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Research Article

Comparison in essential oil of *Rosa damascena* Mill. during harvest period under calcareous soil conditions

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Abstract

***Rosa damascena* Mill. is the most important scented rose species which cultivated for essential oil production in the world. The objective of the present study was to understand how different harvest dates influenced the essential oil yields and chemical compounds in petals of *Rosa damascena* Mill. planted for the first time under calcareous soil conditions, Sa′adat Shahr region. In the current survey, flowers were collected four times with three replications from four years old plants between 05:00 and 06:00 AM (2nd, 9th, 16th and 23rd of May, 2020). The essential oils of samples were extracted by hydro-distillation system (Clevenger apparatus) and were analyzed by a combination of GC-FID and GC-MS techniques, to check for chemical variability. The essential oil content of flowers harvested at different times ranged from 0.046% to 0.082% (w/w). The number of identified compounds were 30, of which 21 were common. The highest content of citronellol + geraniol (60.9 %), which are the most important constituents of its essential oil, was observed when the flowers were harvested in the 2nd of May. However, the lowest content of them (40.6 %) was found in the flowers of the fourth week of harvesting. *n*-nonadecane and *n*-heneicosane continuously increased from the first week (17.4 %) to the fourth week (34.8 %) of the harvest. This experiment revealed that different harvest dates can significantly modify the performance and composition of Damask rose' essential oil. Moreover, calcareous soil conditions could be used to obtain essential oil of *Rosa damascena* Mill.**

Keywords: Chemical variation, Citronellol, Essential oil, Geraniol, Harvest period

Introduction

Rosa genus consists of more than 200 species but not many are being used for essential oil construction (Khaleghi and Khadivi, 2020). The famous and popular hybrid between *Rosa gallica* and *Rosa phoeinica* is *Rosa damascena* Mill. (generally known as Damask rose) which belongs to Rosaceae family (Venkatesha *et al*., 2022). This species is called as king of flowers and Iranians are the first to realize the oral and therapeutic properties of rose from the ancient past as well as it is exported to European countries. Nowadays, Iran is one of the largest producers of *Rosa damascena* Mill. in the world. Furthermore, the major buyers of Iranian roses are Persian Gulf countries and to a lesser extent, European countries (Ersan and Basayigit, 2022; Toluei *et al*., 2019). *Rosa damascena* Mill. is generally preferred for its highly prized rose oil. In the perfume industry, *Rosa damascena* Mill. is the most important species for the producing of attar of rose, made by distilling volatile oils from the flowers. This species is also used widely in the manufacture of cosmetics, beverages, soft drinks, ice-creams and as a fragrance component in ointments and lotions. Besides its major application in perfume and cosmetic industries, *Rosa damascena* Mill. essential oil has been reported with valuable pharmacological properties such as antibacterial, antioxidant, and cytotoxic activities. The essential oil of the damask rose is a more beneficial commodity often regarded as 'liquid gold' traditionally known as 'attar' or 'otto' (Peron *et al*., 2021; Onder *et al*., 2021).

Essential oil is located in the upper part of the petals and inside the cells with a prominent appearance in the flowers of *Rosa damascena* Mill. It has been identified

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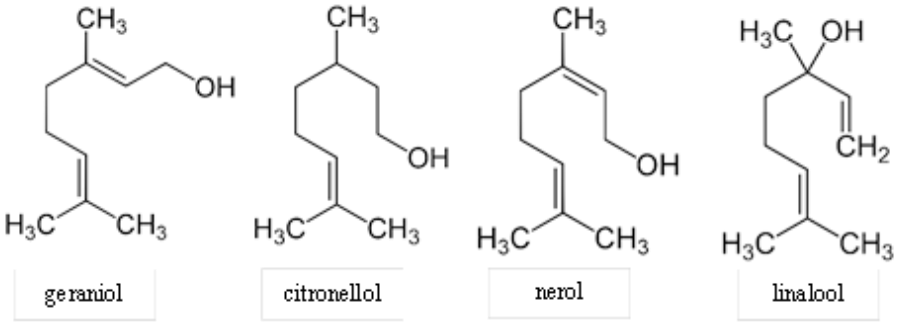

