples were collected from depths of 0 – 20 cm. Samples were then analyzed for chemical and physical properties. The top 0 – 20 cm of the soil layer was used for the lime treatments. Soil nutrient analysis was done using the ammonium acetate extraction method (1 N NH₄OAc). Results of the physical and initial chemical analyses are presented in Table 6.1.

Table 6.1: Chemical and physical properties of soil from the experimental site before lime treatments

Depth (cm)	Clay (%)	pH (H ₂ O)	P ^z	K	Ca	Mg	Na	Cu	Fe	Mn	Zn
(mg·kg ⁻¹)											
0-20	19.5	4.8	80.1	67	156	24	6	4.98	67.2	88.2	4.44
20-40	20.5	4.5	37.9	60	217	27	11	4.23	77.7	142.5	2.04

^z Bray I: soil test solution (0.03 M NH₄F + 0.025 M HCl) to determine the available P in soils

At the time of harvesting, top mature leaves just below the growing tip on the stem were sampled (from 20 plants per treatment per replication) to estimate leaf nutrient accumulation and samples were dried in an oven at 65°C, ground to pass a 20-mesh sieve (2 mm), and digested with a ternary mixture of distilled deionized water,

concentrated nitric acid (HNO_3 , 65%), and perchloric acid (HClO_4 , 60%). Analysis for Al, Ca, Fe, and Mn was made by atomic absorption spectroscopy and P was determined calorimetrically, by using ammonium molybdate - ammonium vanadate reagents. An internal standard of 1% lanthanum oxide (La_2O_3) was used for the Al and Ca determinations.

Experimental design and treatment details

The experiment was established in December 2004 as a randomised complete block design with four treatments (including a control). Each treatment was replicated four times. An experimental plot was 10 m long and 5 m wide, with a 1 m spacing between rows, which gave a total area of 50 m² per plot (Fig. 6.1). From the five rows per treatment, the three centre rows were used for data collection, while the two rows on either side acted as border rows (Fig. 6.1).



Figure 6.1: Illustration of experimental layout on lime trial of rose-scented geranium (January 2005 to April 2006)

The plots were treated with fine-crushed ($\pm 95\% < 106 \mu\text{m}$) dolomitic lime ($\text{CaMg}(\text{CO}_3)_2$) at four different rates: 0, 2, 4 and $6 \text{ t}\cdot\text{ha}^{-1}$. The composition of the lime used was 30.4% Ca, 3.4% Mg, 9% SiO_2 , 0.8% Fe_2O_3 , with $< 10\%$ moisture and 91% CCE (calcium carbonate equivalent). Based on the treatment requirement of each plot, lime and a complete fertilizer blend (2:3:2 (22), containing 6.3% N, 9.4% P and 6.3% K) giving $63 \text{ kg}\cdot\text{ha}^{-1}$ N, $94 \text{ kg}\cdot\text{ha}^{-1}$ P and $63 \text{ kg}\cdot\text{ha}^{-1}$ K were incorporated into the soil to a depth of 20 cm using a rotovator (tractor driven). One month after treatment application (January 2005), healthy and rooted cuttings (45 days old) of rose-scented geranium (*Pelargonium capitatum* x *P. radens* raised in seedling trays from stem cuttings under a mist bed; obtained from a commercial nursery) were transplanted into the experimental plots. Plants were spaced 0.63 m within rows and 1 m between rows, to give a total of 16 000 plants per ha (suggested by the industry). Soil water content was measured weekly to a depth of 60 cm using a neutron probe (Neutron water meter, Model 503 DR, CPN Corporation, CA, USA). When the soil water content dropped below 60% of plant available water, the profile was refilled to field capacity using a drip irrigation system.

Levels of lime were selected based on the chemical analysis of the soil (Table 6.1) through the base saturation method (Eq. [6.1]) (Sparks *et al.*, 1996) and buffering capacity of the soil (van der Waals & Claassens, 2002). The cation exchange capacity (CEC) of the soil was determined on $\approx 2 \text{ g}$ of air-dried soil according to Sparks *et al.* (1996). The treatments were aimed at increasing the base saturation of the 0 – 20 cm soil layer to above 35% and raising the soil pH from 4.8 to 5.6, 6.5 and to 7.1, depending on the treatment. This required application of 2, 4 or $6 \text{ t}\cdot\text{ha}^{-1}$ dolomitic lime.

$$\% \text{ Base saturation} = \frac{100 \times \text{basic cations}}{\text{CEC}} \quad [6.1]$$

The crop was harvested four times at four-monthly intervals. The four growth periods were from January to April 2005 (summer/autumn), May to August 2005 (autumn/winter), September to December 2005 (spring/summer) and January to April

2006 (summer/autumn). At each harvest, plants were cut to a height of 20 to 25 cm above ground level and fresh herbage yield, oil yield and oil composition were determined. Oil content of the foliage (a sample of 10 kg from each treatment) was determined by steam distillation using a custom-made unit. The total essential oil yield per hectare was calculated per treatment according to the herbage yield, oil content and harvested area. Oil content was also correlated with the maximum and minimum temperature differences ($^{\circ}\text{C}$) during the growing periods. Temperature differences were calculated by computing the difference between mean daily maximum and minimum temperature, then computed to an average for each harvesting period.

The oil samples were analysed by gas chromatography (GC). Samples from the same treatments were pooled together to get a representative sample (Eiasu *et al.* 2008). For GC analysis, an Agilent GC (FID) model 6890N (Agilent Technologies Inc., Santa Clara, CA, USA) fitted with 30 m $\times$ 0.25 mm fused silica capillary column and film thickness of 0.25 μm was used, with helium as carrier gas. The temperature programming was 50 $^{\circ}\text{C}$ to 200 $^{\circ}\text{C}$, with ramp amount of 5 $^{\circ}\text{C}$ per min and with detector and injector temperatures of 220 $^{\circ}\text{C}$. The compounds were identified by comparing their retention times and retention indices (Adams, 2004).

After each harvest, soil samples were taken at a depth of 0 – 20 cm from each treatment to determine the soil pH and nutrient balance of the experiment. To obtain a representative sample, soil samples were taken from the three middle rows using a soil auger with 8 cm diameter. Six samples were taken from each row, with three beneath the drippers and the other three between the drippers, giving a total of 18 samples per treatment. These samples were combined and thoroughly mixed; thereafter a representative sub-sample (1 kg) was taken for analysis. Nutrients were added at a rate of 100:60:90 $\text{kg}\cdot\text{ha}^{-1}\cdot\text{year}^{-1}$ (N:P:K respectively, limestone ammonium nitrate (28%N), superphosphate (10.5% P) and potassium chloride (50% K)) as top dressing and worked into the soil after each harvest. Standard cultivation and plant protection practices were followed to control weeds, insects and diseases during the experimental period.

Statistical analysis

Statistical analysis and interpretations were based on comparison of treatment means. The collected data were subjected to analysis of variance (ANOVA) and analysis was carried out using the SAS package (Statistical Analysis System Institute Inc. 1999-2001). Regression analysis of the data was done using MS Excel (linear and polynomial regression) and curve fitting system for Windows (Hyams, 1997). To determine the relationship between fresh biomass production per plant and soil pH, the values were fitted into a Gompertz Relation using a curve fitting (Eq. [6.2]); where Y was fresh biomass per plant (kg); a, b and c were coefficients.

$$Y = a * e^{-e^{(b-cx)}} \quad [6.2]$$

6.3 RESULTS AND DISCUSSION

Records of daily maximum and minimum temperatures and total monthly rainfall from an automatic weather station, installed close to the experimental site, are presented in Appendix D.

Response of soil pH to liming

There was no response of soil pH to applied lime for any of the treatments at the time of the first harvest (Table 6.2). This could be due to slow reactivity of the liming material used. Generally, dolomite is believed to dissolve slower than other liming materials (Waliyar *et al.*, 2003). Liming increased the soil pH for all treatments at the time of the second, third and fourth harvests (Table 6.2). The greatest increase, from an initial pH of 4.8 to 6.8, was observed at the fourth harvest with an application of 6 t·ha⁻¹ lime (Tables 6.1 and 6.2). However, the yield that was obtained at 6 t·ha⁻¹ was not significantly different from that at 4 t·ha⁻¹.

Table 6.2: Soil pH (0 – 20 cm depth) at harvest for the four harvesting periods, i.e. first harvest (summer/autumn 2005), second harvest (autumn/winter 2005), third harvest (spring/summer 2005) and fourth harvest (summer/autumn 2006)

Lime ($\text{t}\cdot\text{ha}^{-1}$)	Soil pH (H_2O)			
	Harvest			
	1	2	3	4
0	5.0	5.1 ^a	4.9 ^a	5.0 ^a
2	4.7	5.5 ^a	5.5 ^b	5.5 ^b
4	4.8	6.0 ^b	6.2 ^c	6.3 ^c
6	5.0	6.2 ^b	6.7 ^c	6.8 ^c
LSD ($\alpha = 0.05$)	NS	0.4	0.5	0.4

Means in a column followed by the same letter are not significantly different

($P \geq 0.05$), using Tukey's comparison test. NS = Nonsignificant; $P \geq 0.05$

Response of herbage and essential oil yield to liming

In terms of herbage yield, rose-scented geranium did not respond significantly to 2 and 4 $\text{t}\cdot\text{ha}^{-1}$ of liming, compared to the control at the time of the first harvest (Table 6.3). However, it responded significantly to a higher rate (6 $\text{t}\cdot\text{ha}^{-1}$) compared to the control and 2 $\text{t}\cdot\text{ha}^{-1}$ of lime. The lack of plant response to the lower levels of lime could be due to the use of dolomitic lime, since dolomitic lime is believed to dissolve slower than other liming materials (Tisdale *et al.*, 1993; Waliyar *et al.*, 2003; Bovi *et al.*, 2004). It could also be due to an antagonistic effect between lime and applied fertilizer, since they were incorporated into the soil at the same time (Waliyar *et al.*, 2003; Bovi *et al.*, 2004). Essential oil yield was lowest at 2 $\text{t}\cdot\text{ha}^{-1}$ lime as compared to the control and the other liming treatments.

