

Effect of Leaf Age on Essential Oil Yield and Composition in Rose-scented Geranium

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Abstract. Knowledge of essential oil content and composition of leaves of different ages could be used as a guide for the right herbage harvesting stage in rose-scented geranium. Change in essential oil yield and composition with leaf age in rose-scented geranium was investigated in a glasshouse of the University of Fort Hare, during the 2012 and 2014 crop seasons. The topmost five pair of leaves on shoots were separately harvested as treatments. Leaf fresh and dry mass were significantly lower in the topmost and the oldest leaf pair. Essential oil in the topmost pair was colorless; but with advance in leaf age, the oil tended to have a blue-green color. Oil content (on a dry mass basis) from the topmost to the bottom most were about 7.0%, 4.9%, 3.2%, 2.4%, and 1.9%, respectively. Oil yield was consistently the highest in the second youngest pair of leaves, and it progressively declined with leaf age. Contributions of the five leaf pairs from the topmost to the bottom most, in respective order, to the total yield were 19.3%, 22.0%, 17.71%, 12.03%, and 8.5%. The citronellol:geraniol ratio was lower in the young leaves than in the old leaves. Linalool and geranyl formate concentrations were the highest in the youngest leaves, and the opposite was true of isomethone. The current results indicate shorter regrowth cycles would increase essential oil yield and quality of rose-scented geranium, provided an efficient harvesting technique was innovated.

Rose-scented geranium (*Pelargonium* species) is a shrubby, herbaceous, perennial plant that belongs to the Geraniaceae family. In South African rural communities, the *Pelargonium* species are known for their use in herbal medicine (Lis-Balchin, 2002). However, at a commercial level, the rose-scented geranium is cultivated for its essential oil, which is produced in glands (trichomes) throughout the green parts of the plant, mainly on the leaves (Rhind, 2012). Rose-scented geranium essential oil is an important component of the different products used in the perfumery, aromatherapy, pharmaceutical, and food-processing industries (Prins et al., 2010; Singh, 1999).

Rose-scented geranium essential oil is composed of more than 120 compounds that belong to different organic compound classes, acids, alcohols, aldehydes, esters, and ketones (Demarne and Van der Walt, 1993; Williams

and Harborne, 2002). The major constituents of the oil are citronellol, geraniol, iso-metone, citronellyl formate, and geraniol formate (Peterson et al., 2006; Weiss, 1997). Market value of rose-scented geranium essential oil is determined by composition (the proportion of the compounds, mainly the citronellol-to-geraniol ratio) and the quantity supplied.

Today the demand for geranium oil worldwide is estimated to be around 600 tons per year (Eiasu, 2009; Shawl et al., 2006). About 20–25 tons of geranium oil are required to close the world's demand for essential oil (Demarne, 2002; Eiasu, 2009). According to Schwab et al. (2008), the international trade of essential oil increases annually on average by 10% a year.

It is well documented that plant developmental stages as well as leaf ages have a strong effect on the volatile composition of aromatic plants. For instance, in sage (*Salvia officinalis*), higher camphor content was found on young growing leaves (Croteau et al., 1981). Havkin-Frenkel and Belanger (2008) also concluded that there are dramatic differences among the leaves of the same plant, depending on their age and position. According to the authors, the lower leaves contain a lower concentration of volatiles compared with the upper, younger leaves—and also their composition differs. Singh et al. (1989) confirms that young, expanding leaves are biogenetically more active than mature leaves. Furthermore, an experiment

on peppermint showed that there is little de novo synthesis of monoterpenes from 14 CO₂ in mature leaves, and more in immature leaves that are still expanding (Croteau et al., 1981). Research on *Cistus ladanifer* showed that young leaves produce more flavonoids and diterpenes (Masa et al., 2016). Thus, leaf age is an important factor affecting essential oil content and composition.

Information on essential oil yield and composition as affected by leaf age and position in rose-scented geranium is limited. Literature does reveal that essential oil and composition depend on the shoot age of aromatic plants (Motsa et al., 2006). Therefore, an investigation into leaf age (position) would help rose-scented geranium producers to develop efficient harvesting cycles. Hence, the main objective of the current research was to examine rose-scented geranium oil yield and quality as affected by leaf age and position. We aimed to quantify the contribution of each leaf age group.

