

Diurnal and intra-plant variation in volatile oil content and constituents of *Salvia rosmarinus* Spenn. leaves

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Abstract

Diurnal conditions, genetic background, and climatic factors, together with within-plant microenvironments, can alter the accumulation of secondary metabolites, leading to variability in plant volatile oils (VOs). In this study, the effects of time of day and vertical canopy position on VO yield and chemical profile were investigated in *Salvia rosmarinus* Spenn. (syn. *Rosmarinus officinalis* L.) leaves at the onset of flowering. Leaves were collected at 06:00, 12:00, and 18:00 from the lower, middle, and upper thirds of the canopy; composite samples were prepared per canopy stratum and sampling time, hydrodistilled, and analyzed by GC–MS. VO yield showed a clear positional pattern, increasing from the lower to the upper canopy across all sampling times, and ranged from 0.43% to 0.75% (v/w, DW basis). Overall, 71 constituents were identified, and the number of detected compounds varied across treatments (55–68), with the lowest richness observed in the upper third at midday. Across all samples, the VO profile was consistently dominated by oxygenated monoterpenes and monoterpene hydrocarbons, whereas sesquiterpene-related fractions exhibited more pronounced time- and stratum-dependent variation. Among major constituents, camphor remained prominent but varied substantially (6.71–15.59%), with a marked minimum in the middle third at midday and a maximum in the upper third in the morning. Other abundant constituents also showed treatment-dependent redistribution, including *cis*-verbenone (10.48–17.94%), borneol (8.60–12.79%), α -pinene (4.91–11.61%), and 1,8-cineole (5.77–9.55%). These findings indicate that both harvest time and canopy sampling height are key determinants of *S. rosmarinus* VO yield and compositional consistency, underscoring the need for standardized harvest specifications when composition-oriented profiles are targeted.

Keywords: Canopy, Diurnal Variability, GC/FID–MS, Hydrodistillation, Rosemary

INTRODUCTION

Salvia rosmarinus Spenn. (syn. *Rosmarinus officinalis* L.), a member of the Lamiaceae, is an aromatic shrub native to the Mediterranean region and is widely used in the food, fragrance, and pharmaceutical industries. Its volatile oil (VO) is commercially valued due to its monoterpene-rich profile, and in many reports, key constituents such as 1,8-cineole, camphor, and α -pinene have been described as major components (Borges et al., 2018). Industrial value is determined by VO yield and the quantitative balance of key constituents. Recent evidence further indicates that *S. rosmarinus* VO can exhibit significant month-to-month variation in its constituent profile, underscoring that its chemical composition is not static even within the same region and species (Bejenaru et al., 2025). GC–MS-based profiling of *S. rosmarinus* accessions has grouped samples into distinct chemotypes (e.g., β -myrcene-, 1,8-cineole-, and camphor-rich profiles), and the European Pharmacopoeia monograph likewise distinguishes two *S. rosmarinus* VO types (Spanish vs. Moroccan/Tunisian), together underscoring that chromatographic profiles should be interpreted in the context of genotype as well as sampling/harvest conditions (Barak et al., 2023; Khan et al., 2025).

Secondary metabolite biosynthesis and accumulation are inherently dynamic and show pronounced plasticity under abiotic and biotic influences (Flamini et al., 2002). In medicinal and aromatic plants, volatile abundance arises from the interplay among biosynthesis, storage, and emission, and this balance is modulated diurnally by light regime, temperature, water status, and air humidity (Escobar-Bravo et al., 2023). Evidence from Lamiaceae also indicates that monoterpene synthase expression can vary rhythmically over the day, as reported in *Lavandula angustifolia* flowers (Seira et al., 2023). This may

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help contextualize within-day shifts in terpene profiles in Lamiaceae aromatics. Consequently, the time of harvest becomes a critical variable. For example, diurnal variation in *S. rosmarinus* VO yield has been reported, with differences observed between pre-sunrise, midday, and post-sunset harvests (Rofouei et al., 2021). These within-day differences indicate the need for time-resolved sampling at representative morning, midday, and late-afternoon time points.