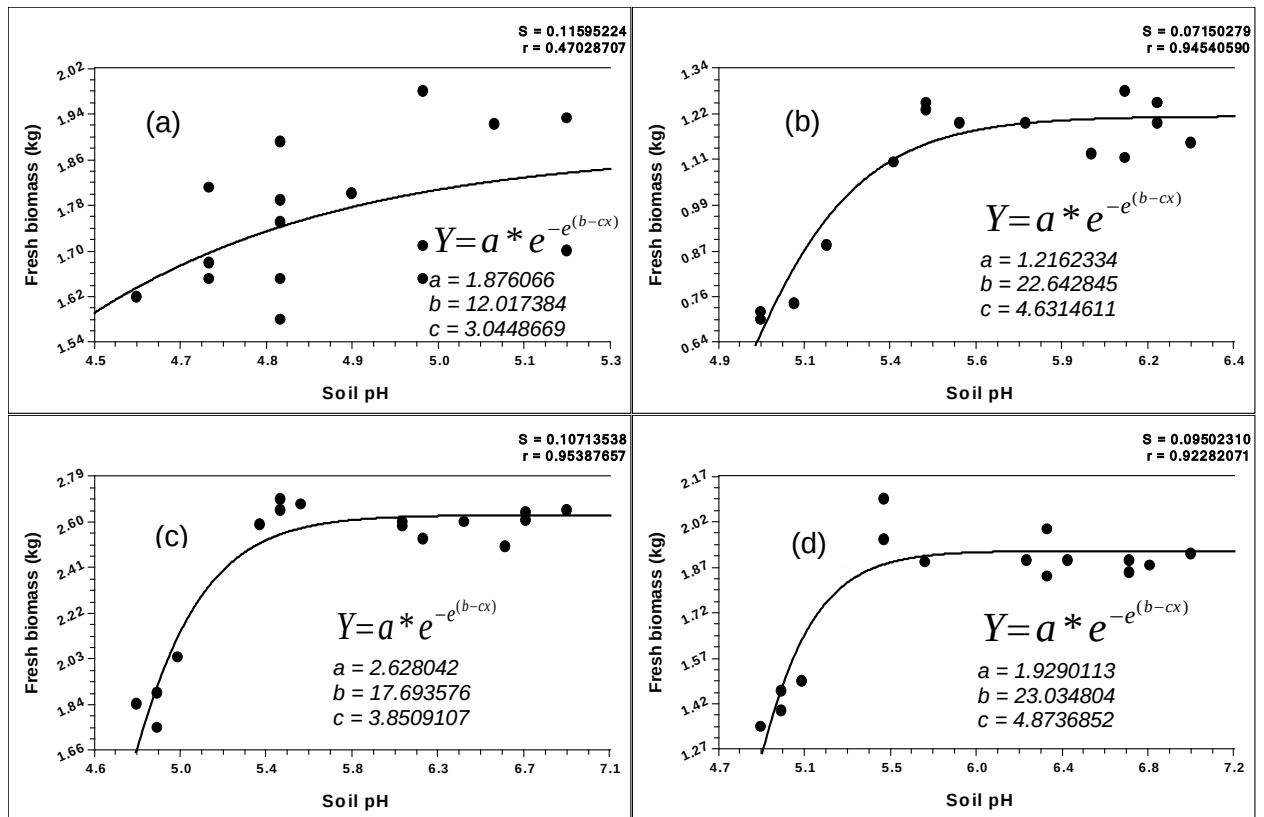
Table 6.3: Influence of liming on fresh herbage yield ($\text{t}\cdot\text{ha}^{-1}$), oil content (% fresh mass basis) and essential oil yield ($\text{kg}\cdot\text{ha}^{-1}$) of rose-scented geranium for the four harvesting periods, i.e. first harvest (summer/autumn 2005), second harvest (autumn/winter 2005), third harvest (spring/summer 2005) and fourth harvest (summer/autumn 2006)

Lime ($\text{t}\cdot\text{ha}^{-1}$)	Herbage yield ($\text{t}\cdot\text{ha}^{-1}$)					Essential oil content (%) ^z				Essential oil yield ($\text{kg}\cdot\text{ha}^{-1}$)				
	Harvest					Harvest				Harvest				
	1	2	3	4	Cumulative	1	2	3	4	1	2	3	4	Cumulative
0	26.3 ^a	10.8 ^a	30.3 ^a	22.5 ^a	89.9 ^a	0.14	0.06	0.08	0.16	36.8 ^a	6.5 ^a	23.3 ^a	36.0 ^a	102.6 ^a
2	26.4 ^a	19.8 ^b	43.1 ^b	31.5 ^b	120.8 ^b	0.11	0.06	0.08	0.16	29.0 ^b	11.8 ^b	34.5 ^b	50.4 ^b	125.7 ^b
4	28.6 ^{ab}	19.4 ^b	42.1 ^b	30.5 ^b	120.6 ^b	0.13	0.05	0.07	0.15	37.2 ^a	9.7 ^b	29.5 ^b	45.8 ^b	122.2 ^b
6	30.6 ^b	17.0 ^b	41.7 ^b	29.4 ^b	118.7 ^b	0.13	0.06	0.08	0.16	39.8 ^a	10.2 ^b	33.4 ^b	47.0 ^b	130.4 ^b
LSD ($\alpha = 0.05$)	3.5	5.0	7.8	4.2	9.9	-	-	-	-	4.4	2.7	6.0	6.4	9.4

Means in a column followed by the same letter are not significantly different ($P \geq 0.05$), using Tukey's comparison test. NS = Non-significant; $P \geq 0.05$.

^zOil content was not statistically analyzed as samples from the same treatments were pooled together to get a representative sample

Plants responded well to applied lime at the second, third, and fourth harvests (Table 6.3). Fresh herbage and essential oil yield increased significantly as a result of liming compared to the control ($0 \text{ t}\cdot\text{ha}^{-1}$). However, limed treatments did not differ significantly from each other in fresh herbage and essential oil yield at these harvesting periods. According to Weiss (1997), under acidic soil conditions a small amount of dolomitic limestone or ground magnesium limestone is required by rose-scented geranium since the plant favours slightly acidic soils (5.5 to 7.0 pH). Findings of this study concur with the above statement. A study done on other essential oil producing plants such as Sachalin mint (*Mentha sachalinensis* Briq.) and peppermint (*Mentha piperita* L.) reported increases in fresh herbage yield due to liming compared to the control (Abbas, 2005). In the present study, the positive response of the plant to applied lime could be related to a rise in pH of the acidic soil (Table 6.2), since there were positive correlations between soil pH and fresh biomass per plant during the last three growing periods (Fig. 6.2). This shows that the plant is sensitive to acidic soil conditions and has preference for a pH of 5.5 and higher to ensure maximum fresh herbage and essential oil production. This is in agreement with a report by Ram *et al.* (1997) where fresh herbage and essential oil yields of *Pelargonium graveolens* L. were higher in a calcareous sandy loam soil of pH 8.4 than in acidic soils of pH 4.9 to 5.1. The sensitivity of this plant to acidic soils could also be related to low availability of certain nutrients, such as P and Ca, which are responsible for healthier plant growth. Tisdale *et al.* (1993) reported that most of the mineral nutrients that are responsible for optimum plant growth are available in the pH range of 5.5 to 6.5. According to Van der Watt *et al.* (1991) acidic soils also have a negative impact on plant-root development of 'moderate soil acidity requiring' plant species.



s^2 = standard error

r = correlation coefficient

Figure 6.2: Fresh biomass yield per plant in response to soil pH for four harvesting periods, i.e. (a) first harvest (summer/autumn 2005) (b) second harvest (autumn/winter 2005), (c) third harvest (spring/summer 2005) and (d) fourth harvest (summer/autumn 2006)

Although no statistical analysis was performed, oil content (% fresh mass basis) of the plant was apparently not affected by application of lime or by an increase in soil pH (Table 6.3). A potted trial on geranium indicated that application of magnesium increased herbage yield without any effect on oil content of the plant (Weiss, 1997). Similarly in other essential oil producing plants (Sachalin mint and peppermint), oil content was not affected by liming or soil pH (Abbas, 2005). A study by Motsa *et al.* (2006) suggested that rose-scented geranium oil content might be more dependent on other factors such as weather conditions (Appendix D) (e.g. mean day and night temperature differences). In the present study, there was a negative linear relationship ($R^2 = 0.97$) between oil content of the plant and day/night temperature differences

(Fig. 6.3). Oil content was higher when the temperature differences between day and night were low (summer/autumn growing period) and lower with higher differences (autumn/winter growing period). This suggests that oil content of this plant was more dependent on air temperature fluctuations than on the applied lime treatments, similar to the results reported by Motsa *et al.* (2006). Generally, the essential oil contents (% fresh mass basis) achieved in the present study fell within the range (0.04 to 0.2%) of values reported for this crop by different authors (Weiss 1997; Sabina Aiyanna *et al.*, 1998; Motsa *et al.*, 2006; Eiasu *et al.*, 2009). Economic sustainability or profitability of the crop is more dependent on fresh herbage yield per hectare and oil quality than essential oil content (Weiss, 1997). Different authors (Kumar *et al.*, 2001; Eiasu *et al.*, 2009) have also suggested that, under most conditions, high essential oil content can not compensate for low biomass production per hectare.