Fig. 1. Chemical structures of geraniol, citronellol, nerol and linalool

more than 300 volatile constituents in damask rose oil which belongs to a various group of compounds including monoterpenes, long-chain hydrocarbons, and metabolites from carotenoid degradation (Nunes and Miguel, 2017; Pal *et al*., 2013). The most important constituents of its essential oil are geraniol with formula $C_{10}H_{18}O$, citronellol with formula $C_{12}H_2O$, nerol with formula $C_{10}H_{18}O$ and linalool with formula $C_{10}H_{18}O$ (Figure 1) (Kumar *et al*., 2013).

The amount of essential oil in rose petals is about 0.03-0.05 %. However, due to the lack of artificial and natural alternatives, it is one of the most expensive essential oils in the global market (Shahbazi and Yousefi, 2020). Nevertheless, there are diverse elements apart from genotype that also play an important function in determining the quality of rose oil such as propagation method, growth condition, harvesting procedures, method of distillation, time and level of pruning, time of flowers harvesting, storage of plant material and prevailing climatic conditions (Venkatesha *et al*., 2022; Thakur *et al*., 2019; Ucar *et al*., 2017).

Fars province is one of the most suitable provinces for planting and developing *Rosa damascena* Mill. in Iran. *Rosa damascena* Mill. essential oil in Fars province has succeeded in obtaining the approval of international standards and currently there are exporting to China and throughout the Islamic world (Karami *et al*., 2012). The role of damask rose flowers and products in the economy of rural households is very important and has abundant applications in various pharmaceutical, cosmetic and health industries. This species adapts to different ecological conditions and produces flowers. Several studies have illustrated the impact of ecological conditions on the quantity and quality of essential oils (Kumar *et al*., 2016; Kovatcheva *et al*., 2011; Tabaei-Aghdayi *et al*., 2005). Harvesting and post-harvesting steps are important for herbal and aromatic plants to obtain higher essential oil content and better quality. Time of harvesting and processing are important non-monetary agronomic inputs which influences the essential oil yield and composition (Ucar *et al*., 2017).

Since the economic value of the essential oil of *Rosa damascena* Mill. is determined by the type and amount of constituents in it, in order to use this species optimally and economically, it is necessary to study the flower yield, quantity and quality of essential oil in different ecological conditions, and the most suitable time of flowers harvesting select for each area. As a rational consequence, the recognition of genotypes and the classification of essential oils according to their quality can be highly crucial for the processing and production of products with higher quality. Therefore, the objective of the present study was to understand how different harvest dates influenced the essential oil yields and chemical compounds in petals of *Rosa damascena* Mill. planted for the first time under calcareous soil conditions, Sa′adat Shahr region, Fars province.

Materials and methods

Plant material and habitat specifications: Investigation was conducted on four years old plantation of *Rosa damascena* Mill. at Horticulture garden, Sabz Dasht Agriculture Company, where the average size of garden was 28 hectares. The study area was located at Sa′adat Shahr, 105 km northeast of Shiraz, in Fars Province, with geographical coordinates of 30˚05′ 43″ North and 53˚04′ 03″ East. It was 1770 meters above sea level and has Mediterranean temperate climate. The petals of *Rosa damascena* Mill. were collected during the peak of flowering period (four weeks), which began in May, on four specified dates (in the 2nd, 9th, 16th and 23rd of May, 2020). Sampling was done in the first hours of the day and before sunrise, between 05:00 and 06:00 AM. The samples were freshly used to extract the essential oil. Furthermore, the hourly temperature and relative humidity values of the harvest times were obtained for the nearest Meteorological Station where the *Rosa damascena* Mill. garden was located, and are demonstrated in Table 1.

