CHAPTER 4

RESPONSE OF ROSE-SCENTED GERANIUM GROWTH, ESSENTIAL OIL YIELD AND OIL COMPOSITION TO A ONE-MONTH IRRIGATION-WITHHOLDING PERIOD

4.1 ABSTRACT

Responses of plant growth, essential oil yield and oil composition of rose-scented geranium to a one-month irrigation-withholding period at different times of regrowth cycles were investigated at the Hatfield Experimental Farm of the University of Pretoria, South Africa, during 2004 to 2007, in an open field and a rain shelter. No-stress (control) and a one-month irrigation withholding period in the second, the third and the fourth month of regrowth were applied as treatments. Herbage yield showed a significant reduction when the water stress period was imposed during the third or fourth month of regrowth. Essential oil yield was reduced when the plants were stressed during the fourth month of regrowth cycles. Essential oil content (percentage oil on fresh herbage mass basis) apparently increased in the stressed treatments, but total oil yield dropped due to lower herbage mass. Essential oil composition changes in response to irrigation-withholding treatments were not consistent. Water-use efficiency was not significantly affected by withholding irrigation in the second and in the third month of regrowth. With a marginal oil yield loss, about 330 to 460 m^3/ha of water could be saved by withholding irrigation during the third month of regrowth cycles. Hence, in water-scarce situations, withholding irrigation during either the second or the third month of regrowth in rose-scented geranium could improve water productivity.

Keywords Herbage mass; essential oil content; essential oil composition; *Pelargonium* species; water use; water-use efficiency, water stress period

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4.2 INTRODUCTION

Rose-scented geranium (*Pelargonium* species) is a perennial herb that is cultivated for its high-value essential oil. Rose-scented geranium oil, commonly referred to as 'geranium' oil, is widely used in the perfumery, cosmetics and aromatherapy industries (Rajeswara Rao *et al.*, 1996). Trade in essential oils is expected to expand in the future as a result of a growing number of consumers and their preferences, and continuous discovery of new uses for the oil constituents (Lis-Balchin *et al.*, 1998; Dorman & Deans, 2000; Lis-Balchin & Roth, 2000; Sangwan *et al.*, 2001; Deans, 2002).

According to Weiss (1997), rose-scented geranium performs well in regions that receive an annual rainfall of 1 000 to 1 500 mm, with fairly good seasonal distribution. The author stated that long, dry seasons resulted in poor plant growth, low essential oil yield and changes in oil composition. Gauvin *et al.* (2004) also mentioned that on Réunion Island, the crop is successfully cultivated in areas that receive an annual rainfall of about 1500 mm. Similarly, Rajeswara Rao *et al.* (1996) found that higher rainfall seasons favoured vegetative growth and essential oil yield.

The available information also indicated that South African rose-scented geranium production is limited to the Mpumalanga Lowveld, KwaZulu-Natal and Limpopo provinces (SANDA, 2006), where annual rainfall is relatively high, about 510 to 1 000 mm in the summer season (Davies & Day, 1998). Since most arable land in South Africa falls within an arid or semi-arid climate, introducing rose-scented geranium production to those dry regions would only be possible under irrigation. Hence, searching for irrigation strategies, which could increase rose-scented geranium essential oil yield and maximise productivity of scarce irrigation water, is a foremost priority.

Under a deficit irrigation technique, Singh *et al.* (1996) suggested that applying 30 mm of irrigation when the cumulative pan evaporation reaches 50 mm could maximise irrigation water-use efficiency in rose-scented geranium fields. Subsequent irrigation trials by Singh (1999) confirmed that supplementary irrigation at 60% of IW:CPE ratio (irrigation water applied to cumulative pan evaporation ratio) increases profitability of rose-scented geranium production in the semi-arid tropical climate of India.

Withholding irrigation during certain crop growth stages that are not sensitive to water stress is one of the several irrigation strategies often applied to improve water productivity (Jalota *et al.*, 2006). Kang *et al.* (2000) suggested that water stress at the seedling and stem-elongation stages of maize would be the best irrigation strategy in semi-arid areas. Research results reported by Çakir (2004) also revealed that water stress during the vegetative stage of corn reduced total biomass, without a significant reduction in grain yield.

Geranium oil is extracted mainly from leaves and, to a certain extent, from stems and flowers by hydrodistillation techniques (Rajeswara Rao *et al.*, 2002). Hence, severe reduction in herbage yield due to water stress could result in a significant decline in essential oil yield, as reported in aromatic compounds of tea plants (Panrong, Chunyan & Kebin, 2006). A certain water stress level could also trigger conversion of primary to secondary metabolites, such as essential oils (Simon *et al.*, 1992). In addition, it is known that essential oil yield and composition depend on the shoot age of aromatic plants (Marotti, Piccaglia & Giovanelli, 1994; Sangwan *et al.*, 2001; Kothari *et al.*, 2004; Lattoo *et al.*, 2006; Motsa *et al.*, 2006). Hence, it was hypothesised that the timing of water stress could influence rose-scented geranium essential oil yield, oil composition and water productivity. In the current work, therefore, the effects of withholding irrigation for a one-month period at different times of plant regrowth were investigated.

4.3 MATERIALS AND METHODS

4.3.1 Site description

The experiments were conducted in an open field and in a rain shelter at the Hatfield Experimental Farm of the University of Pretoria, Pretoria, South Africa (latitude 25° 45'S and longitude 28° 16'E; altitude of 1372 m), from October 2004 to February 2007. The experimental site is situated in a region with an average annual rainfall of 670 mm, mainly in the summer season (October to March). Highest long-term maximum and lowest long-term minimum temperatures are about 30°C in January and 1.5°C in July, respectively (Annandale *et al.*, 1999).

4.3.2 Plant culture

Rose-scented geranium (*Pelargonium capitatum* x *P. radens* cv. *Rose*) was used as plant material. About 45-day-old plantlets (raised from stem cutting by commercial nursery) were transplanted to the field on 28 October 2004. For the rain shelter trial, healthy stem cuttings (taken from the open-field trials) were planted in seedling trays (filled with peat) on 25 August 2005, and raised at high relative humidity (in a mist bed) in a greenhouse at the Hatfield Experimental Farm. Starting three weeks after planting, a complete nutrient solution was applied once a week. The plantlets were transplanted on 1 October 2005.

In the open field, the plants were allowed to grow for about seven months and on 3 June 2005 they were cut back to about 15 cm above the ground to start the irrigation treatments. Due to technical problems experienced in the rain shelter, irrigation treatments were applied only after one year.