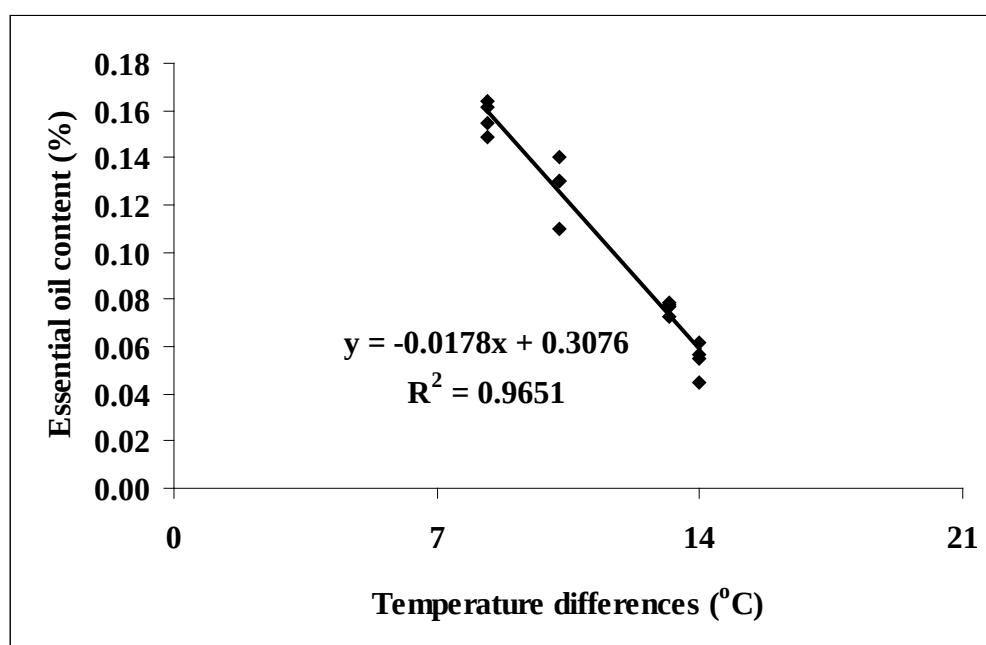


Figure 6.3: Oil content (% fresh mass basis) as influenced by mean day/night temperature differences (°C) throughout the growing period, i.e. first harvest (summer/autumn 2005), second harvest (autumn/winter 2005), third harvest (spring/summer 2005) and (d) fourth harvest (summer/autumn 2006)

The cumulative fresh herbage and essential oil yields of rose-scented geranium from the four harvests also increased with liming (Table 6.3). The most pronounced increase in fresh herbage and essential oil yields occurred when liming rate increased from 0 and 2 t·ha⁻¹. Fresh herbage yield increased by 34.4%, while essential oil yield increased by 22.5%. Increasing liming rate beyond 2 t·ha⁻¹ had no further significant contributions to increasing fresh herbage and essential oil yields.

At the end of the experiment the soil base saturation at a depth of 0 – 20 cm was 35, 55, 64 and 77% for the 0, 2, 4 and 6 t·ha⁻¹ lime treatments, respectively (Table 6.4). By increasing the base saturation (%) of the soil above that of the control, plant growth was improved, and this resulted in higher biomass and essential oil yields. Better plant growth could have been due to an increase in available nutrients from the soil, which are very important for optimum plant growth. Leaf plant tissue analysis indicated that the uptake of calcium (Ca) substantially increased after liming (Fig. 6.4a) as compared to the control but the differences among the liming rates were not significant. Leaf phosphorus (P) (Fig. 6.4b) and nitrogen (N) (Fig. 6.4f) uptake also significantly increased with liming while the concentration of aluminium (Al), iron (Fe) and manganese (Mn) (Fig. 6.4c, d and e, respectively) significantly reduced after liming. However, leaf potassium (K) (Fig. 6.4f) content was not significantly influenced with liming. According to the soil analysis results (Table 6.4), it appears that higher liming rates generally resulted in higher extractable levels of essential nutrients. Soils with higher base saturation are believed to be the best for optimum growth of plants and are less likely to require liming (Brady & Weil, 2002).

Table 6.4: Soil chemical analysis at the end of the experimental period for the 0 – 20 cm soil layer, i.e. first harvest (summer/autumn 2005), second harvest (autumn/winter 2005), third harvest (spring/summer 2005) and fourth harvest (summer/autumn 2006)

Lime (t·ha ⁻¹)	P ^z	Ca	K	Mg	Na	Cu	Fe	Mn	Zn	Soil base saturation (%)
mg·kg ⁻¹ of soil										
0	94.4	159.0	64.0	36.0	19.0	3.6	65.0	85.5	3.6	35
2	112.0	264.0	64.0	62.0	11.0	3.7	54.2	72.0	3.7	55
4	107.7	325.0	55.0	78.0	17.0	4.5	59.0	118.5	3.2	64
6	96.2	511.0	51.0	88.0	18.0	4.2	101.9	94.5	4.2	77

^z Bray I: soil test solution (0.03 M NH₄F + 0.025 M HCl) to determine the available P in soils

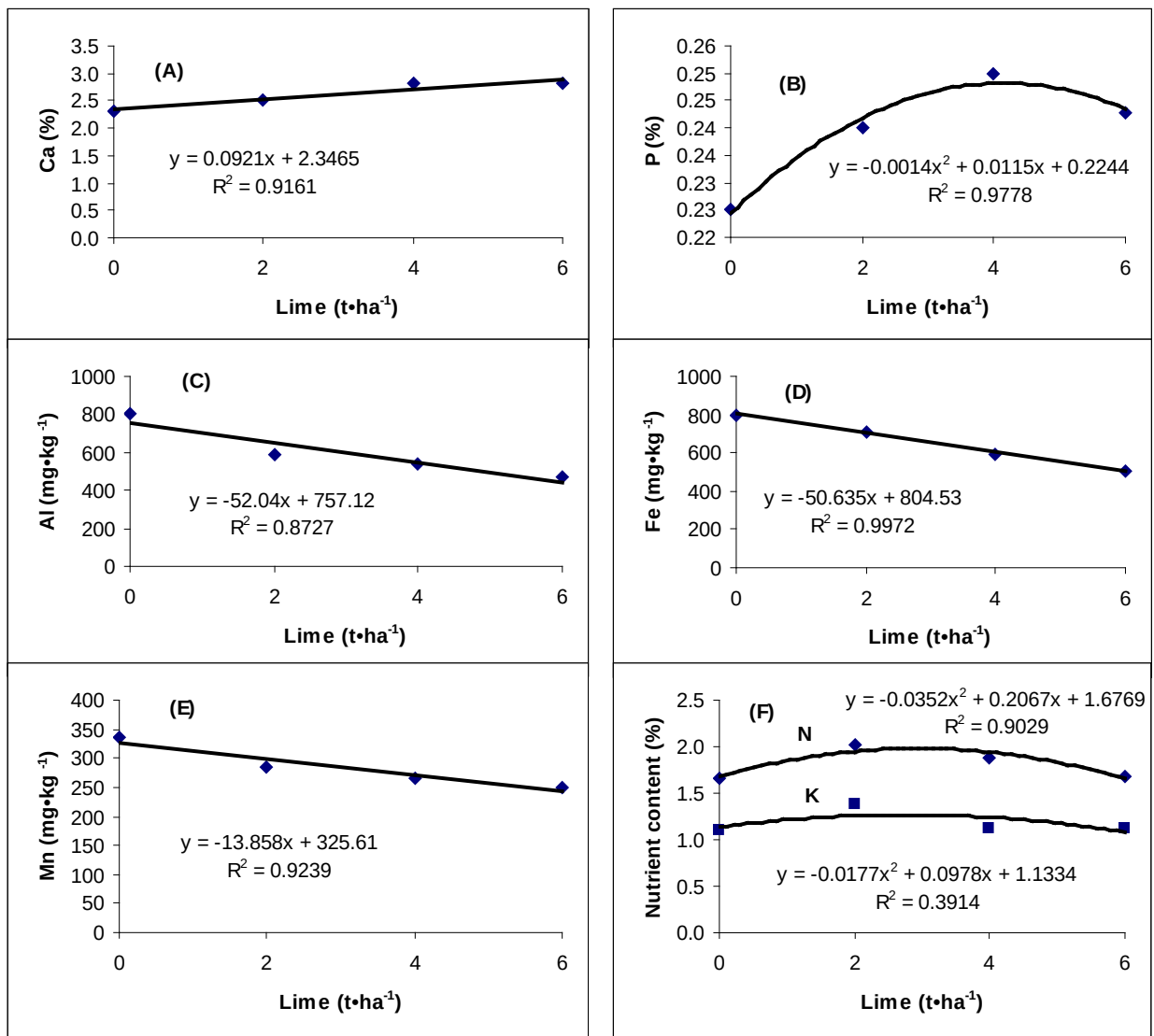


Figure 6.4: Effect of liming on rose-scented geranium leaf nutrient concentration during the growing period: (A) Calcium, (B) Phosphorus, (C) Aluminium (Al), (D) Iron (Fe), (E) Manganese (Mn) and (F) Nitrogen (N) and Potassium (K). Values presented are means ($n = 16$) compared using Tukey's test at $P \geq 0.05$

Response of essential oil composition to liming

The main constituents of rose-scented geranium essential oil are citronellol, geraniol, iso-menthone, citronellyl formate, geraniol formate (Swamy *et al.*, 1960; Weiss, 1997; Miller, 2002; Peterson *et al.*, 2006) and guaia-6,9-diene (Rajeswara Rao, 2000) and of these the most important ones are citronellol, geraniol and guaia-6,9-diene. Oil composition data from this study is shown in Table 6.5. The values presented are compositions of pooled samples per treatment in each harvest, and thus they were not statistically analysed. However, the characteristic of the essential oil was within the range of internationally accepted (Lis-Balchin, 1995) rose-scented geranium oil (Fig. 6.5). The reason for lack of consistency in the essential oil composition, regardless of the harvesting season, was most probably due to seasonal effects as reported by Motsa *et al.* (2006).