Materials and Methods

Growing system and planting culture. The experiments were conducted in Jan.–May 2012 (Harvest 1) and June–Oct. 2014 (Harvest 2) in a glasshouse at the University of Fort Hare, Alice Campus, South Africa (a latitude and longitude of 25°45' S and 28°16' E, respectively, and an altitude of 535 m above sea level). Light penetration through the polycarbonate roofing sheet was about 90%. A computer-controlled cooling (wet pad and fan) system was set to regulate the temperature when it elevated to more than 25 °C. This was because temperatures could surge up to 35 °C for few hours during the hottest days. Mean minimum and maximum temperatures for Harvest 1 (summer–autumn) were 21 and 33 °C, respectively, and that of Harvest 2 (winter–spring), 12 and 26 °C, respectively.

Healthy stem cuttings of rose-scented geranium (*Pelargonium* spp.), taken from healthy growing plants (in a tunnel), were raised in seedling trays (filled with Hygromix, a commercial rooting media) in a mist bed for 40 d. Uniform, healthy plantlets were transplanted to 10-L pots filled with Luvisol soils, collected from the University of Fort Hare Research Farm. For uniform growth, the plants were grown for 3 months. Then the plants were cut back and allowed to regrow for about 4 months.

Treatments and research design. The rose-scented geranium regrowths were harvested at about 4 months of age (at 12-leaf stage). On average, the age difference between two leaves on consecutive nodes was about 1 week. During harvesting, the five top pairs of leaves were separately collected and used as treatments as follows: in Treatment 1 (T1), the topmost pair of leaves (open but still not fully expanded) or youngest pair; in Treatment 2 (T2), the second topmost pair of leaves (just fully open), or second youngest; in Treatment 3 (T3), from the top the third pair of leaves, or third youngest; in

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Treatment 4 (T4), from the top the fourth pair of leaves, or second oldest; and in Treatment 5 (T5), the fifth pair of leaves from the top, or oldest pair.

The experiment was a randomized complete block design (RCBD) replicated four times. Within each of the four blocks, the different leaf pairs were positioned vertically on the plants. From each plot, 15 plants were harvested, which gave about 0.5 kg of each leaf pair.

Agricultural practices. Each potted rose-scented geranium plant received 3 g nitrogen (N), 4.5 g phosphorus (P), and 3 g potassium (K) [in the form of 2:3:2 (22) NPK fertilizer granules] as a split application in Week 1 and Week 7 of each regrowth cycle. In addition, 1 g N (as ammonium nitrate) and 1 g K (as potassium chloride) were applied to each pot in Week 9 of each regrowth cycle. To avoid possible high nutrient (K and N) levels, plants were over-irrigated on the first and second day of each regrowth cycle, before the application of new fertilizer (Eiasu, 2009). Plants were well watered on well-drained soil (a sandy clay loam). Municipality water (with 7.4 pH and 38.5 mS/m electrical conductivity) was used as source of irrigation water. A spaghetti drip irrigation system with Gardena Timer (Electronic C 14e, Germany) was used. The irrigation system was set to water the plants three times a day, around 1000, 1400, and 1700 HR. Irrigation duration ranged between 2 and 8 min per irrigation event (that is 150 mL to 600 mL), depending on the growth stage and temperature of the growing system.

Data collected and statistical analysis. Immediately after harvesting, the pairs of leaves from the stem were put in separate plastic bags. The total sample mass was then taken, and the number of leaves per sample (replication) was determined. Later the data were used to determine the average oil content per fresh weight and yield per leaf. Leaf area was measured using an LI 3100 belt-driven leaf area meter (LI-COR, Lincoln, NE).

The leaves of each replication were distilled using the hydro-distillation technique, in a 10 L capacity bottle flask, mounted on a heating mantle equipped with a thermostat. The leaves were boiled for 2 h until there were no traces oil recovery. The condensed oil was separated with a Clevenger receiver and stored in amber-colored bottles to reduce the possible effect of light. After the hydrosol was drained from the distillation flask, the leaves were oven-dried at about 68 °C until a constant mass was achieved, to determine leaf dry matter.

To determine composition, samples of essential oil from each treatment replications were sent to the Dohne Laboratory of the Department of Agriculture and Rural Development, Stutterheim, Eastern Cape. Gas-chromatography (GC) analysis procedure was used to identify and quantify the essential oil constituents. The analysis method used an Agilent GC (FID) model 6890N (Agilent Technologies, Inc., Santa Clara, CA) fitted

with a 30-m × 0.25-mm fused silica capillary column and a film thickness of 0.25 µm. Helium gas was the carrier. The GC-analysis temperature was set to increase from 50 to 200 °C with increments of 5 °C·min⁻¹. In addition, a temperature of 220 °C was used for the detector and injector. Essential oil constituents were identified by comparing their retention time and retention indices to standard values in the library of the GC-analyzer machine (Adams, 2004).