Isolation of the essential oils: The distillation method with water was employed to extract the essential oils and to determine percentages. Consequently, 400 g of the fresh flowers of each sample was added to 2 Liter of distilled water using the distillation method (Jalali *et al*., 2021). The essential oils of samples were extracted by hydro-distillation for 3 h with three replications for each sample, using a Clevenger-type apparatus (5 L volume; Shot, Germany) according to the

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Table 1. Meteorological values of Sa′adat Shahr

Harvest times (May)	Temperature (ºC) 06:00 am	Relative humidity (%) 06:00
2	19	42
9	20	37
16	23	28
23	25	23

method recommended in British Pharmacopoeia (Pharmacopoeia, 2007). Finally, the essential oil yield was calculated based on the fresh weight of the plant material, using Equation (1) (Medjahed *et al*., 2016).

$$\text{Essential oil yield } (\%) = \frac{(\text{Essential oil weight} \times 100)}{\text{Plant material weight}} \quad (1)$$

Anhydrous sodium sulfate was used to remove the existing moisture in the resulting essential oils and they were stored at 4◦C in sealed glass containers for analysis in the refrigerator (Binava *et al*., 2020).

Separation and identification of essential oil constituent compounds: Gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) devices were used to identify and separate the constituent compounds of the essential oils, in the Medicinal Plant Research Department of Iran Research Institute of Forests and Rangelands, Tehran, Iran. After separation, the percentages of the constituent compounds of each essential oil were calculated along with the inhibition index. To conduct a qualitative examination (identification), the mass spectrums related to the compounds of essential oils were obtained. The spectrums were identified by calculating Kovats retention indices, carried out by injecting normal hydrocarbon (C_7-C_{25}) under the same conditions as the injection of essential oils; then, they were compared against values published in various references. Mass spectrums were also examined to identify the compounds and the identifications were confirmed based on standard compound mass spectrums and different library sources. The relative percentages of each essential oil constituent compound were obtained for the area under the curve in the gas chromatography spectrum and then compared with values in different reference by considering the Kovats index (Shibamoto, 1987). The Quantitative analysis mass fraction was calculated as following formula:

Content of each component (μg g^{-1}) = [Peak area of each component/ Peak area of internal standard × concentration of internal standard (μg μL^{-1}) × volume of internal standard]/sample weight (g).

Gas chromatography device (GC): The employed GC device (Model: Agilent 7890A, USA) was equipped with a Chrom-card 2006 processor and capillary column with a length of 30 m, internal diameter of 0.25 mm, and a stationary phase layer thickness of 0.25 μ, under the commercial name, 'DB -5'. The temperature planning of the column entailed a starting temperature of 60˚C and adding 3˚C to the temperature every 3 minutes until reaching 220˚C. Then, the temperature was increased by 20˚C every minute and stopped at 260˚C for 10 minutes. The injection chamber and detector temperatures were adjusted as 280 and 300˚C, respectively. The type of detector used in the GC device was FID (Flame Ionization Detector); nitrogen gas was used as the carrier gas and its input pressure to the column was set as 0.7 ml/min (Davies, 1990).

Gas chromatography-mass spectrometry device (GC/MS): A GC device installed to MS (Agilent A/5975C, GC/MS, USA) was employed. The used column was a DB-5 type with a length of 30 m, an internal diameter of 0.25 mm, and a stationary phase layer thickness of 0.25 μ. The temperature planning involved 60-220˚C with temperature raising pace of 3˚C per minute, and the temperature of the injection chamber and transfer line were 260˚C and 280˚C, respectively, with helium used as the carrier gas. The helium gas speed was 30.6 cm/s, the ion trap detector ionization energy was 70 eV, the scanning time was 1s and the mass range was 40-300 (Adams, 2011).

The data obtained from the essential oil yield of different harvesting times to variance analysis were analyzed in a completely random design, with three replications, involving a comparison of mean average performance using Duncan's multiple range test at 1% probability level via SAS ver. 9.4 software.