4.3.3 Field layout and treatments

Field layout

In the open field, plots were 7.5 m long and 5 m wide. There was a buffer strip of 1.5 m between two adjacent blocks. Spacing between rows was 1 m and plants within a row were 0.62 m apart. Each experimental plot consisted of five rows. Data were recorded on plants in the three middle rows. In the rain shelter, seedlings were planted at narrower spacings of 0.75 m inter-row and 0.45 m intra-row due to limited space. Plastic sheets were installed vertically to a depth of 80 cm to avoid lateral water movement and root growth between adjacent plots. Each experimental plot consisted of four rows of 6 m long. In both experiments, treatments were replicated four times and arranged in a randomised complete block design (RCBD).

Treatments

Rose-scented geranium has no definite phenological stages because the plant (1) is commonly established from stem cuttings, (2) is grown as a perennial crop, and (3) rarely flowers and does not bear fruits or seeds due to male sterility (Tokumasu, 1974; Demarne, 2002). A regrowth duration period of four months was, therefore, decided upon in accordance with local commercial farmers' practices. Motsa *et al.* (2006) also reported that a four-month regrowth cycle produced the highest essential oil yield per harvest in this region.

For the first month of regrowth (beginning of each experiment), plants were allowed to regenerate under full irrigation to ensure recovery after harvesting injury. Irrigation treatments, therefore, started from the 31st day of each regrowth cycle. The following predefined irrigation treatments were applied:

1. No water stress throughout the growth cycle (NNNN or control);
2. Withholding irrigation during the second month of regrowth cycles (NSNN);
3. Withholding irrigation during the third month of regrowth cycles (NNSN);
4. Withholding irrigation during the fourth month of regrowth cycles (NNNS);

4.3.4 Irritation monitoring

Since these trials and the maximum allowable depletion level experiments were carried out on the same site and shared common soil characteristics, for the detailed information on neutron probe calibration and irrigation-monitoring procedures, see Chapter 3.

Non-stressed treatments were irrigated to field capacity when about 20% of the available soil water was depleted. During the stress period, irrigation was withheld completely. Soil water status was monitored every second day using a neutron probe (Model 503 DR, CPN Corporation, CA, USA). Measurements were taken at 0.2 m depth increments from 0 to 1.2 m soil depth. A computer-controlled drip irrigation system (with water discharge rate of 1.6 ℓ/hr and at pressure range of 120-200 kPa) was used in both experiments. Dripper lines were spaced 0.5 m apart, and the distance between drippers (emitters) within a line was 0.3 m. Evapotranspiration (ET) for each regrowth cycle was calculated using Equation 3.4 (Chapter 3).

The water stress treatment during the last regrowth month (NNNS treatment) of Harvest 2 in the open field was disrupted by continuous heavy rainfall (248 mm) (Appendix B). Hence, plant water-use efficiency and total evapotranspiration of that particular regrowth period could not be determined. Regrowths of Harvests 1 and 3 (in the open field) were in a dry season (negligible effective rainfall), and in the rain shelter (Harvest 4), rainfall was successfully screened out. Hence, runoff and deep percolation of water in these harvests were assumed to be zero because the irrigation depth was always equal to the measured soil water deficit (ET loss), and application rate did not exceed soil infiltration rate.

4.3.5 Agronomic practices

During establishment, plants received 60 kg/ha nitrogen (N), 90 kg/ha phosphorus (P) and 60 kg/ha potassium (K). In the second week of each regrowth cycle, N, P and K were applied at rates of 30, 15 and 30 kg/ha, respectively. Hoeing was done during the first month of each regrowth cycle. Hand-weeding was practised, and standard pest and disease control measures were taken when necessary.

4.3.6 Data recorded

Data for three regrowth cycles from the open field, Harvest 1 (02 June to 1 Oct 2005), Harvest 2 (2 October 2005 to 1 February 2006), Harvest 3 (12 July to 11 November 2006), and for one growth cycle from the rain shelter, Harvest 4 (27 October 2006 to 26 February 2007) were collected. For further information on data collected, instruments used, procedures followed, and statistical analysis, see to Chapter 3.

4.4 RESULTS AND DISCUSSION

4.4.1 Soil water content during the irrigation-withholding periods

Soil water status during the irrigation withholding periods is depicted in Figure 4.1. The data showed that for all the treatments, plants extracted the most water from the top 0.4 m soil layer, indicating that the most active roots were concentrated in this soil layer. This highlights that the water below this soil layer was not readily available to the plants. The results imply that deep irrigation could be helpful only when it is intended to keep the plants alive during a prolonged drought condition. Based on similar observations, it was suggested that only the 0.45 m top root zone should be considered in irrigation scheduling for maize (Panda *et al*., 2003) and wheat (Panda *et a*l., 2004).

The soil water depletion rate, especially in the top 0.4 m root zone, tended to increase with increase in shoot age for which irrigation was withheld. Consequently, at the end of the irrigation-withholding period, the highest and lowest soil water contents were recorded for the NSNN and NNNS treatments, respectively. Higher soil water depletion levels during the later regrowth stages (e.g. in the fourth month of regrowth cycles) could be associated with larger foliar canopies since evapotranspiration loss is directly related to canopy size (Wright & Smith, 1983; Karam *et al*., 2005).