Data analysis. The data were statistically analyzed using a two-way analysis of variance by SPSS software, and the comparison of the means of yield and constituent for essential oil was determined with least significant difference at $P \leq 0.05$ level.

Results and Discussion

Total leaf fresh weight per growth. The results of the leaf fresh weight for both harvests are presented in Fig. 1. The overall fresh leaf mass was higher in Harvest 1 (regrowth during summer season) than in Harvest 2 (regrowth during winter season), which supports the reports that indicated warm season enhances vegetative growth in rose-scented geranium (Eiasu et al., 2012; Motsa et al., 2006). Generally, there was a difference on the leaf fresh weight of different treatments (leaf age). The results suggest that although the leaves were collected from the same shoot, they differed in fresh weight with change in age and/or position.

Fresh leaf mass was the lowest in the youngest leaf pair (T1). This is because the first two pairs of leaves would have just opened, and they were small in size as well as in dry matter content. Fresh weight progressively increased with leaf age, reaching the highest at the second oldest pair of leaf (T4). The slight declining tendency in fresh leaves on the fifth pair (T5) could be as a result of translocation of nutrients to the

young leaves (Dixit and Srivastava, 2000). Bhakta-Guha and Ganjewala (2009) also indicated that the slight decline in fresh weights of leaves determine the beginning of leaf senescence in old leaves.

Dry matter accumulation. Dry matter content of the harvested rose-scented geranium leaves followed similar trends to that of fresh leaf mass (Fig. 2). The lowest dry matter content was recorded for the topmost pair of leaves, and it progressively increased. It reached the maximum at the second pair from the bottom (Treatment 4). The largest dry matter content difference was observed between T1 and T2 (especially in Harvest 1, T2 had an increase of about 10%).

Essential oil content. The results of the study also indicate that the oil content (% on leaf dry mass basis) shows declining trends as leaf age advanced (Fig. 3). The highest declining rate was observed between the topmost pair (T1) and the second topmost leaves, and this drastic declining rate could be attributed to different sizes of leaves. These trends indicate that the oil is produced at early growth stage in a limited time period, as reported by Gupta and Ganjewala (2015) on *Cymbopogon flexuosus*. On the other hand, dry matter continued to build up throughout the leaf expansion and thickening stages until the senescence stage approached, as recorded in Figs. 1 and 2. Moreover, it was observed that up to certain leaf age, the decline in oil percentage may not imply a decrease in the actual oil content; rather, it is a reflection of continuous increases in dry mass deposition.

In addition, it emerged from the result of the study that, although at slower rate, the oil content declining rate continued up to the bottommost (oldest) pair of leaves. In both harvests, oil content was lower in the fully expanded (older) leaves. According to Eiasu et al. (2012), the essential oil per leaf remains the same. The authors suggested that the number of oil glands and oil yield per leaf

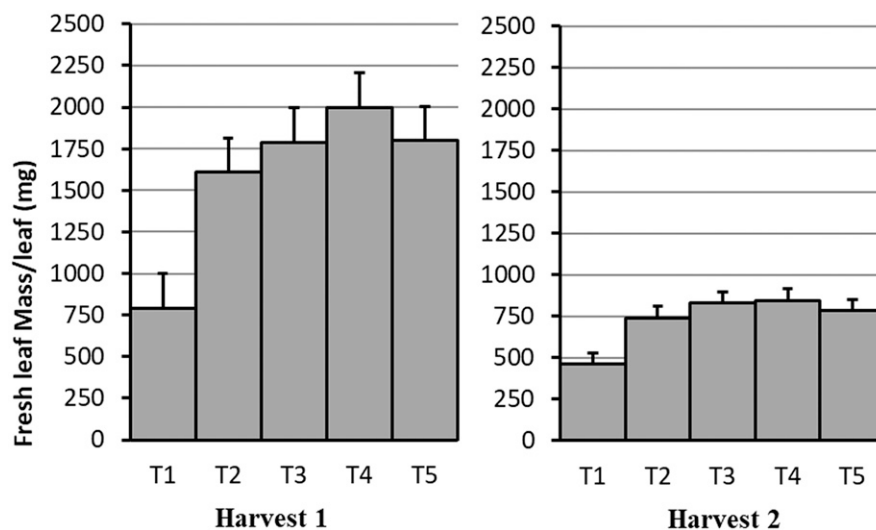


Fig. 1. Leaf fresh weight of rose-scented geranium. The vertical error bars are least significant difference ($\alpha = 0.05$); T1, T2, T3, T4, and T5 represent the topmost, second topmost, middle, second bottom most, and fifth pair of leaf, respectively.