Table 6.5: Liming effects on oil composition of rose-scented geranium during the experimental period, for the first harvest (summer/autumn 2005), second harvest (autumn/winter 2005), third harvest (spring/summer 2005) and the fourth harvest (summer/autumn 2006)

Harvest	Lime (t/ha)	Essential oil components (%)							
		Linalool	Iso-menthone	Citronellol (C)	Geraniol (G)	Citronellyl formate	Geranyl formate	Guaia-6,9-diene	C:G ratio
1	0	0.82 ^z	4.69	29.21	13.51	15.92	7.11	6.62	2.16
	2	1.02	4.80	29.84	13.78	16.49	7.66	6.12	2.17
	4	1.30	4.90	29.19	15.76	15.96	8.22	6.12	1.85
	6	1.16	4.85	29.52	14.77	16.23	7.94	6.12	2.00
2	0	0.89	6.11	32.89	11.66	20.77	7.89	4.32	2.82
	2	0.77	6.10	32.35	10.83	21.84	8.06	4.78	2.99
	4	0.72	4.61	33.70	10.45	22.43	7.71	4.67	3.22
	6	0.82	4.15	31.10	11.22	20.95	8.21	4.93	2.77
3	0	0.73	1.64	31.62	11.95	18.78	6.76	6.46	2.65
	2	0.59	3.55	28.60	12.32	17.31	6.68	6.22	2.32
	4	0.62	3.12	28.10	11.91	17.10	6.57	6.40	2.36
	6	0.66	3.70	27.87	11.00	17.64	6.43	6.83	2.53
4	0	0.90	3.27	26.27	12.72	15.57	8.62	6.54	2.07
	2	0.76	4.89	26.53	13.71	15.06	8.48	5.99	1.94
	4	0.72	4.08	25.64	13.36	15.73	9.32	6.02	1.92
	6	0.57	3.25	27.85	12.51	16.30	8.57	6.17	2.23

^z Values are pooled means per treatment

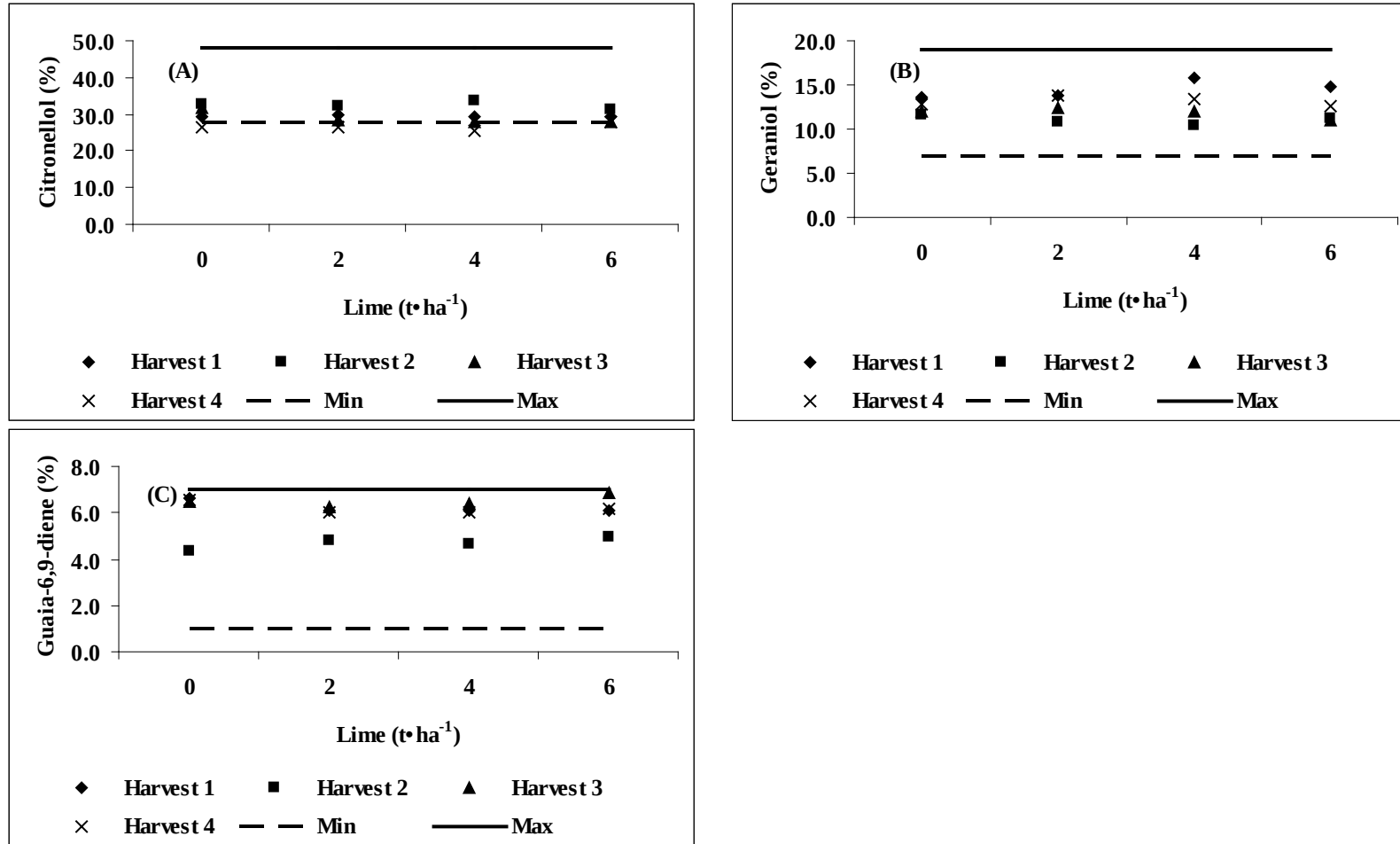


Figure 6.5: Rose-scented geranium essential oil composition in the present study vs the minimum and maximum accepted standard values presented by Lis-Balchin (1995): (a) Citronellol (b) Geraniol and (c) Guaia-6,9-diene during the four harvesting periods

Citronellol and geraniol are among the most important components of rose-scented geranium essential oil and the essential oil quality is characterized by their ratio (C:G ratio) (Doimo *et al.*, 1999; Motsa *et al.*, 2006). The desirable C:G ratio is 1.0 to 3.0 (Bauer *et al.* 1990; Anistescu *et al.* 1997). In this investigation, the C:G ratio (Table 6.6) was influenced by seasonal differences in maximum temperatures (Fig. 6.6). The C:G ratio was low (most desirable; Doimo *et al.*, 1999) in both summer/ autumn harvests (mean maximum temperature between 23.9 and 26.0 °C) while it was high (less desirable) in the autumn/ winter harvest (mean maximum temperature of 21.7 °C) followed by the spring/ summer harvest (mean maximum temperature of 28.0 °C). This is in agreement with Doimo *et al.*, (1999) and Motsa *et al.*, (2006) where C:G ratios were highest in winter and late winter/ spring harvests.

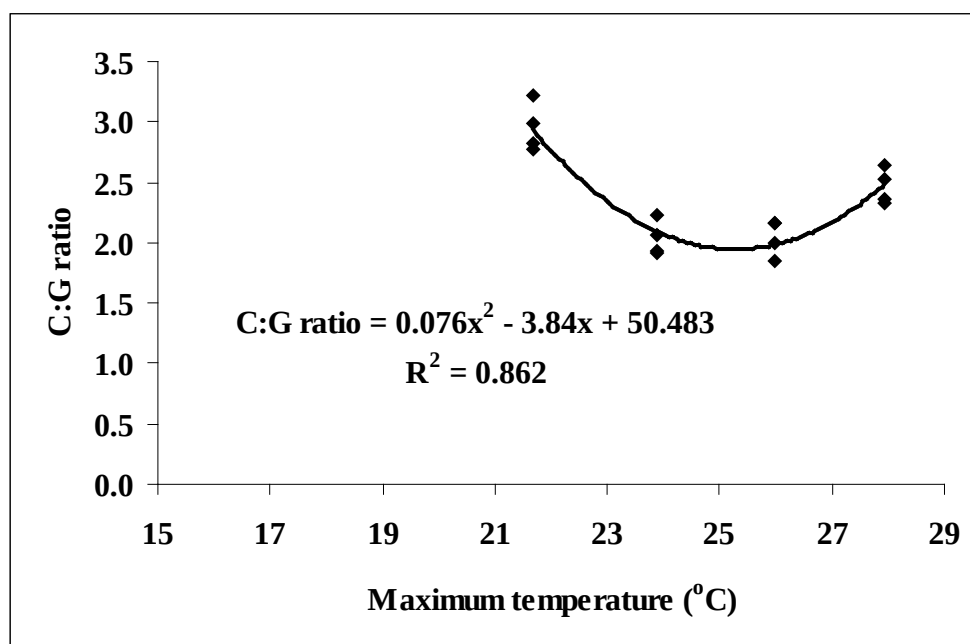


Figure 6.6: Citronellol:geraniol (C:G) ratio versus mean maximum temperatures during the growing period, for the first harvest (summer/autumn 2005), second harvest (autumn/winter 2005), third harvest (spring/summer 2005) and the fourth harvest (summer/autumn 2006)

6.4 CONCLUSIONS AND RECOMMENDATIONS

This investigation found that optimum growth of rose-scented geranium can be achieved by application of lime when the plant is grown on acidic soils in South Africa. Liming significantly increased the uptake of Ca and P by the plant, which might favour optimum growth, while excessive uptake of elements such as Al, Fe and Mn were reduced. The implication of this study is that lime application on acidic soils increased fresh herbage and essential oil yield of rose-scented geranium, without any notable effects on the composition of the essential oil under South African conditions. The plant attained maximum growth when the soil pH was above 5.5 and when the base saturation was above 55%, and for this study, it was achieved by applying at least $2 \text{ t} \cdot \text{ha}^{-1}$ dolomitic lime. These findings imply that the hypothesis for this study could be accepted, as liming of acidic soil did improve herbage and essential oil yield of rose-scented geranium.