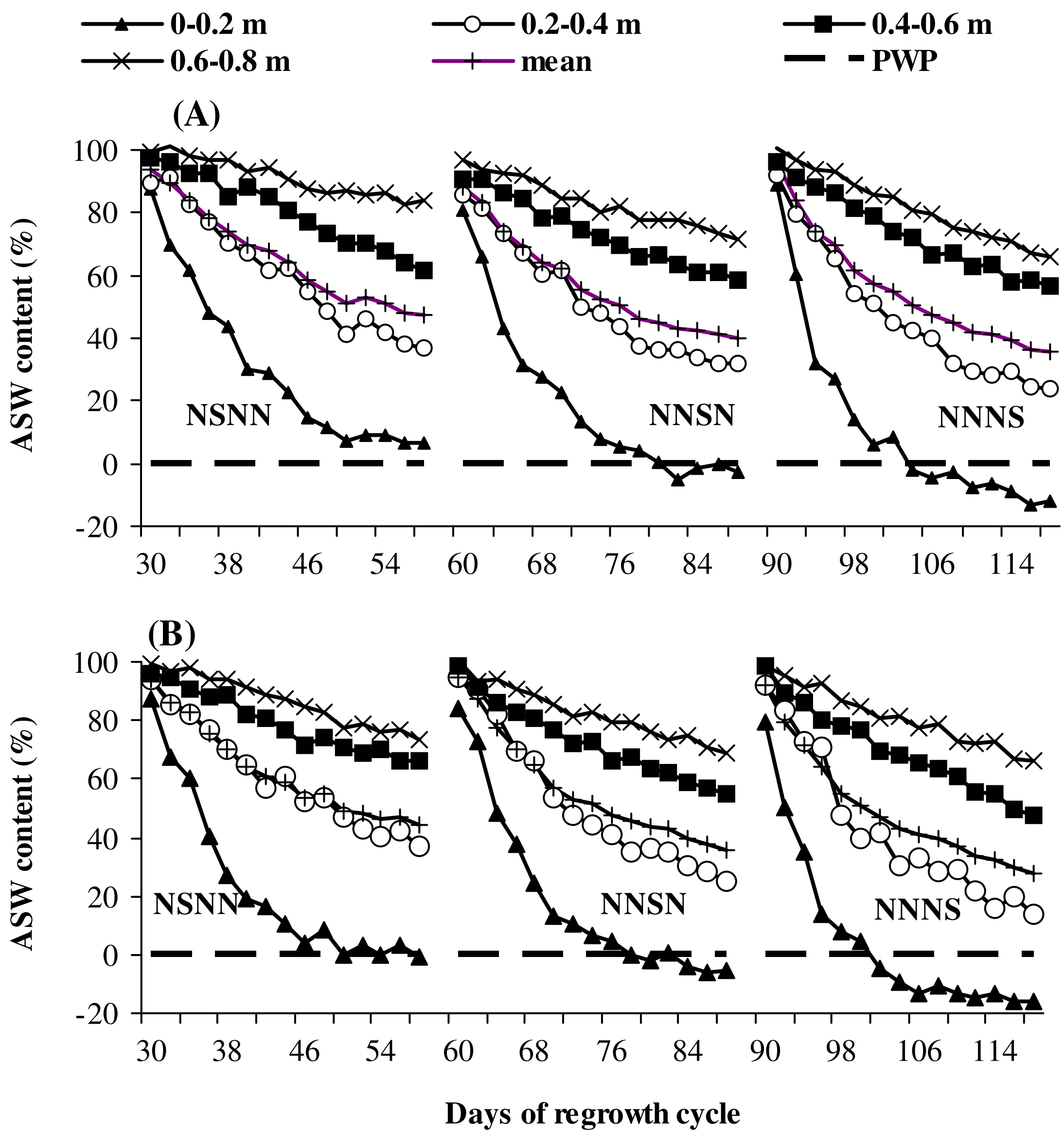

Figure 4.1: Available soil water content per soil layer in the root zone of rose-scented geranium during the one-month irrigation-withholding periods. NSNN, NNSN and NNNS represent irrigation-withholding treatments in the second, third and fourth month of regrowth cycles; (A) Harvest 1 and (B) Harvest 4 were conducted in October 2005 and February 2007, respectively

4.4.2 Herbage growth parameters

Leaf area index (LAI) accumulation during regrowth period

The data presented in Figure 4.2 show that the LAI values obtained differed substantially for the regrowth cycles of Harvests 1 and 4. The LAI in the regrowth cycle for Harvest 1 was very low compared to that of the regrowth cycle for Harvest 4. The major sources for this variation were probably difference in season and plant density. The regrowth for Harvest 1 was during a cool season (25 and 8°C mean maximum and minimum temperature, respectively). The regrowth cycle for Harvest 4, on the other hand, was during a warm to hot season (mean maximum and minimum temperatures of 30 and 16°C, respectively). Plant density was also lower (16000 plant/ha) for Harvest 1 (open field trial) than that for Harvest 4 (rain shelter trial, about 29600 plants/ha).

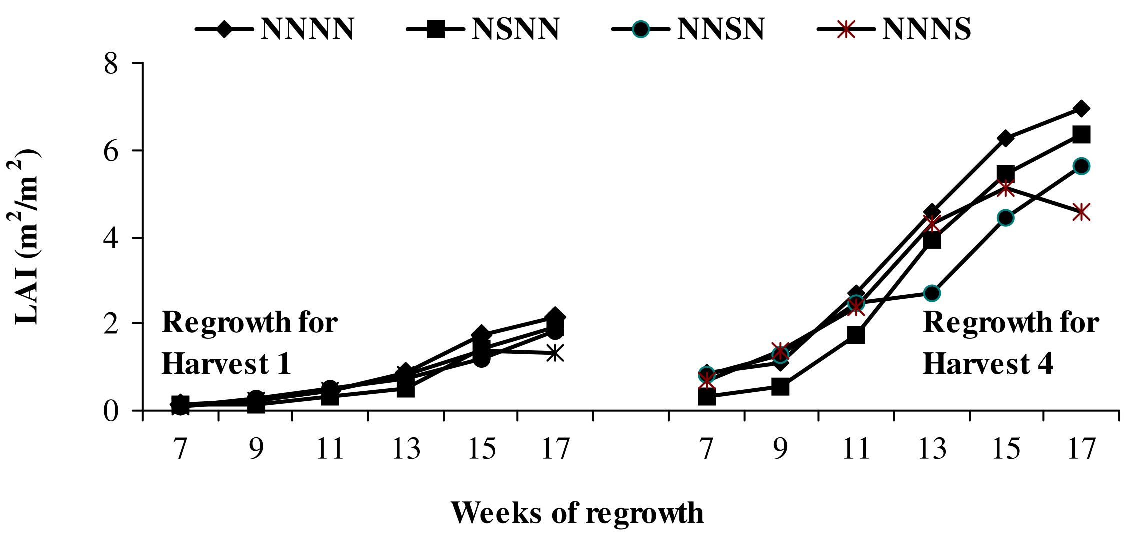

Figure 4.2: Leaf area index growth trends of rose-scented geranium that was water-stressed for one month at different regrowth stages. NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation during the second, third and fourth regrowth months, respectively; Harvests 1 and 4 were conducted in October 2005 and February 2007, respectively

The effect of the irrigation-withholding treatments on LAI development could be clearly seen in the regrowth cycle for Harvest 4. The data show that the impact of the one-month irrigation-withholding period on LAI was affected by the shoot age at which the water stress was imposed. Water stress in the second month of the regrowth cycle resulted in a temporary decline in LAI development. In most cases, irrigation withholding in the third or fourth month of regrowth cycles resulted in a significant reduction in LAI per regrowth cycle (Table 4.1). The reduction in leaf area for the NNNS treatment (compared to the control, NNNN) for Harvests 1, 3 and 4 was 39, 36 and 34%, respectively.