Results

The results of variance analysis showed a significant difference in terms of the essential oil yield at 1% level among four different times of flowers harvesting in *Rosa damascena* Mill. under examination (Table 2).

The essential oil content of *Rosa damascena* Mill. petals harvested at different times ranged from 0.046 to 0.082 (w/w). Results obtained by comparing the oil yield obtained from the different *Rosa damascena* underlined that the highest was obtained using the flowers from the first week (0.082 ± 0.0010 %) and the lowest using the plants from the fourth week (0.046 ± 0.0010 %) (Table 3).

A comparative study of volatile components of *Rosa damascena* Mill. flower oil harvested at different times demonstrated significant variation in terms of type and percentage of constituents of essential oil (Table 4). In *Rosa damascena* Mill. essential oil collected at different times of flower harvesting, 30 different compounds were identified, 21 of which were common. The highest number of identified compounds was found in the essential oil of *Rosa damascena* Mill. harvested at both the first and third weeks (26 compounds) of harvesting period followed by the essential oil from the fourth week (26 compounds) and the second week (23 compounds).

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Table 2. Analysis of variance for essential oil percent of *Rosa damascena* Mill. at different times of flowers harvesting

Source of variation (S.O.V)	df	Mean of Squares
Essential oil yield	3	0.041642**
Error	8	0.004287

** **Significantly different at the 1 % probability level**

Table 3. Average of the essential oil content (w/w) of *Rosa damascena* Mill. at different times of harvesting

Time of flowers harvesting	1st week	2nd week	3rd week	4th week
Essential oil yield (%)	0.082 ± 0.0010[a]	0.078 ± 0.0011[a]	0.053 ± 0.0011[b]	0.046 ± 0.0010[c]

* **Means with different letters are significant according to the Duncan's multiple range test at P ≤ 0.01. The values are mean of three replicates ± standard deviation (SD).**

Table 4. Essential oil compositions of *Rosa damascena* Mill. at different times of flowers harvesting