CHAPTER SEVEN

GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

Worldwide there are over 3000 plant species that can be exploited for their essential oils (Lintu, 1995). However, only 300 of these species are commercially important to date (Lintu, 1995) and among these is rose-scented geranium (*Pelargonium capitatum* × *P. radens*), which belongs to the family Geraniaceae and genus *Pelargonium*. Rose-scented geranium is a highly fragrant perennial aromatic shrub, with a multi-harvest, high value, commercially important essential oil (Weiss, 1997; Lis-Balchin, 2002). The essential oil is extracted from the herbage of this plant through steam distillation and is widely used in the fragrance and cosmetics industries (Rajeswara Rao *et al.*, 1990a,b; Ram, Ram & Roy, 2003). The essential oil is stored in special structures called glands on the tender shoots and leaf surfaces (Lis-Balchin, 2002). During the steam distillation process, membranes covering the glands (cuticles) get ruptured with heat and consequently the essential oil becomes released. This makes the above ground biomass (leaves and stem) yield an important yield factor in rose geranium production (Oosthuizen & Coetzee, 1984; Demarne & van de Walt, 1989; Weiss, 1997).

Worldwide demand for rose-scented geranium essential oil is increasing. South Africa, despite being the centre of origin of the genus, produces only small amount of geranium oil (about 3 ton per annum), but potentially it could contribute about 50 ton per annum of geranium essential oil to the world markets (FRIDGE, 2004). Historically, China, Egypt, Réunion Island and India are the leading rose geranium essential oil producing countries (Rajeswara Rao *et al.*, 1990a,b; Ram, Ram & Roy, 2003). Oil from each region has a unique chemical composition and, therefore, a unique position in the market. Réunion oil (Bourbon) has typically demanded the highest price (Weiss, 1997).

Essential oil production of crops is reported to be influenced by several factors (Rajeswara Rao *et al.*, 1990a; Araya *et al.*, 2006), such as environmental conditions

which are known to affect the biomass, oil content and essential oil composition of the plant, which indirectly also influences its market value. Due to this, certain regions of the world are well known for the production as well as for their dominance in the world market of rose geranium essential oil. This is why the essential oil of this plant is characterized and marketed with an inclusion of its growing region, such as rose geranium oil from Grasse, France or Réunion Island ‘Bourbon type’ (Weiss, 1997; Lis-Balchin, 2002). South Africa is also a country with different growing regions and many of these are believed to have good potential for rose geranium production. However, in order for production of the crop to be successful in the region, research inputs are important.

In an effort to improve local production of the crop, a study was carried out on rose-scented geranium to study the effects of nitrogen rate, source, season of production and liming on oil yield and quality. This study was conducted under field conditions at the Hatfield Experimental Farm, University of Pretoria. The investigated features were: sources of N, namely conventional (limestone ammonium nitrate or LAN, 28% N) and organic fertilizers (blend of four organic fertilizers) at three levels (100, 200 and 300 kg·ha⁻¹ N·year⁻¹) and a control (no N) (Chapter three), time of N application and re-growth (Chapter four), influence of N fertilization timing (winter, spring, summer and autumn) and estimation of foliar N content using a SPAD-502 chlorophyll meter (Chapter five), as well as influence of liming (0, 2, 4 and 6 t·ha⁻¹) on acidic soils (Chapter six) were studied.

According to different studies (Rajeswara Rao *et al.*, 1990a; Weiss, 1997; Araya *et al.*, 2006), herbal plants (such as rose-scented geranium) in general are reported to require larger quantities of the essential in plant nutrients, such as nitrogen, phosphorus and potassium, for optimum growth. Among these essential elements, nitrogen plays an important role in yield and growth of rose-scented geranium, especially after every harvest. Since most of the above-ground biomass is removed at harvest, fertilization of the crop becomes essential. Nitrogen requirements of rose-scented geranium is reported to vary with growing area and a rate that was found advantageous for optimum growth in one area might not be appropriate for another area (Weiss, 1997).

Under the South African conditions, rose-scented geranium responded well to source and amount of N. The first harvest after N fertilization (summer/autumn), there was no significant effect of conventional N on fresh herbage and oil yield, probably due to leaching of N by rainfall. However, organic N at $100 \text{ kg}\cdot\text{ha}^{-1}$ increased fresh herbage and oil yields about 58% and 48% over the control, respectively. In the second harvest (spring/summer), fresh herbage yield increased by 46% (conventional N) and 60% (organic N) at $100 \text{ kg}\cdot\text{ha}^{-1}$ compared to the control. Compared to the control, $100 \text{ kg}\cdot\text{ha}^{-1}$ conventional and organic N also increased essential oil yields by 94% and 129%, respectively. For both N sources, NUE and LAI decreased with an increase in N level, and organic N gave highest essential oil production efficiency and LAI. Essential oil content (% fresh mass basis) also varied between the harvests, and was greater in the second harvest (September to December 2005; spring/summer) than the first harvest (February to May 2005; summer/autumn). This was due to different environmental conditions occurred between the harvesting periods. N level and source were found to have no noticeable effect on essential oil composition of the plant. This study further revealed that rose-scented geranium produced higher fresh herbage and essential oil yield when organic fertilizer was used as a source of N. Results of this trial indicated that fresh herbage yield, essential oil yield and NUE of the crop can be improved with organic N at $100 \text{ kg}\cdot\text{ha}^{-1}$. On the other hand, increased N showed a tendency to increase citronellol levels, particularly in the autumn months and to decrease guaia-6,9-diene. This trend may, for certain commercial applications, negate the benefit of increased yield with increased N application.

Delaying nitrogen topdressing (conventional N in the form of LAN; N 28%) after harvest to between the 7th and 9th week after cut back (Chapter four), was found to have a significant positive effect on biomass and essential oil production. This shows that, N management in terms of rate and time of application is important in rose-scented geranium production. Essential oil content of the plant, however, did not show any response to a delay in nitrogen topdressing. A delay in nitrogen topdressing during the first re-growth resulted in a lower citronellol to geraniol (C:G) ratio, which favour essential oil quality of the crop. Generally, the characteristic of the essential oil was within the range of internationally accepted rose-scented geranium essential oil. In addition, production of rose-scented geranium during cooler periods is not

advisable due to limited biomass production, which may also encourage leaching of applied nitrogen.

The trial on the response of rose-scented geranium to conventional and organic N in four seasons (Chapter five), showed that organic N at $300 \text{ kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$ increased herbage and essential oil yield in spring and summer but further increases in conventional N levels had no significant effect. N application either in winter or autumn did not improve biomass production, which indicates that response of the crop to N is season dependent and application of more N than what is required for optimum growth of the plant had no positive effect on essential oil production. The net benefits from N application (NUE) is dependent on the growing period and in the present study spring and summer were more beneficial than winter and autumn. Application of organic N also resulted in higher N use efficiency than conventional N. The essential oil contents (% fresh mass basis) achieved in the present study generally fell within the reported range of 0.04 to 0.2%. Most desirable essential oil (low Citronellol to geraniol ratio; low C:G ratio) was attained in spring, summer and autumn harvesting seasons and less desirable oil was attained in winter harvests. The relationship between SPAD-502 chlorophyll meter readings (SPAD units) and leaf N content (% dry weight basis) indicated the possibility of this instrument to estimate the foliar N in the crop. That is, SPAD-502 chlorophyll meter readings (SPAD units) matched well with that of leaf N concentration data of rose-scented geranium. Regardless of the factors that affect the readings, this instrument has potential to be used as an indication of leaf N status of rose-scented geranium. Therefore, it can be concluded that N rate, source and season of production should be considered to ensure optimal rose-scented geranium production.