Leaf position is closely linked to leaf age and light exposure within the canopy and can generate intra-plant variation in both VO yield and composition. In a Lamiaceae species, higher VO content was reported for upper leaves compared with middle and lower leaves (Özel & Tekin, 2021). Leaf ontogeny can influence glandular (globular) trichome density. For instance, in *Origanum majorana*, trichome density decreased with leaf maturity (Baâtour et al., 2016). In *L. angustifolia*, VOCs are synthesized and stored in glandular trichomes (Zhang et al., 2023a), highlighting glandular trichomes as key structures in the biology of volatile compounds. Moreover, vertical canopy gradients can create warmer upper layers and more humid lower layers during daytime (Zhang et al., 2023b). These vertical microclimatic differences provide a physiological basis for position-dependent variation in volatile biosynthesis and volatilization. Accordingly, leaves from different vertical canopy strata may differ within the same plant and day not only in VO yield but also in the relative distribution of key constituents.

The effects of geography, genotype, and broad climatic factors on *S. rosmarinus* VO have been widely reported. Nevertheless, studies that jointly assess short-term (within-day) diurnal variation and within-plant (vertical canopy) leaf position remain limited in a manner directly relevant to harvest optimization. Therefore, practical harvest protocols should specify not only the harvest date but also time of day and canopy position to improve comparability and support the production of more consistent, composition-oriented oils.

In this context, the present study aimed to quantify the effects of sampling time (06:00, 12:00, and 18:00) and intra-plant leaf position (lower, middle, and upper thirds) on VO yield and chemical composition in *S. rosmarinus* leaves. Understanding these sources of variability may support more precise harvest timing and canopy-targeted collection practices to obtain VOs enriched in desired constituents. The findings may also support the standardization of sampling/harvest specifications and improve comparability among studies targeting *S. rosmarinus* VO profiles.

MATERIALS AND METHODS

Plant material, location, and treatments

Three-year-old *Salvia rosmarinus* Spenn. (syn. *Rosmarinus officinalis* L. / rosemary) plants were under cultivation at the Afyonkarahisar Medicinal and Aromatic Plants Center (Afyonkarahisar, Türkiye; 38°46' N, 30°30' E; 1057 m a.s.l.) under a sustainable agriculture management system, with a planting density of 50 × 70 cm. The site lies in Türkiye's Inner Aegean transitional zone, influenced by Mediterranean, Central Anatolian, and Aegean climatic regimes. The region is characterized by precipitation concentrated mainly in winter and spring, hot and dry summers, and cold winters with occasional snowfall (Soltanbeigi & Maral, 2022). A summary of the main meteorological variables for the study area is provided in Table 1, and the principal physical and chemical properties of the experimental soil are presented in Table 2.

Table 1. Some local meteorological data for experimental year (2021)

Month	Temperature (°C)			Precipitation (mm)	Mean Relative Humidity (%)	Insolation (h)
	Minimum	Maximum	Mean			
January	-0.1	8.4	3.7	57.5	71.3	1.82
February	-0.7	10.0	4.1	20.2	64	3.26
March	-0.2	10.2	4.4	66.1	63.8	4.31
April	5.5	17.1	11	33.6	56.1	4.89
May	10.4	25.4	17.8	13.7	44.3	8.63
June	11.7	24.1	17.5	84.2	61.9	6.94
July	16.4	30.5	23.5	19.3	46.7	9.87
August	16.1	31.7	24	0	40.3	10.55
Total				294.6		

5th Regional Directorate of Meteorology, Afyonkarahisar, Türkiye

To assess diurnal and within-plant (vertical canopy) variability, leaf material was collected on 10 August 2021 at three sampling times (06:00, 12:00, and 18:00), coinciding with the onset of flowering (first blossoms visible across the field). The mean plant height at sampling was 80 ± 10 cm. At each sampling time, five plants were randomly selected. Each plant was harvested individually, measured, and then divided into three equal vertical sections (lower, middle, and upper thirds). Leaves from the same canopy section were then pooled across the five plants to generate one composite sample per canopy position at each sampling time. The resulting samples were spread as a thin layer and dried in a natural-convection drying chamber (Binder ED 720) at 37 °C for 72 h, after which they were immediately subjected to hydrodistillation.