No.	Compound name	Class of Compounds	RI**	Oil content (%)				Identification*
				1st week	2nd week	3rd week	4th week	
1	*α*-pinene	MH	941	0.9	Tr	1.1	0.7	MS, RI
2	*β*-pinene	MH	985	Tr	Tr	0.3	0.2	MS, RI
3	myrcene	MH	989	0.1	Tr	0.5	Tr	MS, RI
4	1,8-cineole	OM	1040	0.4	Tr	Tr	Tr	MS, RI
5	linalool	OM	1102	0.2	0.3	0.3	0.5	MS, RI
6	*cis*-rose oxide	OM	1111	0.1	Tr	Tr	Tr	MS, RI
7	phenyl ethyl alcohol	Aromatic alcohol	1115	0.5	Tr	0.3	Tr	MS, RI
8	citronellol	OM	1230	34.4	31.2	26.7	22.1	MS, RI
9	geraniol	OM	1258	26.5	23.5	20.6	18.5	MS, RI
10	citronellyl acetate	OM	1346	0.5	Tr	0.4	1.1	MS, RI
11	eugenol	OM	1355	1.6	0.5	0.7	0.3	MS, RI
12	geranyl acetate	OM	1376	1.4	0.8	2.1	1.2	MS, RI
13	*β*-elemene	SH	1385	0.2	0.3	0.3	0.3	MS, RI
14	methyl eugenol	OM	1404	0.5	0.5	0.4	0.2	MS, RI
15	*E*-caryophyllene	SH	1415	0.4	0.6	0.6	0.7	MS, RI
16	*α*-guaiene	SH	1442	0.7	0.7	0.9	0.8	MS, RI
17	*α*-humulene	SH	1460	0.2	0.4	0.6	0.5	MS, RI
18	germacrene D	SH	1485	0.4	1.5	1.7	1.3	MS, RI
19	*α*-bulnesene	SH	1506	0.3	0.6	0.3	0.2	MS, RI
20	*n*-heptadecane	Alkane	1700	1.1	1.3	0.8	2.3	MS, RI
21	(*E*,*Z*)-farnesol	OS	1718	1.8	1.1	1.7	1.2	MS, RI
22	*n*-hexadecanol	Fatty alcohol	1875	1.6	3.2	2.3	3.7	MS, RI
23	*n*-nonadecane	Alkane	1900	11.2	13.1	18.4	19.4	MS, RI
24	*n*-eicosane	Alkane	2000	1.1	1.9	1.7	2.1	MS, RI
25	*n*-heneicosane	Alkane	2100	6.2	7.7	12.1	15.4	MS, RI
26	*n*-docosane	Alkane	2200	Tr	0.2	Tr	0.2	MS, RI
27	9-tricosene	Alkene	2291	Tr	0.3	0.2	0.1	MS, RI
28	*n*-tricosane	Alkane	2300	1.3	3.7	1.1	2.6	MS, RI
29	*n*-pentacosane	Alkane	2500	0.4	1.8	0.7	0.8	MS, RI
30	*n*-hexacosane	Alkane	2600	0.5	1.7	0.8	1.1	MS, RI
	Monoterpene Hydrocarbons (MH)			1.0	0.0	1.9	0.9	
	Oxygenated Monoterpens (OM)			65.5	56.8	51.2	43.9	
	Sesquiterpene Hydrocarbons (SH)			2.2	4.1	4.4	3.8	
	Oxygenated Sesquiterpens (OS)			1.8	1.1	1.7	1.2	
	Long-chain Hydrocarbons (Alkane)			21.8	31.7	35.8	44.0	
	Others			2.1	3.2	2.6	3.7	
	Total identified			94.7	96.9	97.6	97.5	

***) Mode of identification: retention index (RI), mass spectrometery (MS), and co-injection (CoI) with some available authentic compounds.**

****) RI: retention indices determined in the present work relative to C_7-C_{25} *n*-alkanes on the DB-5 column.**

Tr: traces

The identified constituents from the flowers of the first week accounted for 94.7 % of the total essential oil. The major constituent compounds of the flowers from the first week in *Rosa damascena* Mill. were included

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citronellol (34.4 %), geraniol (26.5 %), *n*-nonadecane (11.2 %) and *n*-heneicosane (6.2 %). Other compounds constitute less than 3 % of the essential oil, which are listed in Table 4. The identified compounds in the damask rose flowers of the second week constituted 96.9 % of the essential oil. The pre-eminent compounds of this sample were citronellol (31.2 %), geraniol (23.5 %), *n*-nonadecane (13.1 %), *n*-heneicosane (7.7 %), *n*-tricosane (3.7 %) and *n*-hexadecanol (3.2 %). 97.6 % of the whole essential oil was identified in the third week's essential oil and the important compositions were found to be citronellol (26.7 %), geraniol (20.6 %), *n*-nonadecane (18.4 %) and *n*-heneicosane (12.1 %). Finally, 97.5 % of the whole essential oil was identified in the essential oil of the damask rose flowers of the fourth week and the main compounds entailed citronellol (22.1 %), geraniol (18.5 %), *n*-nonadecane (19.4 %), *n*-heneicosane (15.4 %) and *n*-hexadecanol (3.7 %). The constituent compounds of the essential oil in *Rosa damascena* Mill. at different times of flowers harvesting were classified in terms of chemical formula which are listed in Table 4.

Considering the different compounds identified in the essential oil of these four samples, it was shown that oxygenated monoterpenes were the major group except the fourth week (43.9 %) that constituted the first week (65.5 %), the second week (56.8 %) and the third week (51.2 %). The second main group obtained from *Rosa damascena* Mill. essential oil was long-chain hydrocarbons (alkane) which organized the fourth weeks (44.0 %), the third week (35.8 %), the second week (31.7 %) and the first week (21.8 %), followed by sesquiterpene hydrocarbons and oxygenated sesquiterpenes as well as monoterpene hydrocarbons, respectively (Table 4).