In a study conducted to ameliorate soil acidity using liming (Chapter six), soil pH was not influenced by liming for any of the treatments at the first harvest after liming (January to April 2005, summer/autumn), whereas at the second (May to August 2005, autumn/winter), third (September to December 2005, spring/summer) and fourth (January to April 2006, summer/autumn) harvests an increase in pH was observed for all the limed treatments. At the time of the first harvest, plants did not respond significantly to 2 and $4 \text{ t} \cdot \text{ha}^{-1}$ of liming, but responded positively to $6 \text{ t} \cdot \text{ha}^{-1}$, with higher herbage yield compared to the control and $2 \text{ t} \cdot \text{ha}^{-1}$ lime treatments. At the

second, third and fourth harvests fresh herbage and essential oil yield increased significantly due to liming. The differences among lime treatments were not significant for fresh herbage and essential oil yield. Cumulative fresh herbage and essential oil yields of all harvests were higher on the limed treatments as compared to the control. Soil pH above 5.5 and soil base saturation above 55% increased fresh herbage and essential oil yield (per ha), which corresponded in this case with 2 to 6 t·ha⁻¹ of lime application. Oil content (%) was not significantly affected by application of lime or by an increase in soil pH but was more dependent on air temperature fluctuations. Similarly, no clear differences were observed in the composition of the essential oil throughout the experimental period due to lime. The ratio between citronellol and geraniol (C:G) was correlated with seasonal differences in maximum temperatures and not with liming. The implication of this study is that optimum growth of rose-scented geranium can be achieved by application of lime when plants are grown on acidic soils, but without any effect on oil content and essential oil composition.

Based on the above-mentioned findings, the following recommendations can be made to rose-scented geranium growers:

- Increasing the N level generally increased fresh herbage and essential oil yield of rose-scented geranium.
- The response to N was more pronounced for organic than for conventional fertilizers. When conventional N is used maximum yield can be expected at about 100 kg·ha⁻¹· year⁻¹ N, but when organic N is used, yield response can be expected at rates up to 300 kg·ha⁻¹· year⁻¹.
- The crop re-growth is slow until 8 weeks after the previous harvest and most rapid between 9 and 12 weeks. It is therefore important to delay N application to about 7 to 9 weeks after harvest to ensure best growth response and highest essential oil yield.
- Better return on N application from both sources (organic or conventional) can be attained if applied in spring and summer months.
- Autumn seasons tend to favour higher oil content (% oil), but this does not guarantee higher essential oil production due to low yields in autumn.

- The SPAD 502 chlorophyll meter can be used as indicator of leaf N status of rose-scented geranium.
- Rose-scented geranium production can be improved by liming when the crop is grown on acidic soils ($\text{pH} < 5.5$).
- Liming should not have any significant effect on the oil composition.

Recommendation for future research are as follows:

- To make results useful to other conditions (soils and climates), a modelling angle could be considered.
- Nutrient and water mass balances should be conducted to explore certain responses, for example why organic fertilizers consistently outperformed conventional (inorganic) fertilizers.

These prospects can be considered in order to try to explain why certain responses occurred (for example, organic vs inorganic)

RESEARCH OUTPUTS RESULTING FROM THIS PROJECT

This has been presented or published in part as follows:

ARAYA, H.T., SOUNDY, P. & STEYN, J.M., 2011. Liming improves herbage yield, essential oil yield and nutrient uptake of rose-scented geranium (*Pelargonium capitatum* x *P. radens*) on acidic soils. *New Zealand Journal of Crop and Horticultural Science* 39 (2): 1-12.

ARAYA, H.T., SOUNDY, P. & STEYN, J.M., 17 to 21 January 2011. Nitrogen effects on rose-scented geranium (*Pelargonium capitatum* x *P. radens*) production. University of Pretoria, Pretoria. Poster presented at the Combined Congress 2011 of the Soil Science Society of South Africa, the South African Society of Crop Production and the Southern African Society for Horticultural Sciences.

ARAYA, H.T., SOUNDY, P. & STEYN, J.M., 22 to 25 January 2008. Herbage yield, essential oil yield and composition of rose-scented geranium (*Pelargonium capitatum* x *P. radens*) as influenced by liming. Rhodes University, Grahamstown. Paper presented at the Combined Congress 2008 of the Soil Science Society of South Africa, the South African Society of Crop Production and the Southern African Society for Horticultural Sciences.

ARAYA, H.T., SOUNDY, P. & STEYN, J.M., 22 to 25 January 2008. Glandular and non-glandular trichome density of rose-scented geranium (*Pelargonium* sp.) as influenced by source of nitrogen. Rhodes University, Grahamstown. Poster presented at the Combined Congress 2008 of the Soil Science Society of South Africa, the South African Society of Crop Production and the Southern African Society for Horticultural Sciences.

ARAYA, H.T., SOUNDY, P. & STEYN, J.M., 2007. Influence of conventional and organic nitrogen fertilizer on herbage yield, essential oil yield and composition of rose-scented geranium (*Pelargonium* sp.). *Proceedings, HortScience* 42 (4): pp 939.

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APPENDIX A

SOIL AND WEATHER DATA DURING THE EXPERIMENTAL PERIOD

1. SOIL DATA

Table A1: Soil chemical properties (0-30 cm) at the end of the first harvest (February to May 2005; summer/autumn) (Chapter one)

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Source	Treatment N (kg·ha ⁻¹ ·year ⁻¹)	pH (H ₂ O)	P ^z	K	Ca	Mg	Na	N
			(mg·kg ⁻¹)					(%)
Control	0	5.9	22.3	38	371	123	14	0.04
Conventional	100	5.9	23.2	25	320	150	14	0.04
	200	5.9	24.0	24	358	109	15	0.05
	300	6.0	22.4	25	374	120	15	0.04
Organic	100	7.4	24.0	17	275	95	16	0.04
	200	6.2	23.6	14	392	123	87	0.04
	300	6.1	61.2	11	235	81	11	0.04

^z Bray I: soil test solution (0.03 M NH₄F + 0.025 M HCl) to determine the available P in soils

Table A2: Soil chemical properties (0-30 cm) at the end of the second harvest (September to December 2005; spring/summer) (Chapter one)

Source	Treatment N (kg·ha ⁻¹ ·year ⁻¹)	pH (H ₂ O)	P ^z	K	Ca	Mg	Na	NH ₄	NO ₃
			(mg·kg ⁻¹)						
Control	0	6.2	18.3	28	423	150	18	0.56	18.76
Conventional	100	6.6	14.1	26	427	158	17	0.17	6.72
	200	6.5	18.0	31	428	153	14	0.06	12.49
	300	6.2	13.9	29	432	162	14	0.90	7.62
Organic	100	6.1	41.7	23	401	154	20	1.18	6.50
	200	6.0	32.6	37	386	143	19	1.68	8.12
	300	6.2	46.5	26	362	131	13	1.85	17.08

^z Bray I: soil test solution (0.03 M NH₄F + 0.025 M HCl) to determine the available P in soils

2. WEATHER DATA

Table A3: Mean monthly maximum and minimum temperatures ($^{\circ}\text{C}$) and total monthly rainfall (mm) recorded for the first harvest (February to May 2005; summer/autumn) and second harvest (September to December 2005; spring/summer) growing periods

Month (2005)	Harvest	Temperature ($^{\circ}\text{C}$)		Total rainfall (mm)
		Maximum	Minimum	
February	1	28.4	17.4	48.8
March		25.5	15.4	48.4
April		22.5	12.7	76.6
May		22.1	8.8	-
September	2	27.9	12.1	-
October		28.7	14.8	13.0
November		27.6	16.3	81.8
December		27.6	15.9	62.1

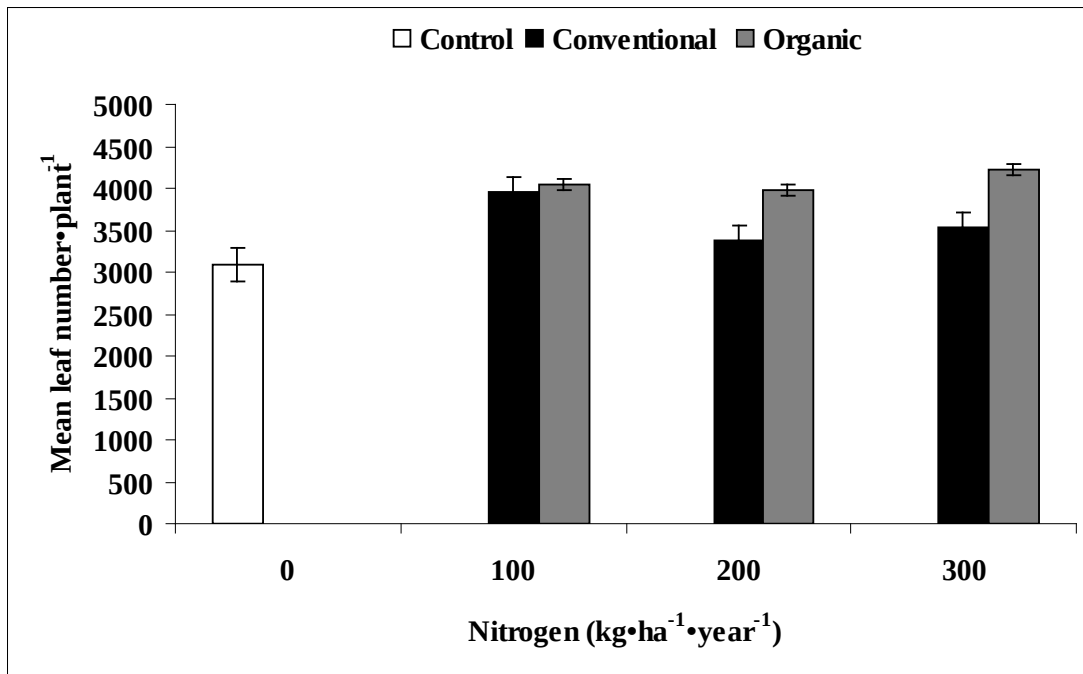


Figure A1: Mean leaf number of rose-scented geranium as influenced by nitrogen rate (0 to 300 kg·ha⁻¹·year⁻¹) and source (control, conventional or organic fertilizers)