Table 2. Selected physicochemical properties of the experimental soil

Elements (ppm)			Properties (%)		
Depth (cm)	0-30	30-60	Depth (cm)	0-30	30-60
Ca	3952	4999	Organic matter	0.34	0.27
Mg	624	747	Total N	0.10	0.08
K	344	307	Sand	52.99	48.61
Na	50	838	Clay	32.70	32.89
Fe	1.07	1.08	Silt	14.31	18.50
P	73	49	Lime	1.88	2.01
Cu	0.75	0.62			
Zn	1.12	0.86			
Mn	6.03	3.50			

EC (mS cm⁻¹): 0.15 and 0.17; pH: 8.44 and 8.74 at depths of 0–30 and 30–60 cm, respectively

Volatile oil hydrodistillation

Volatile oil (VO) was extracted by hydrodistillation using a Clevenger-type apparatus. Briefly, 50 g of dried leaf sample was mixed with distilled water at a 1:10 (sample-to-water) ratio and hydrodistilled for 3 h. Hydrodistillation was performed twice on the same sample material as technical extraction replicates. The VO yield was determined volumetrically and expressed as mL 100 g⁻¹ dry weight (DW), which is numerically equivalent to % (v/w):

$$VO \text{ (mL 100 g}^{-1} \text{ DW)} = 100 \times \frac{VO \text{ (mL 100 g}^{-1} \text{ DW)}}{100} = VO \text{ (\% v/w)}$$

The obtained VO was dried over anhydrous sodium sulfate and stored in amber vials at 4 °C until analysis.

GC/FID–MS identification of volatile oil constituents

A gas chromatography–mass spectrometry system (Shimadzu GCMS-QP2010 SE) equipped with a flame ionization detector (FID) was used to identify the chemical constituents of the VO. VO sample preparation, column characteristics, and instrumental and injection conditions are summarized in Table 3.

Retention indices (RI) were calculated using a standard mixture of C7–C30 n-alkanes (Merck/Supelco) analyzed under the same chromatographic conditions as the VO samples. VO constituents were identified by comparing RI values and mass spectra with the NIST and Wiley libraries, published reference data (Adams, 2017), and an in-house database. Relative amounts of the detected constituents were expressed as percentage peak area based on FID peak-area normalization relative to the total peak area of all identified constituents; no response factors were applied.

Table 3. GC-MS operating conditions, column specifications, and preparation details of VO samples.

GC/FID-MS analysis conditions	
Instrument	Shimadzu GC-MS QP2010 SE
Stationary phase	Teknokroma, TRB-WAX 60 m × 0.320 mm inner diameter and 0.25 µm film thickness
Carrier gas / Flow	Helium (99.999%) / 2.06 mL min ⁻¹
Sample preparation	1 mL VO diluted 1:50 in n-hexane
Injection volume	1 µL (split mode, 40:1)
1 st stage temp. planning & gradient	70 °C after injecting with 5 min hold-up time, after raised to 160 °C with a 3 °C min ⁻¹ heating ramp and 10 min hold-up time
2 nd stage temp. planning & gradient	Increased to 250 °C with 6 °C min ⁻¹ heating ramp and 20 min hold time
Detector temp.	250 °C
Injector temp.	250 °C
Ion source temp.	250 °C
MS scan range (m/z)	35-550 atomic mass units (AMU)
Electron impact ionization	70 eV

RESULTS

Volatile oil content (%)

Table 4 shows that VO content varied with both canopy position and sampling time, ranging from 0.43% to 0.75% (v/w, DW basis). Across all time points, VO content increased consistently from the lower to the upper plant section. The lowest value was recorded in the lower-third leaves harvested at 06:00 (0.43%). In the lower third, VO increased from 0.43% at 06:00 to 0.55% at 12:00 and remained unchanged at 18:00 (0.55%). In the middle third, VO showed a modest midday

maximum (0.55%) compared with 06:00 (0.5%), followed by a slight decrease at 18:00 (0.53%). The upper third exhibited the highest VO levels at all sampling times, increasing from 0.68% at 06:00 to 0.75% at both 12:00 and 18:00.

Table 4. Volatile oil content of *S. rosmarinus* leaves under diurnal and intra-plant variation (%)

Lower third			Middle third			Upper third		
06:00	12:00	18:00	06:00	12:00	18:00	06:00	12:00	18:00
0.43	0.55	0.55	0.5	0.55	0.53	0.68	0.75	0.75

Overall, the largest contrast was observed between canopy positions, with upper-third leaves showing higher VO contents than middle- and lower-third leaves throughout the day; the maximum VO (0.75%) occurred in the upper third at 12:00 and 18:00, whereas the minimum (0.43%) occurred in the lower third at 06:00. These position- and time-dependent patterns may reflect differences in microenvironmental conditions (e.g., light exposure and temperature) along the canopy, which can influence the accumulation of volatile metabolites.