Table 4.1: Maximum LAI of rose-scented geranium that was water-stressed for one month at different regrowth stages

Treatment	Open field		Rain shelter
	Harvest 1	Harvest 3	Harvest 4
NNNN	2.15 a[†]	4.61 a	6.96 a
NSNN	1.90 b	4.44 a	6.34 a
NNSN	1.83 b	3.09 b	5.64 ab
NNNS	1.32 c	2.96 b	4.57 b
Grand mean	1.80	3.778	5.89
CV (%)	6.41	11.58	17.38
LSD (α = 0.5)	0.185	0.7	1.63

[†]Values with the same letter in a column are not significantly different; NNNN, NSNN, NNSN and NNNS represent control and irrigation withholding in the second, third and fourth month of regrowth cycles; Harvests 1, 3 and 4 were conducted in October 2005, November 2006 and February 2007, respectively

The severe negative effect of water stress imposed during the fourth month of regrowth could probably partially be attributed to hastened defoliation of older leaves (data not presented). The general LAI response to irrigation withholding is comparable to the results reported by Karam *et al.* (2005). According to the authors, lag in leaf area growth due to water stress in the earlier growth stages could be compensated for by a stress-free period in the later growth stages of soybean.

Dry matter accumulation trends

The dry matter accumulation trends for the different regrowth cycles (Figure 4.3) were comparable with trends observed for LAI. The higher dry matter accumulation rate during the warmer season (regrowth for Harvest 4) confirms that rose-scented geranium favours warmer temperature regions (Kumar et *al.*, 2001; Lis-Balchin, 2002b; Motsa *et al.*, 2006).

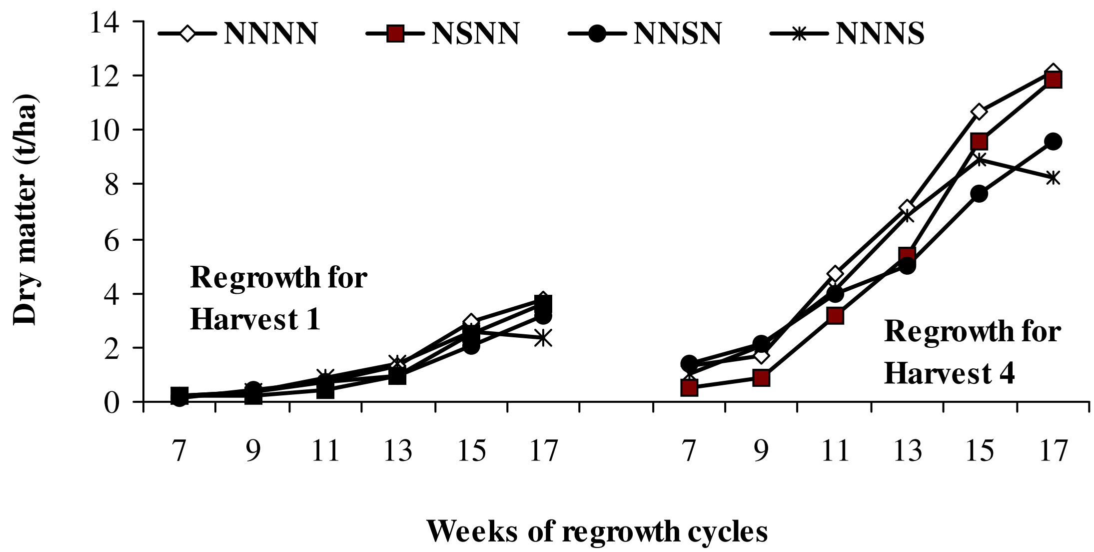

Figure 4.3: Dry matter accumulation of rose-scented geranium that was water-stressed for one month at different regrowth stages. NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation during the second, third and fourth month of regrowth cycles, respectively; Harvests 1 and 4 were conducted in October 2005 and February 2007, respectively

The results presented in Table 4.2 indicate that leaf and stem dry matter contents were not affected by water stress applied during the second or third month of regrowth. In most cases, a significant increase in dry matter content was recorded only for the NNNS treatment, for which the plants were harvested while still in a water-stressed condition. In addition, the data showed that lower dry matter content (%) was recorded for stems than for leaves in the same treatment and harvest.

Table 4.2: Dry matter content (%) of rose-scented geranium that was water-stressed for one month at different regrowth stages

Treatment	Open field				Rain shelter	
	Harvest 1		Harvest 3		Harvest 4	
	Dry leaf mass (%)	Dry stem mass (%)	Dry leaf mass (%)	Dry stem mass (%)	Dry leaf mass (%)	Dry stem mass (%)
NNNN	17.48 a[†]	14.16 a	18.14 c	15.22 a	16.27 c	13.96 b
NSNN	17.80 a	14.45 a	18.74 bc	15.95 a	17.15 b c	14.50 b
NNSN	17.92 a	15.55 a	19.00 b	16.42 a	17.64 b	14.79 ab
NNNS	18.31 a	16.07 a	20.09 a	16.5 a	19.54 a	16.32 a
Grand mean	17.87	15.06	18.99	16.02	17.65	19.89
CV	3.57	8.67	2.62	4.91	4.06	6.59
LSD ($\alpha = 0.5$)	NS	NS	0.797	1.25	1.146	1.57

[†]Values followed by the same letter in a column are not significantly different; NNNN, NSNN, NNSN and NNNS represent control and irrigation withholding in the second, third and fourth month of regrowth cycles, respectively; Harvests 1, 3 and 4 were conducted in October 2005, November 2006 and February 2007, respectively

Total herbage yield per regrowth cycle

The effects of a one-month irrigation-withholding period in different months of regrowth cycles on fresh herbage yield are illustrated in Figure 4.4. In general, withholding irrigation during any of the three regrowth months tended to reduce fresh herbage yield in all harvests, except for the NNNS treatment in Harvest 2. For this regrowth period (fourth month of Harvest 2) the irrigation-withholding period was interrupted by high (248 mm) and well-distributed rainfall (Appendix B). In Harvests 1, 3 and 4, significant fresh herbage mass reductions were recorded for treatments NNSN and NNNS. The yield losses for treatment NNNS, compared to the non-stressed control (NNNN), were 25, 33 and 41%, in Harvests 1, 3 and 4, respectively.