are determined at the leaf initiation (primordial) stage (no new oil glands are produced when the leaves continue to expand). In agreement with the above statement, Motsa et al. (2006) reported that younger shoots had higher oil percentage. However, the opposite was observed on the dry mass accumulation. Oil content showed a fast drop between Treatment 1 and 2 of this study, which could be attributed to the increase in dry mass. A study by Turner et al. (2000) indicates that once the essential oil trichome is formed, it fills within hours. However, their observation differs from that of Eiasu et al. (2012) because they (Turner et al., 2000) claimed that new trichome formation continues through the leaf expansion period in peppermint.

Essential oil yield. Figure 4 shows essential oil yield of rose-scented geranium as affected by leaf age. The two first topmost pairs leaves (T1 and T2) were active in accumulating essential oil, as it showed an increasing trend up to the second topmost leaf pair (T2). Consistently, the highest essential oil yield was obtained from the second topmost leaf pair. In both experiments, oil yield tended to significantly reduce more in the older leaves than in the second topmost pair of leaves. These results could be attributed to loss of the volatile oil to the atmosphere and low (or no) further biosynthesis of the volatile oils.

The current results, to certain extent, are commensurate with the findings of Motsa et al. (2006). These researchers attempted to look at effect of shoot age (not individual leaves). They discovered that younger shoots gave more essential oil yield per fresh weight in rose-scented geranium. In agreement with the above reports, Gebremeskel (2014) discovered that total oil yield per hectare continues to increase until reaching a maximum at the age of 120 d after transplant. Plants harvested later than 120 d start to lose their oil yield (Rocha et al., 2014). On the other hand, no significant differences were observed on essential oil yield of lemon grass (*Cymbopogon citratus*) that were harvested at different ages (3, 6, 9, and 12 months after transplant).

Essential oil composition. Geranium essential oil consists of several compounds that are responsible for its odor. The major components are linalool, iso-methane, citronellol, geraniol, and geranyl and citronellyl formates (Eiasu, 2009; Verma et al., 2010). These essential oil components are present in the oil at different concentration levels (Rajeswara Rao et al., 1996). The results of the current experiment showed that rose-scented geranium oil composition changed as leaf age advanced (Table 1).

The most sensitive essential oil components to leaf age were citronellol and geraniol, and their respective formates. Geraniol and geranyl formate were at their highest level at the topmost leaf pair and progressively declined as the leaf age advanced. The level (%) of citronellol and citronellyl formate showed an increasing trend as the leaf age advanced. Similarly, Gupta and Ganjewala

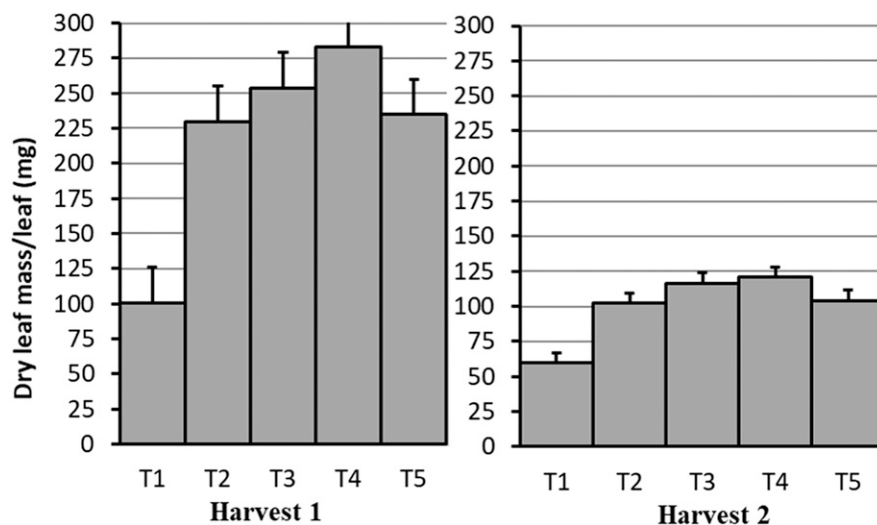


Fig. 2. Rose-scented geranium leaf dry matter content (%) as affected by leaf age. The vertical error bars are least significant difference ($\alpha = 0.05$); T1, T2, T3, T4, and T5 represent the topmost, second topmost, middle, second bottom most, and fifth pair of leaf, respectively.