Volatile oil constituents

Table 5 summarized the GC–MS results, in which 71 constituents were identified overall. The number of detected constituents shows observable differences across sampling times and canopy positions. In the lower third, compound counts range from 64 to 68; in the middle third, from 60 to 68; and in the upper third, from 55 to 61. Notably, the upper third at 12:00 shows the lowest number of detected constituents (55), pointing to potential diurnal and within-plant differences in the detectable volatile profile. These patterns may be related to changes in environmental conditions (e.g., temperature and light intensity) that can affect volatilization and detectability of volatile constituents. Accordingly, harvest time and canopy position should be carefully considered when aiming for a consistent and targeted *S. rosmarinus* volatile profile. Across all canopy positions and sampling times, the summed contribution of the reported chemical classes accounted for 99.09–99.70% of the VO (Table 5).

Table 5. Volatile oil constituents of *S. rosmarinus* leaves under diurnal and intra-plant variation

RT ¹	R ²	R ³	Constituents (%)	G.C. ⁴	Lower third			Middle third			Upper third		
					06:00	12:00	18:00	06:00	12:00	18:00	06:00	12:00	18:00
8.459	1016	1020 ^a	Tricyclene	MH ⁵	0.11	-	0.08	0.05	-	0.1	-	0.08	-
8.727	1028	1032 ^a	α -Pinene	MH	11.53	4.91	10.88	11.29	6.84	11.61	7.68	11.6	9
9.742	1074	1080 ^a	Camphene	MH	2.2	0.92	1.84	2.23	1.32	2.05	1.56	2.25	1.62
10.816	1117	1121 ^a	β -Pinene	MH	0.43	0.22	0.41	0.57	0.39	0.53	0.7	0.89	0.7
11.285	1132	1136 ^a	Verbenene	MH	0.96	0.45	0.96	0.96	0.62	1	0.44	0.71	0.58
11.978	1154	1158 ^a	δ 3-Carene	MH	1.68	1.22	1.8	1.72	1.55	1.72	1.26	1.57	1.44
12.287	1164	1168 ^a	β -Myrcene	MH	0.87	0.65	0.91	1.02	0.86	1.01	0.93	1.07	0.98
12.504	1171	1212 ^b	α -Phellandrene	MH	0.26	0.21	0.3	0.31	0.3	0.33	0.36	0.43	0.41
12.922	1184	NDF ^a	1,3,5-Cycloheptatriene, 3,7,7-trimethyl-	MH	0.11	-	0.1	0.11	-	0.1	-	-	-
12.985	1186	1181 ^b	α -Terpinene	MH	0.38	0.37	0.4	0.42	0.47	0.43	0.47	0.58	0.52
13.643	1206	1206 ^b	Limonene	MH	3.62	2.79	3.64	3.66	3.58	3.43	2.87	3.26	3.04
14.026	1216	1220 ^a	1,8-Cineole	OM ⁶	9.44	6.85	7.52	7.26	5.77	9	9.23	9.55	8.27
15.325	1250	1240 ^b	γ -Terpinene	MH	0.32	0.31	0.35	0.42	0.52	0.42	0.51	0.6	0.59
15.622	1258	1262 ^a	3-Octanone	K ⁷	0.17	0.13	0.15	0.17	-	0.21	0.21	0.19	0.18
16.326	1277	1281 ^c	o-Cymol	MH	2.12	1.61	1.89	1.68	1.49	1.47	0.85	0.91	0.81
16.767	1288	1279 ^b	α -Terpinolene	MH	0.54	0.57	0.62	0.72	1	0.76	1.13	1.23	1.27
23.032	1443	1447 ^a	p-Cymenene	MH	0.2	0.22	0.21	0.18	0.22	0.17	0.11	0.12	0.11