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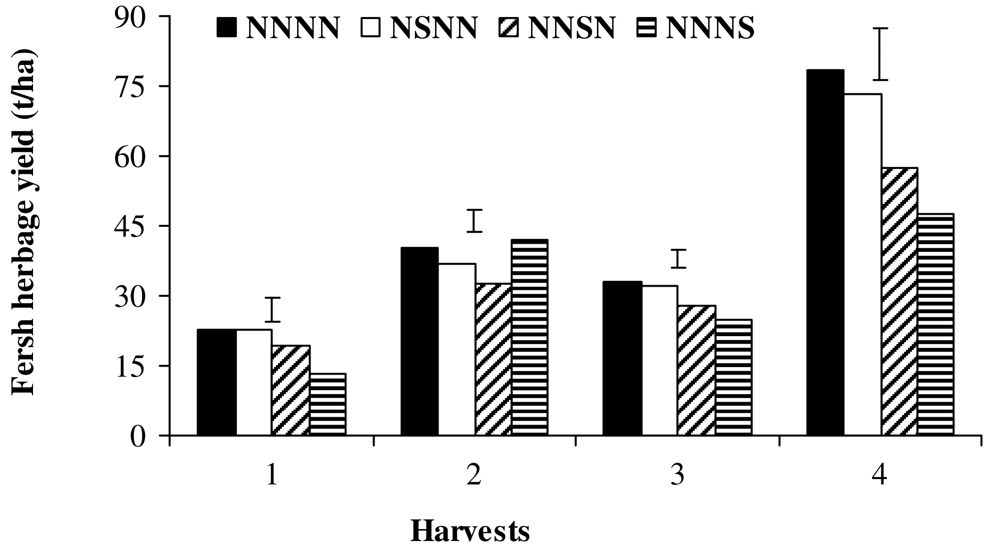

Figure 4.4: Fresh herbage yield of rose-scented geranium that was water-stressed for one month at different regrowth stages. The vertical bars are LSD at α = 0.05); NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation during the second, third and fourth month of regrowth cycles, respectively; Harvests 1, 2, 3 (open field) and 4 (rain shelter) were conducted in October 2005, February 2006, November 2006 and February 2007, respectively

The minor reduction in herbage yield of plants that were water-stressed in the second month of regrowth (NSNN) could be explained by the relatively small canopy size during this early regrowth stage. In such a situation, transpiration rate was presumably low, which could have given the plants a better chance to readjust their physiological processes with relatively slow development of water stress. Withholding water in the later stages (when plants had well-developed canopies) had more serious consequences because transpiration demand was high (Brady & Weil, 1999; De Medeiros, Arruda, Sakai & Fujiwara, 2001). In such conditions, most of the readily available soil water probably was depleted within a short period of time, before the plants had a chance to make physiological adjustments to cope with the water stress

(Bray, 1997). This probably affected plant growth negatively. In agreement with the current results, studies conducted on *Cryptantha flava* revealed that larger plants were more sensitive to drought than smaller plants (Casper, Forseth & Wait, 2006).

The extremely high herbage yield in Harvest 4 (rain shelter) could possibly be explained by the higher plant density used in the rain shelter. In line with this observation, Rajeswara Rao (2002) reported that rose-scented geranium fresh herbage mass increased by 134.4% when it was planted at a 0.6 m x 0.3 m inter- and intra-row spacing, compared to a 1.2 m x 0.3 m inter- and intra-row spacing. In addition, the higher herbage yield from Harvest 2, a regrowth cycle during a warm season (mean maximum and minimum temperatures of 29 and 16°C, respectively), indicates that rose-scented geranium grows better in warm to hot seasons (Weiss, 1997; Motsa *et al*., 2006).

Contribution of leaves and stems to total fresh herbage yield was affected by the irrigation-withholding treatments (Table 4.3). The contribution of leaves to the total herbage yield increased as water stress was imposed later in the regrowth cycle. Thus, it became more noticeable when irrigation was withheld in the last regrowth month (NNNS treatment), except in Harvest 2 (where the NNNS treatment was not successfully applied).

Both the higher percentage fresh leaf mass (out of the total herbage yield) and higher dry matter content of leaves (compared to stems of the same treatment) (Table 4.3), at least partly, imply that rose-scented geranium plants have succulent stems. The extra water stored in the stems could possibly be reallocated to the leaves to balance the presumably lower water potential developed as a result of evapotranspiration losses. This might help plants to overcome brief water-stress conditions. The succulent nature of stems could also be among the long-term water-stress tolerating mechanisms in the *Pelargonium* species, which possibly enable members of the species to follow a crassulacean acid metabolism (CAM) in water-stressed conditions (Jones *et al.*, 2003).

Table 4.3: Fresh leaf mass to total fresh biomass ratio (%) of rose-scented geranium that was water-stressed for one month at different regrowth stages

Treatment	Open field			Rain shelter
	Harvest 1	Harvest 2	Harvest 3	Harvest 4
NNNN	64.25 c[†]	65.50 b	58.40 c	65.40 b
NSNN	68.40 b	67.35 ab	61.02 b	66.04 b
NNSN	68.36 b	69.54 a	63.06 b	68.41 ab
NNNS	71.55 a	64.98 b	65.28 a	71.57 a
Grand mean	68.15	66.84	61.938	67.85
CV (%)	2.68	2.24	2.15	3.71
LSD (α = 0.05)	2.92	2.43	2.13	4.02

[†]Values with the same letter in a column are not significantly different; NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation in the second, third and fourth month of regrowth cycles, respectively; Harvests 1, 2, 3 and 4 were conducted in October 2005, February 2006, November 2006 and February 2007, respectively

4.4.3 Essential oil yield and quality parameters

Essential oil content

Change in essential oil content (percentage oil on fresh herbage mass basis) was not consistent (Figure 4.5). The overall result, however, indicated that oil content tended to be higher in the water-stressed treatments. Except for Harvest 1 (essential oil content was highest in the NNNS treatment), maximum increase in essential oil content was observed when irrigation was withheld during the third month (the NNSN treatment).