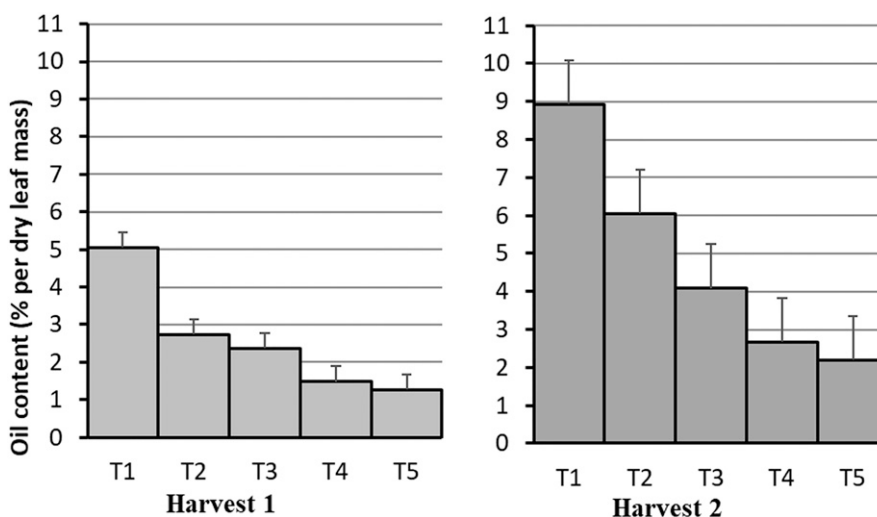


Fig. 3. Rose-scented geranium essential oil content (% on dry mass basis) as affected by leaf age. The vertical error bars are least significant difference ($\alpha = 0.05$); T1, T2, T3, T4, and T5 represent the topmost, second topmost, middle, second bottom most leaf pair, and fifth pair of leaf, respectively.

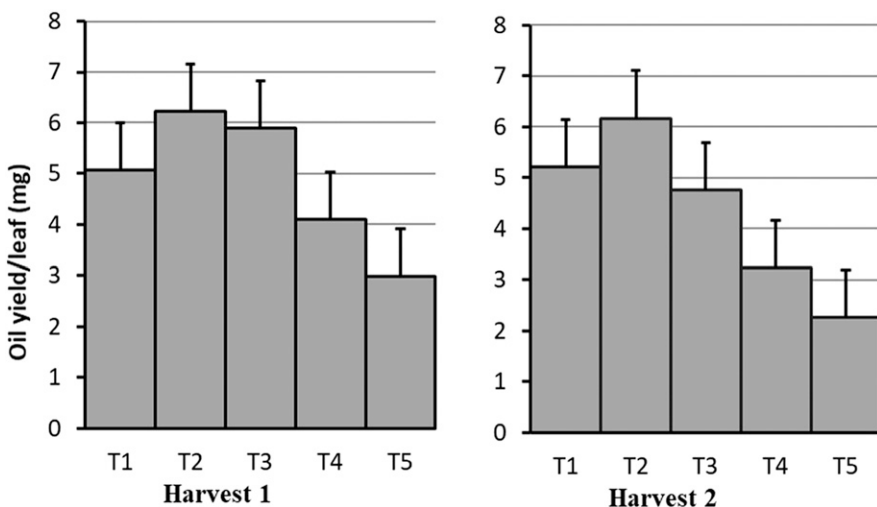


Fig. 4. Essential oil yield of rose-scented geranium as affected by leaf age/position. The vertical error bars are least significant difference ($\alpha = 0.05$); T1, T2, T3, T4, and T5 represent topmost, second topmost, middle, second bottom most, and bottom most leaf pairs, respectively.

Table 1. Percentage composition of the major components of rose-scented geranium (*Pelargonium* spp.) essential as affected by leaf age.