23.193	1447	1445 ^d	Filifolone	OM	0.6	0.4	0.4	-	0.24	0.31	-	-	-
23.25	1448	1451 ^a	1-Octen-3-ol	A ⁸	0.4	0.4	0.36	0.71	0.16	0.56	0.72	0.72	0.66
23.982	1466	1463 ^b	cis-Sabinene hydrate	OM	-	-	-	-	-	-	-	0.18	0.21
25.298	1498	1497 ^d	α -Copaene	SH ⁹	0.19	0.37	0.33	0.21	0.56	0.23	0.22	0.21	0.25
25.985	1515	1510 ^b	Chrysanthemone	OM	1	1.72	1.3	1.25	0.41	1.38	1.19	0.97	0.64
26.414	1526	1530 ^a	Camphor	OM	15.49	15	13.39	12.59	6.71	14.48	15.59	14.67	13.63
27.221	1546	1550 ^a	Linalool	OM	4.48	5.08	4.28	4.43	4.37	5	5.83	5.45	5.07
27.36	1550	1563 ^c	trans- β -Terpineol	OM	-	-	-	-	-	-	0.3	0.2	0.23
27.576	1555	1559 ^a	Pinocamphone	OM	2.23	2.46	1.99	1.65	1.57	1.72	2.06	1.84	1.69
28.434	1577	1581 ^a	Pinocarvone	OM	0.34	0.4	0.33	0.33	0.26	0.38	0.41	0.39	0.37
28.794	1586	1588 ^b	Bornyl acetate	Es ¹⁰	0.34	0.27	0.22	0.68	1.09	0.58	3.53	3.1	2.73
29.544	1605	1608 ^f	Caryophyllene	SH	2.2	3.99	3.1	2.21	6.51	2.45	2.75	2.51	2.93
31.003	1643	1678 ^g	Methyl geraniate	Es	-	0.14	0.11	0.08	0.22	0.12	0.14	0.13	0.14
31.535	1657	1671 ^h	cis-Verbenol	OM	0.39	0.56	0.44	0.51	0.51	0.59	0.86	0.77	0.85
31.833	1665	1671 ⁱ	p-Mentha-1,5-dien-8-ol	OM	0.12	0.18	0.14	0.13	0.26	0.12	0.21	-	-
32.176	1674	1682 ^d	δ -Terpineol	OM	0.19	0.21	0.19	0.15	0.15	0.19	0.12	0.19	0.2
32.291	1677	1681 ^a	α -Humulene	SH	0.24	0.58	0.42	0.23	0.95	0.26	0.31	0.26	0.36
32.411	1680	1683 ^d	trans-Verbenol	OM	0.8	1.2	1.04	1.24	1.11	1.37	1.8	1.61	1.73
32.731	1689	1681 ^j	Limonen-4-ol	OM	-	-	-	0.16	0.19	0.16	0.2	0.18	0.17
32.926	1694	1698 ^a	γ -Muuroleone	SH	0.15	0.36	0.28	0.19	0.65	0.18	0.23	0.21	0.28
33.103	1699	1703 ^a	α -Terpineol	OM	2.29	2.74	2.27	2.23	2.28	2.21	2.49	2.18	2.3
33.303	1704	1708 ^a	Borneol	OM	9.99	12.79	10.05	10.29	10.19	10.69	9.55	9.15	8.6
33.727	1715	1725 ^d	cis-Verbenone	OM	11.07	10.48	12.27	14.56	11.91	12.7	11.4	11.37	17.94
34.162	1727	1773 ^k	p-Mentha-1(7),2-dien-8-ol	OM	0.64	0.92	0.75	0.69	1.38	0.59	0.67	0.67	0.62
34.476	1735	1739 ^a	α -Citral	OM	0.13	0.17	0.12	0.2	0.24	0.16	0.48	0.34	0.33
35.415	1760	1756 ⁱ	δ -Cadinene	SH	0.21	0.59	0.46	0.25	1.02	0.21	0.28	0.27	0.33
35.483	1762	1757 ^g	Citronellol	OM	0.32	0.27	0.22	0.45	0.43	0.47	0.44	0.47	0.32
35.712	1768	1772 ^h	γ -Cadinene	OM	0.31	0.36	0.32	0.35	0.28	0.31	0.34	0.3	0.3
36.067	1777	1777 ^l	β -Sesquiphellandrene	SH	0.18	0.23	0.19	0.19	0.19	0.18	0.18	0.18	0.16
36.639	1793	1797 ^a	Homomyrtenol	OM	1.89	2.36	1.94	1.97	1.98	1.69	1.99	1.58	1.67
38.356	1833	1845 ^d	trans-Carveol	OM	-	0.14	0.12	0.12	0.14	-	0.13	-	-
38.482	1836	NDF ^a	ξ -Fenchene	MH	1.12	1.51	1.2	1.17	1.72	0.91	1.05	0.79	0.82
38.728	1841	1846 ^a	Geraniol	OM	1.84	2.84	1.92	2.08	2.98	1.65	2.08	1.65	1.55