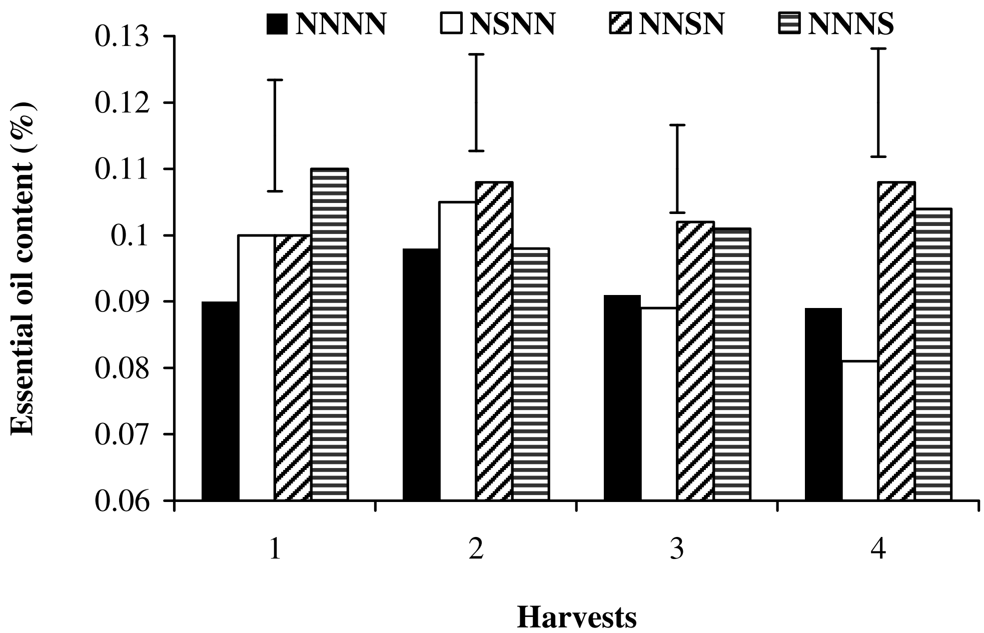

Figure 4.5: Essential oil content (% oil on fresh herbage mass basis) of rose-scented geranium that was water-stressed for one month at different regrowth stages. The vertical bars are LSD at α = 0.05; NNNN, NSNN, NNSN and NNNS represent control, and withholding irrigation during the second third and fourth regrowth months, respectively; Harvests 1, 2, 3 and 4 were conducted in October 2005, February 2006, November 2006 and February 2007, respectively

Similar to the present results, Weiss (1997) reported that essential oil content (percentage oil on fresh herbage mass basis) of rose-scented geranium for a harvests after a three-month wet period was lower than oil content obtained from plants harvested after a three-month dry period. Similarly, aromatic compounds of tea plants increased in water-stressed conditions (Panrong *et al.*, 2006)

Essential oil yield

Figure 4.6 shows the average essential oil yield (kg/ha) for the different treatments. The general response of essential oil yield was similar to that of fresh herbage yield. The present results, therefore, support the report of Srivastava and Luthra (1993), which indicated that secondary metabolites such as essential oils are positively related to primary metabolites. The results of this research also agree with those results reported by Kumar *et al.* (2001) and Motsa *et al*. (2006), which indicated that higher vegetative growth resulted in higher total essential oil yield in rose-scented geranium, even if the percentage oil declined slightly under favourable growing conditions.

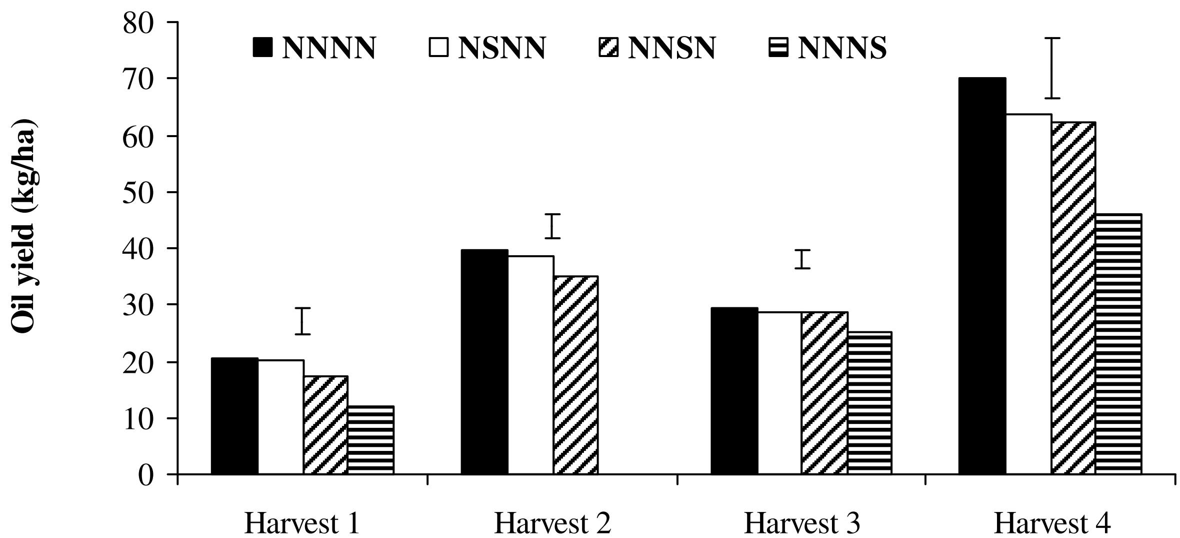

Figure 4.6: Essential oil yield (kg/ha) of rose-scented geranium that was water-stressed for one month at different regrowth stages. The vertical bars are LSD at α = 0.05; NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation during the second, third and fourth month of regrowth cycles, respectively; Harvests 1, 2, 3 and 4 were conducted in October 2005, February 2006, November 2006 and February 2007, respectively

Compared to the fresh herbage yields, essential oil yield was less sensitive to water stress because the latter (essential oil yield) maintained or showed only a marginal reduction when irrigation was withheld in the second or third month of the regrowth cycles. Water stress during the fourth month of regrowth cycles (NNNS treatment) resulted in a significant essential oil yield loss. The losses in essential oil yield caused by irrigation withholding during the fourth month of regrowth in Harvests 1, 2, and 3 (compared to the control, NNNN) were 41, 15, and 34%, respectively.