Treatments ⁱ	Citronellol	Citronellyl formate	Geraniol	Geranyl formate	Linalool	Menthone	Guaia-6, 9-diene
T1	28.51 b	15.42 c	18.463 a	7.72 a	7.28 a	2.87 d	5.64 b
T2	30.82 b	18.02 b	13.40 b	7.02 ab	4.86 b	3.31 cd	6.76 a
T3	32.52 ab	18.91 b	12.63 bc	6.69 bc	4.91 b	3.70 bc	5.81 b
T4	34.09 a	19.03 a	11.12 d	5.38 d	4.63 b	4.14 ab	4.74 c
T5	33.15 a	18.02 b	11.79 c	6.11 dc	4.38 b	4.23 a	5.33 bc
Grand mean	31.8	17.88	13.48	6.58	5.211	3.65	5.65
CV (%)	5.80	3.76	6.62	9.63	13.94	11.79	8.89
LSD ($\alpha = 0.05$)	2.54	0.93	1.23	0.87	1.00	0.593	0.693

ⁱT1, T2, T3, T4, and T5 represent topmost, second topmost, middle, second bottom most, and bottom most leaf pairs, respectively. Mean values in each column that share the same letter are not significantly different at a 5% probability level.

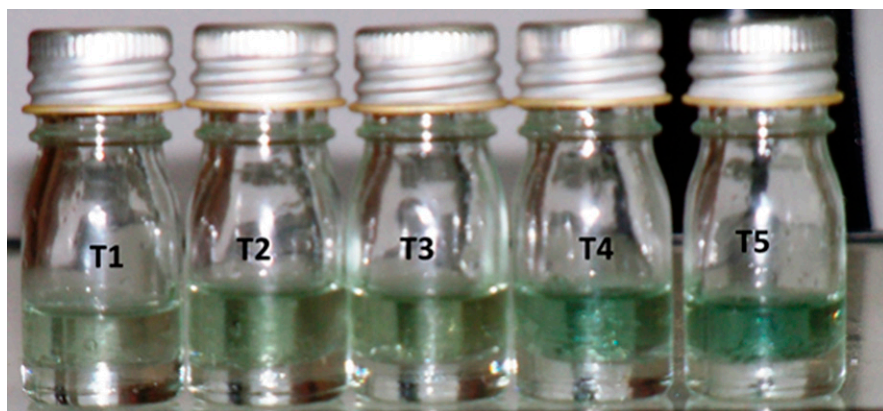


Fig. 5. Color change of rose-scented geranium with leaf age; T1, T2, T3, T4, and T5 represent topmost, second topmost, middle, and second bottom most, and bottom most leaf pairs that were considered as treatments.

(2015) demonstrated that geraniol content decreases as the age of *Cymbopogon flexuosus* progresses. In general, geraniol and citronellol showed an inverse relationship, which is consistent with research findings of Eiasu et al. (2008a, 2008b).

The citronellol to geraniol (C:G) ratio in T1, T2, T3, T4, and T5 were 1.54, 2.30, 2.56, 3.07, and 2.81, respectively. These figures indicate that citronellol reduce (be lost) at a relatively slower rate as compared with geraniol. These results are in line with the Gupta and Ganjewala (2015) findings on *Cymbopogon flexuosus*, which proved that geraniol decrease with the advance in leaf age.

For unknown reasons, the essential oil obtained from different leaf age groups showed different colors. The essential oil from the topmost leaves (youngest pair) were almost colorless. As the leaf age progressed, the color of oil progressively changed to green or blue-green. This phenomenon may need further investigation to identify the compound resulting in the different colors as the age of the leaves progresses (Fig. 5).

It is evident that leaf age (position) plays an important role in determining the quality and quantity of the essential oil of rose-scented geranium. Consistently, essential oil accumulation reaches its highest peak before accumulation of dry matter. Essential oil per leaf reached maximum oil yield at the second

topmost open leaf pair, then it progressively declined as the leaves lost the volatile oil to the air. Biosynthesis and loss of the essential oil components showed the same trends; except citronellol, which was the highest in the second topmost pair of leaves, whereas all the other components continued to decline more or less in a similar rate. Best quality (low C:G ratio) was in the younger leaves than in the older leaves. Therefore, it is recommended that if effective harvesting techniques are to be developed, harvesting young shoots at stage of 5 to 6 open leaf stage would improve yield and composition of rose-scented geranium oil.

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