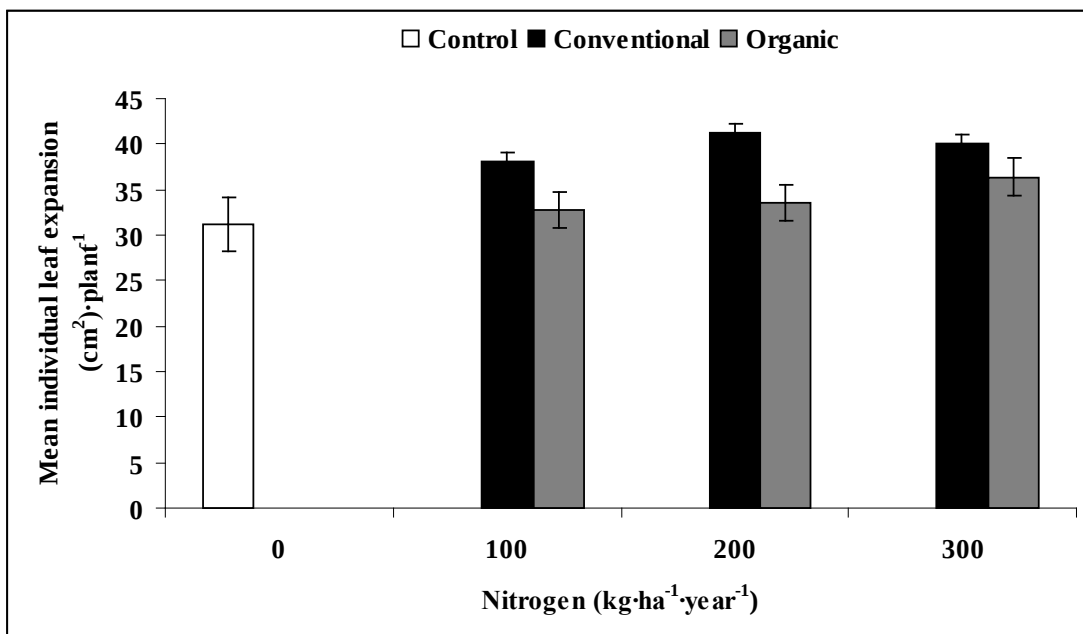


Figure A2: Mean individual leaf expansion (cm²)·plant⁻¹ of rose-scented geranium as influenced by nitrogen rate (0 to 300 kg·ha⁻¹·year⁻¹) and source (control, conventional or organic fertilizers)

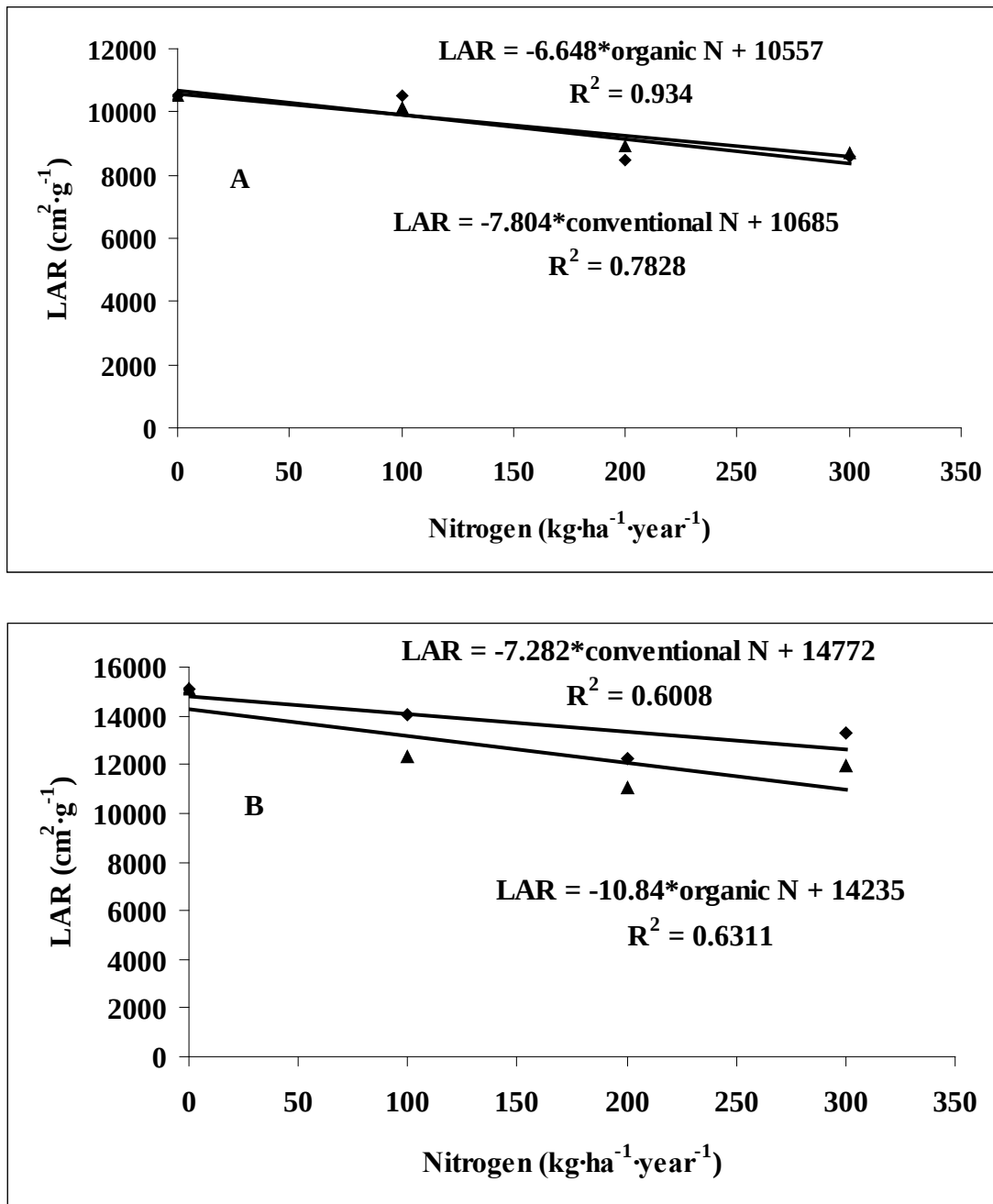


Figure A3: Leaf area ratio (LAR; cm²·g⁻¹) of rose-scented geranium at (A) first (February to May 2005; summer/autumn) and (B) second (September to December 2005; spring/summer) harvests using (♦) conventional or (▲) organic fertilizers. Values presented are means (n = 16)

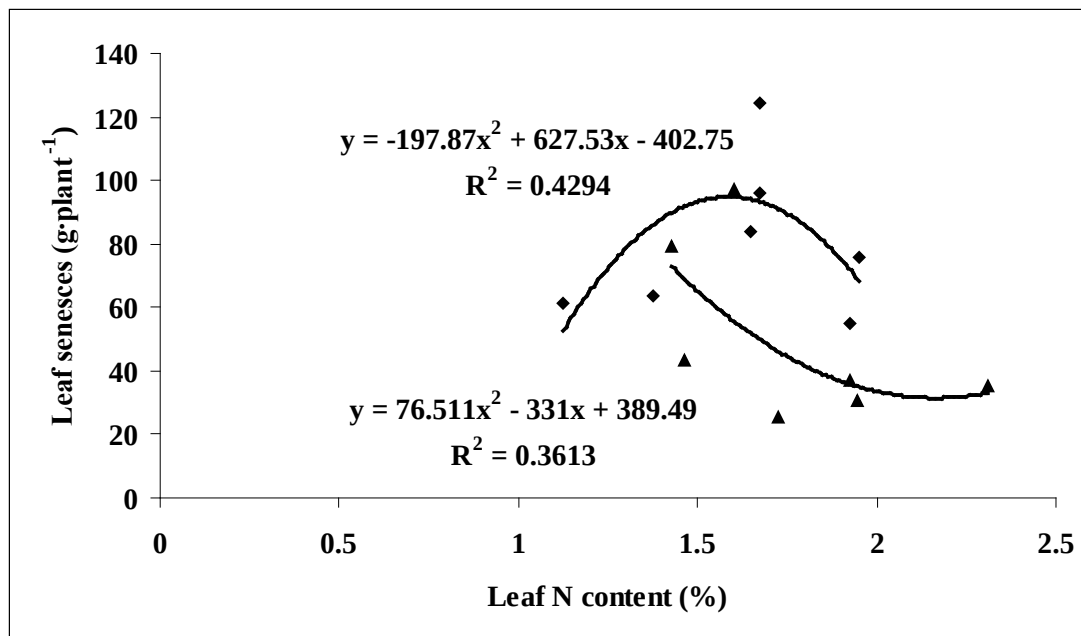


Figure A4: Relationship between leaf senescence (g·plant⁻¹) and leaf N content (%) of rose-scented geranium at first (February to May 2005; summer/autumn) (♦) and second (September to December 2005; spring/summer) harvests (▲) using conventional or organic fertilizers. Values presented are means (n = 16)