The increase in oil content (percentage oil on herbage fresh mass basis) and lower oil yield (kg/ha) in the water-stressed treatments suggest that the apparent increase in essential oil concentration in stressed conditions resulted from reduced leaf sizes and low leaf and stem water content. Such phenomena could lead to a reduction in fresh mass, the denominator in calculating percentage oil content. Similar to the present results, Panrong *et al.* (2006) reported that in water stressed conditions, the relative essential oil content increased, but total essential oil yield reduced due to a decline in herbage yield.

The current results contradict the general understanding that plant secondary metabolites, such as essential oils, are enhanced by water-stressed conditions (Yaniv & Palevitch, 1982; Sangwan *et al.*, 2001; Zobayed, Afreen & Kozai, 2007). Similarly, Simon *et al.* (1992) reported that mild to moderate water stress encouraged essential oil production in sweet basil. Weiss (1997) also documented that rose-scented geranium gave a slightly higher essential oil yield in a dry season than in a wet season, while the reverse was true for herbage yield.

Essential oil composition

Due to some technical problems, essential oil analysis for Harvests 3 and 4 could not be done. Gas chromatography (GC) results of the seven major and total trace essential oil constituents for Harvests 1 and 2 are presented in Figure 4.7. In all samples, regardless of irrigation treatment, citronellol was the highest component of the oils (32 ± 2.8%). Neither withholding irrigation nor the harvesting season affected linalool and guaia-6,9-diene concentrations. The overall result shows that the seven major essential oil constituents comprised 77.8% ± 3.1% of the total extracted oil.

In Harvest 1, there was no clear relationship between geraniol and citronellol. The mild increase in geraniol and citronellol contents in this regrowth cycle seemed to be paralleled by decreases in contents of the trace oil constituents. This could not be fully explained by the stress treatments. It could be attributed to some reversible reaction undergone between alcohols (such as geraniol and citronellol) and their esters (part of the trace constituents) in the distillation processes (Babu & Kau, 2005).

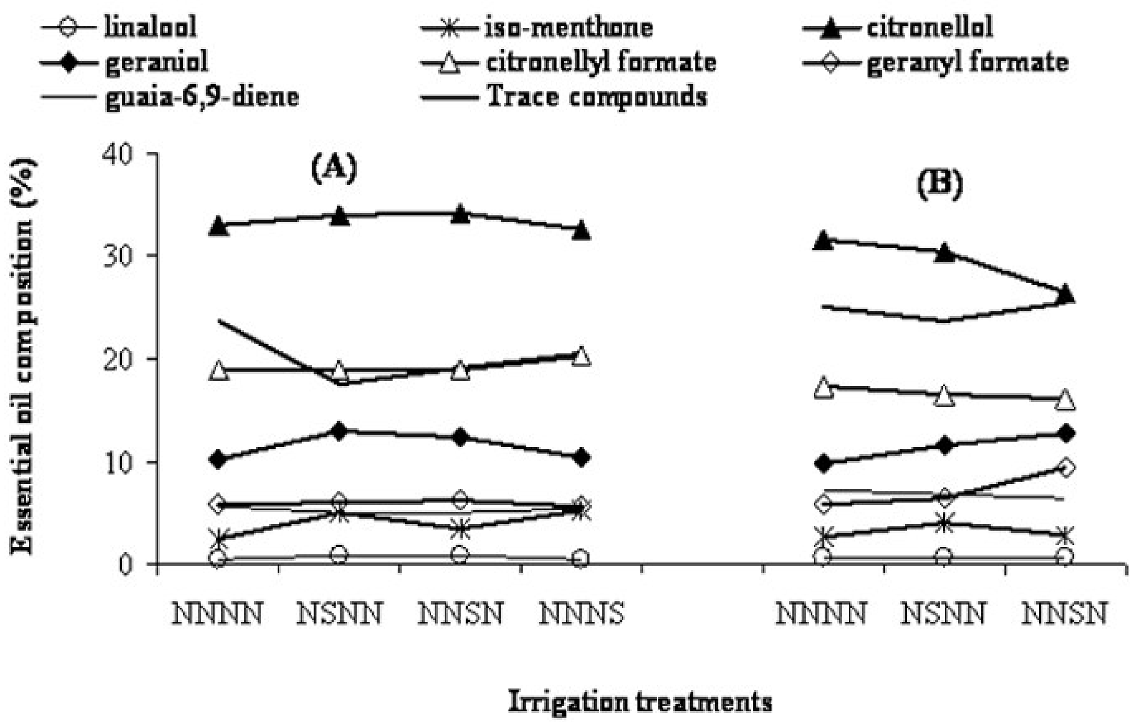

Figure 4.7: Essential oil composition of rose-scented geranium that was water-stressed for one month at different regrowth stages. NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation during the second, third and fourth month of regrowth cycles, respectively; (A) Harvest 1 and (B) Harvest 2 were conducted in October 2005 and February 2006, respectively

In Harvest 2, a progressive increase in the concentration of geraniol and geranyl formate was accompanied by reductions in citronellol and citronellyl formate content in the treatments stressed towards the harvesting. The general relationship among these groups of compounds agree with previous reports (Rajeswara Rao *et al.*, 1996) which indicated that geraniol and geranyl formate were negatively related to citronellol and citronellyl formate. Contrary to the patterns observed in the current results, however, Rajeswara Rao *et al.* (1996) indicated that water stress favoured citronellol and its ester concentrations.

Citronellol and geraniol levels and the ratio of these two components are usually primary indicators of oil quality. Although a C:G ratio in the range of one to three is acceptable, the most desirable in the perfumery and fragrance industries is a 1:1 ratio (Motsa *et al.,* 2006). In both harvests, the C:G ratio was consistently higher in the control (about 3.2) compared to that in the NSNN and NNSN treatments (which ranged between 2.1 and 2.8). The current results, therefore, indicate that water stress in the second or third month of regrowth improved oil quality by reducing the C:G ratio.

4.4.4 Water use and water-use efficiency (WUE)

Results of irrigation applied and evapotranspiration water losses (per harvest) are summarised in Table 4.4. Soil water data for Harvest 2 from the open field are not presented because the NNNS treatment was interrupted by intensive rainfall. Water applied was considerably higher in the non-stressed plots (NNNN treatment), and lowest when the irrigation was withheld in the last regrowth month (the NNNS treatment). These results support earlier reports, which associated evapotranspiration rate with high soil water (Wallace, 2000).