Discussion

Ahead of time Weiss (1997) illustrated that the increase in the temperature causes removal of the essential oils from the trichomes of the petals. Necat Izgi (2022) reported the essential oil yield of five *Rosa damascena* Mill. samples (five samples with three replications, at around one-week intervals, from a four years old cultivated of *Rosa damascena* Mill. garden in the Yaylabasi district at 6:00 AM, in Turkey) and indicated that a significant difference was present between all tested samples. The highest essential oil yield (0.082 % w/w) was observed when the flowers were harvested at the first week of harvesting period in 2nd of May (lower temperature) and subsequently there was reduction from 0.078, 0.053 and 0.046 % w/w in essential oil content when the flowers were harvested at the second (9th of May), third (16th of May) and fourth weeks (23rd of May) (higher temperature), respectively. The temperatures of Sa′adat Shahr region at first, second, third and fourth weeks of the harvesting period were 19 ºC, 20 ºC, 23 ºC and 25 ºC at 06:00 am, respectively. The maximum reduction in essential oil yield was at the fourth week of harvesting (0.046 % w/w) when the temperature was maximum during the harvesting period, 25 ºC at 06:00 am. Similarly, the amount of essential oil in the *Rosa damascena* Mill. flowers harvested from different sites and times has been investigated, confirming the role of the environment and time of harvesting in affecting the amount and accumulation of essential oil, as also reported by others (Meftahizade *et al*., 2022; Sharma and Kumar, 2018; Mirzaei *et al*., 2016; Kumar *et al*., 2013; Yilmaz *et al*., 2011). The reduction in essential oil yield of flowers is due to the increase in temperature, as a result of evaporation of the essential oil content and reduction in weight of flowers (Nunes and Miguel, 2017).

According to the comparison between the constituent compounds of essential oils in the examined samples, there were four main compounds detected in the essential oil (Table 4). The major constituents in the essential oils of the first, second, third and fourth weeks' samples respectively included citronellol, geraniol, *n*-nonadecane and *n*-heneicosane. Citronellol and geraniol are the main aroma substances of *Rosa damascena* essential oil (Antonova *et al*., 2021; Honarvar *et al*., 2010; Tabaei-Aghdayi *et al*., 2005). The highest content of citronellol + geraniol (60.9 %) was observed when the flowers were harvested at the first week. However, the lowest content of them (40.6 %) was found in the flowers of the fourth week harvesting. On the whole, the total of these components declined from the first to the last harvest, which was thought to be attributable to raising temperature and decreasing relative humidity (Table 1).

On the other hand, the percentage of some long-chain hydrocarbons components such as *n*-nonadecane and *n*-heneicosane increased from the first week (17.4 %) to the fourth week (34.8 %) of harvesting, during which the temperature also augmented. Freezing point is one of the important criteria in determining the quality of Damask rose essential oil. High quality *Rosa damascena* Mill. essential oil has a low freezing point. Long-chain hydrocarbons, which contribute an unpleasant scent to essential oils, are undesirable in Damask rose because they increase the freezing temperature (Necat Izgi, 2022).

Conclusions

In general, the results of the present study provided distinctions between different times of *Rosa damascena* flower harvesting in terms of the main constituent compounds of its essential oil, which it was cultivated and collected in a new region of Iran for the first time, Sa′adat Shahr. This experiment revealed that different harvest dates can significantly modify the performance and composition of Damask rose' essential oil. Moreover, calcareous soil conditions could be used to obtain essential oil of *Rosa damascena* Mill. Finally, the performance of *Rosa damascena* Mill. is strongly influenced by the environmental conditions (especially temperature and relative humidity) and how well adapted the genotype is to those conditions.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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