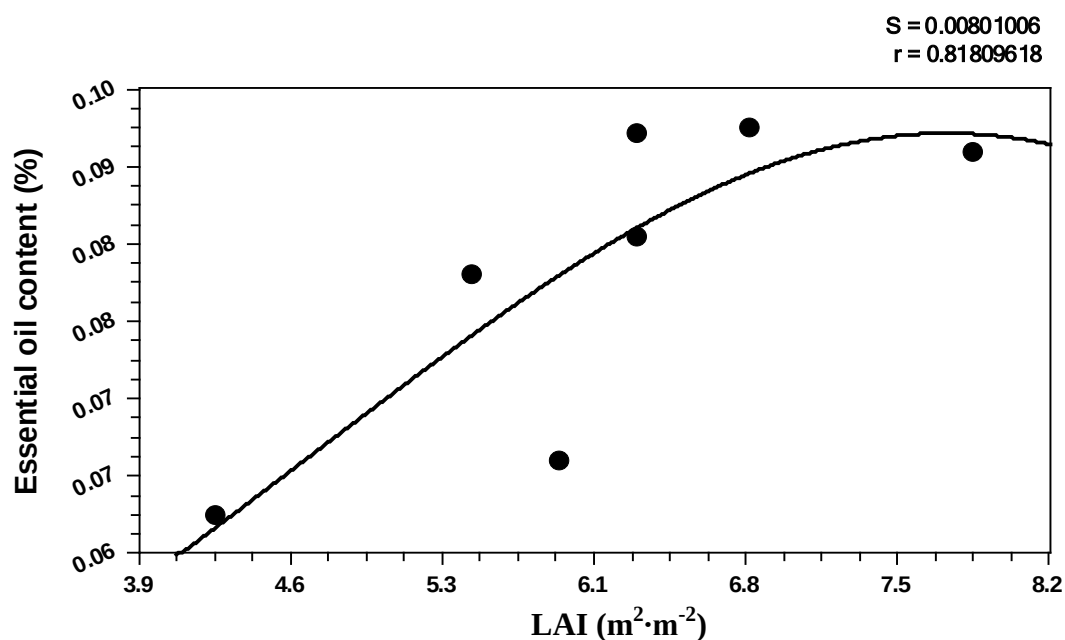


Figure A5: Relationship between leaf area index (LAI; m²·m⁻²) and essential oil content (%) of rose-scented geranium at the second (September to December 2005; spring/summer) harvests using conventional or organic fertilizers

APPENDIX B

WEATHER DATA DURING THE EXPERIMENTAL PERIOD

Table B1: Mean monthly maximum and minimum temperatures (°C) and total monthly rainfall (mm) recorded

Month	Harvest (H) or growth cycle (C)	Temperature (°C)		Total rainfall (mm)
		Maximum	Minimum	
February'05	C1	28.4	17.4	48.8
March		25.5	15.4	48.4
April		22.5	12.7	76.6
May		22.1	8.8	-
June	C2	21.4	6.5	-
July		20.6	6.1	-
August		22.6	9.4	1.4
September		27.9	12.1	-
October'05	H1	30.3	14.2	15.2
November		29.5	15.3	94.4
December		29.4	15.5	65.4
January'06		25.4	17.7	191.9
February		28.4	17.4	48.8
March	H2	25.5	15.4	48.4
April		22.5	12.7	76.6
May		22.1	8.8	-
June		21.4	4.4	-
July	H3	22.4	5.5	-
August		21.3	6.0	36.1
September		27.9	12.1	-
October		28.7	14.8	13.0

APPENDIX C

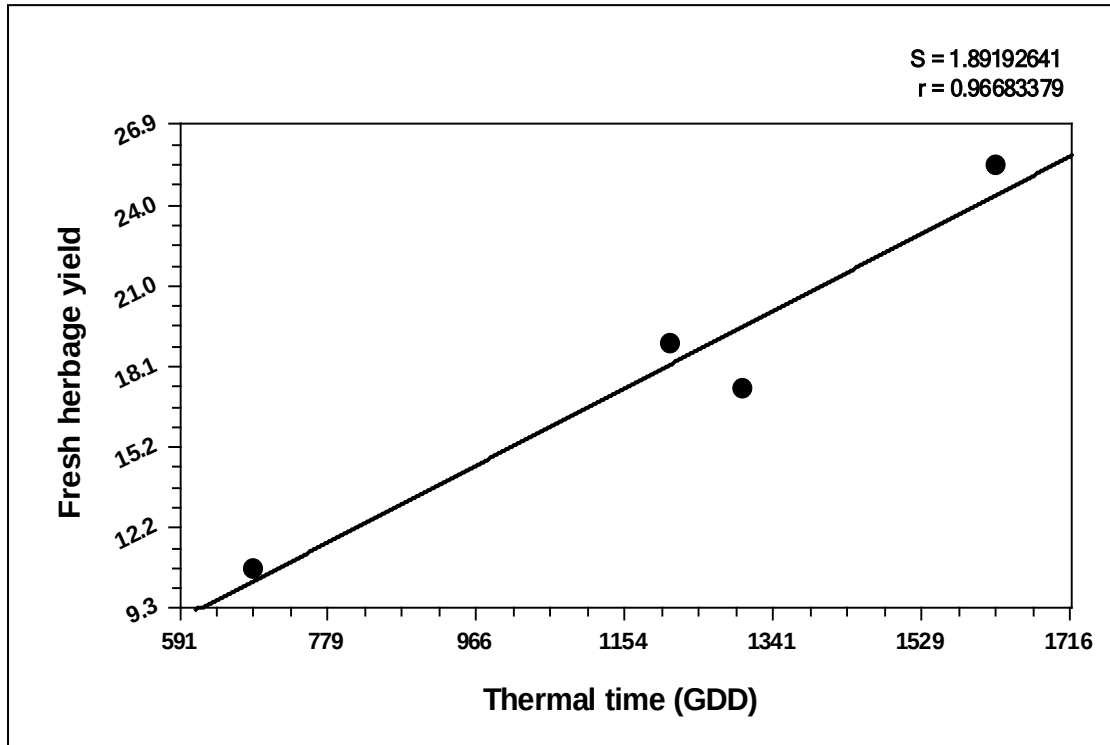


Figure C1: Relationship between fresh biomass accumulation ($\text{t}\cdot\text{ha}^{-1}$) and thermal time accumulated (GDD). Individual values presented are means per season

Table C1: Mean monthly maximum and minimum temperatures ($^{\circ}\text{C}$) and total monthly rainfall (mm) recorded

Month (2005)	Harvest	Temperature ($^{\circ}\text{C}$)		Total rainfall (mm)
		Maximum	Minimum	
Winter	1	21.8	5.2	36.1
Spring	2	28.9	14.2	142.0
Summer	3	30.7	15.4	214.5
Autumn	4	24.6	11.8	216.0

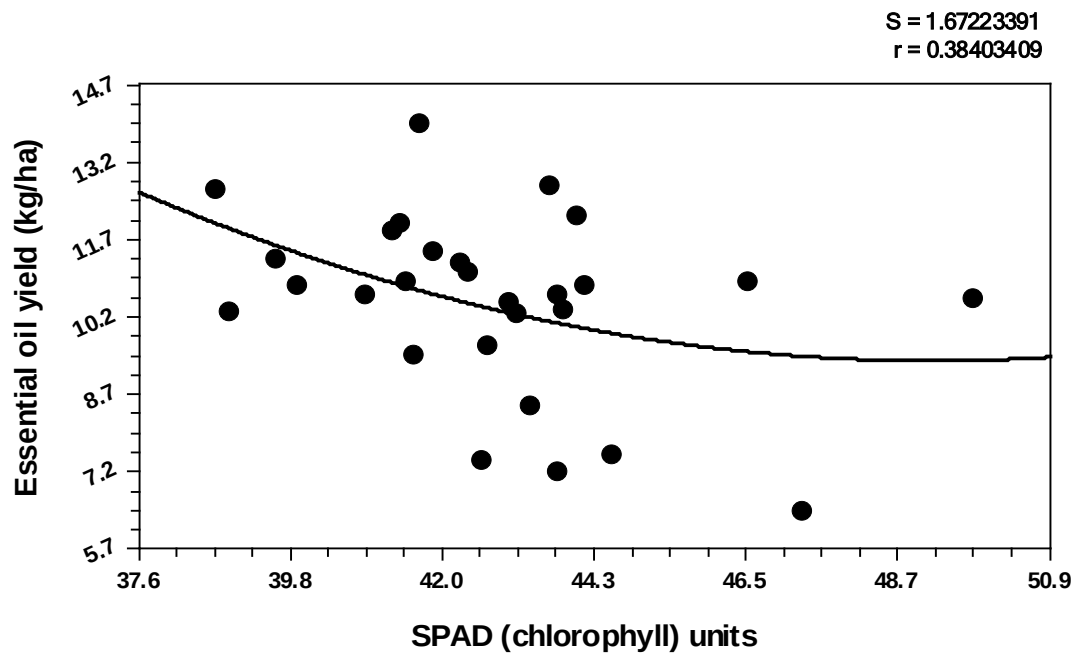


Figure C2: Relationship between leaf chlorophyll meter SPAD values (units) and essential oil yield ($\text{kg} \cdot \text{ha}^{-1} \cdot \text{year}^{-1}$) of rose-scented geranium in autumn season of production in 2006/2007. Individual values presented are means ($n = 16$)

APPENDIX D

Table D1: Mean monthly maximum and minimum temperatures (°C) and total monthly rainfall (mm) recorded for the growing periods.

Month	Harvest	Temperature (°C)		Total rainfall (mm)
		Maximum	Minimum	
January '05	1	27.5	17.4	217.9
February		28.4	17.4	48.8
March		25.5	15.4	48.4
April		22.5	12.7	76.6
May	2	22.1	8.8	-
June		21.4	6.5	-
July		20.6	6.1	-
August		22.6	9.4	1.4
September	3	27.9	12.1	-
October		28.7	14.8	13.0
November		27.6	16.3	81.8
December		27.6	15.9	62.1
January '06	4	25.4	17.7	191.9
February		25.3	17.0	193.9
March		23.4	14.9	111.3
April		21.4	12.6	31.6