The amount of water applied was almost the same as the evapotranspiration for the NNNN, NSNN and NNSN treatments. In the NNNS treatment, a considerable difference was observed between the amount of water applied and used (evapotranspiration). For this treatment (NNNS), the amount of irrigation was substantially less, as the profile (root zone) was not refilled at the end of the season. The amount of irrigation water saved by withholding irrigation in the third month of regrowth (NNSN), with only marginal changes in essential oil yield, ranged between 33 and 46 mm (equivalent to 330 to 460 m^3 of water per hectare per growth cycle).

Table 4.4: Total irrigation applied and amount of water used by rose-scented geranium that was water-stressed for one month at different regrowth stages

Treatments	Open field		Rain shelter
	Harvest 1	Harvest 3	Harvest 4
	------------------Irrigation applied (mm) -----------------------		
NNNN	346 a[†]	362 a	506 a
NSNN	316 a	329 a	467 a
NNSN	313 a	316 a	463 a
NNNS	268 b	259 b	392 b
Grand mean	310.8	316.5	457.0
CV (%)	7.1	9.0	7.8
LSD (α = 0.05)	35.2	45.8	57.0
	------------- Evapotranspiration loss -----------------		
NNNN	355 a	361 a	502 a
NSNN	326 a	330 a	467 a
NNSN	319 a	321 a	457 a
NNNS	318 a	321 a	450 a
Grand mean	329.5	333.3	469.0
CV (%)	9.9	11.1	7.1
LSD (α = 0.05)	NS	NS	NS

[†]Values with the same letter in a column are not significantly different; NNNN, NSNN, NNSN and NNNS represent control (no stress), stress during the second, third and fourth month of regrowth cycles; Harvests 1, 3 and 4 were conducted in October 2005, November 2006 and February 2007, respectively

The results in Figure 4.8 indicate that the overall water-use efficiency (WUE) values for Harvests 1 and 3 (in the open field) were influenced by season. The WUE was higher in

Harvest 3, a regrowth cycle in higher temperatures (mean maximum and minimum of 28 and 12°C, respectively), than in Harvest 1 grown during lower temperatures (mean maximum and minimum of 25 and 8°C, respectively).

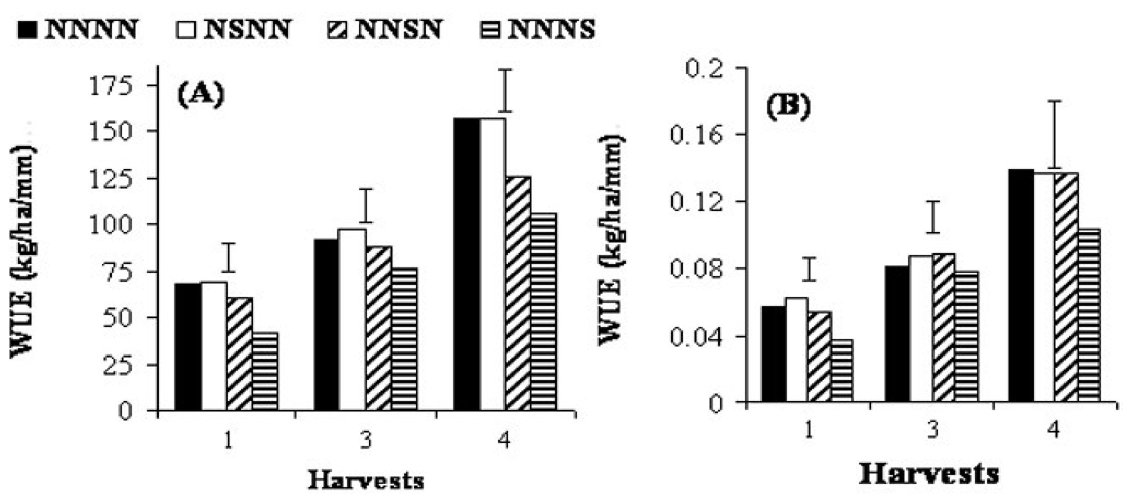

Figure 4.8: Water-use efficiency (WUE) (kg/ha/mm) of rose-scented geranium that was water-stressed for one month at different regrowth stages: (A) on fresh herbage mass and (B) on essential oil yield basis. The vertical bars are LSD at α = 0.05; NNNN, NSNN, NNSN and NNNS represent control and withholding irrigation during the second, third and fourth month of regrowth cycles, respectively; Harvests 1, 3 and 4 were conducted in October 2005, November 2006 and February 2007, respectively

The higher WUE (in terms of herbage yield) recorded for the NNNN and NSNN treatments (Figure 4.8a) was consistent with results reported for alfalfa (Saeed & El-Nadi, 1997) onion (Kadayifci *et al.*, 2005) and cucumber (Şimşek, Tonkaz, Kaçira, Çömlekçioğlu & Doğan, 2005). These findings support the ideas of Bessembinder *et al.* (2005), who stated that well-watered plants would result in higher water-use efficiency, provided that other factors such as soil nutrients are not limiting.

Results presented in Figure 4.8b indicated that WUE, in terms of essential oil produced, considerably reduced only when the water stress was applied in the fourth month of regrowth.

This observation, together with the marginal/negligible reduction in oil yield caused by water-stressed condition during the second and the third months of regrowth, implies that withholding irrigation during these regrowth stages would be possible without compromising essential oil yield. Such irrigation management strategy would save water, which could be used to avoid severe water stress in the fourth month of regrowth of the crop, to expand the irrigated land area or to alleviate water shortages in other economic and social service sectors, where freshwater is a limiting factor (Ali, Hoque, Hassan, & Khair, 2007; Bouman, 2007).

4.5 CONCLUSIONS AND RECOMMENDATIONS

The present study reveals that essential oil yield is positively related to biomass production. Essential oil concentration apparently increased in water-stressed conditions, but its contribution was not large enough to compensate for the essential oil loss as a result of reduction in herbage yield. A significant decline in essential oil yield was observed only when the crop was stressed in the fourth month of regrowth. Hence, farmers are advised to avoid severe water stress during the last month before harvest. In freshwater-scarce regions, withholding irrigation during the second and third months of regrowth of rose-scented geranium could improve water productivity, because the technique would save water that could be used to irrigate the crop during more water-stress-sensitive regrowth stages (fourth month of regrowth cycle), to expand the irrigated land area, or to alleviate freshwater shortage in other economic and social service sectors. Specifically, in cool weather conditions, when rose-scented geranium growth rate is relatively slow, this study suggests that increasing planting density could improve essential oil yield per hectare.