38.848	1844	1833 ^m	Isopiperitenon	OM	0.29	-	0.29	0.36	-	0.27	-	-	0.24
38.997	1848	1852 ^a	<i>p</i> -Cymen-8-ol	OM	0.25	0.28	0.28	0.28	0.27	0.23	0.16	-	-
39.357	1856	1856 ^a	Geranyl acetone	K	0.35	0.53	0.41	0.36	0.89	0.28	0.28	0.21	0.24
40.015	1871	1869 ^o	<i>cis</i> -Myrtanol	OM	-	0.18	0.16	0.17	0.22	-	0.15	-	-
42.367	1930	1934 ^a	Piperitenone	OM	0.36	0.36	0.41	0.32	0.41	0.23	-	-	0.16
44.593	1991	1995 ^a	Caryophyllene oxide	OS	0.88	1.58	1.11	1.07	3.15	0.76	0.84	0.59	0.7
45.302	2014	2019 ^a	Methyl eugenol	Et ¹¹	0.13	0.18	0.17	0.22	0.29	0.15	0.3	0.24	0.29
46.309	2049	2040 ⁱ	Humulene epoxide	OS ¹²	0.24	0.43	0.31	0.24	0.67	0.16	0.13	-	0.11
49.325	2167	2172 ^a	Thymol	OM	0.12	0.16	0.14	0.11	0.2	0.15	-	-	-
49.405	2170	2175 ^a	Eugenol	OM	0.18	0.19	0.21	0.28	0.29	-	0.2	0.17	0.27
49.525	2175	NDF [*]	Bicyclo[4.4.0]dec-1-ene, 2-isopropyl-5-methyl-9-methylene-	SH	0.11	0.24	0.16	0.13	0.4	-	0.12	-	0.1
49.880	2190	2189 ^a	τ -Murolol	OS	-	0.16	0.12	0.09	0.29	-	0.07	-	-
49.977	2194	2194 ^p	γ -Eudesmol	OS	0.13	0.21	0.17	0.1	0.27	-	-	-	-
50.117	2200	NDF [*]	<i>p</i> -Isopropylanisole	Et	0.12	0.16	0.16	0.13	0.3	-	-	-	-
50.378	2213	2219 ^a	Carvacrol	OM	0.1	0.14	0.12	0.11	0.18	-	-	-	-
50.475	2218	2209 ^j	α -Bisabolol	OS	0.17	0.43	0.26	0.17	0.6	0.11	0.15	0.1	0.12
50.864	2237	2239 ^j	α -Cadinol	OS	0.1	0.25	0.16	0.12	0.43	-	0.14	-	0.11
52.203	2304	2296 ⁱ	Caryophylla-4(12),8(13)-dien-5 β -ol	OS	0.29	0.6	0.4	0.27	0.8	0.16	0.16	0.09	0.11
52.907	2343	2359 ^j	Caryophylla-3,8(13)-dien-5 β -ol	OS	0.83	1.77	1.17	0.72	2.3	0.42	0.46	0.3	0.38
53.531	2378	2376 ^q	Farnesyl acetone	K	0.16	0.44	0.25	0.13	0.62	0.09	0.14	0.08	0.11
53.622	2383	2389 ^d	Caryophyllenol-II	OS	0.28	0.66	0.42	0.25	0.96	0.15	0.15	0.08	0.13
Number of constituents					64	65	68	68	65	60	61	55	59
Total (%)					99.18	99.1	99.09	99.7	99.13	99.35	99.31	99.44	99.67

¹Retention time; ²Retention indices calculated against n-alkanes (C7-C30) on Teknokroma, TRB-WAX column; ³Grouped constituents; ⁴Monoterpene hydrocarbon;

⁵Oxygenated monoterpene; ⁶Ketone; ⁷Alcohol; ⁸Sesquiterpene hydrocarbon; ⁹Ester; ¹⁰Ether; ¹¹Oxygenated sesquiterpene;

a: Türk Baydır et al., 2023; b: Verma et al., 2012; c: Soltanbeigi & Maral, 2005; d: Polatoglu et al., 2010; e: Lattoo et al., 2006; f: Soltanbeigi & Ozliman, 2023; g: Gancel et al., 2005; h: Kirimer et al., 1999; i: Kurose et al., 2007; j: Kollmannsberger et al., 1992; k: Blanc et al., 2004; l: Choi & Sawamura, 2000; m: Umano et al., 1994; n: Birtic et al., 2009; o: Gonny et al., 2006; p: Iscan et al., 2006; q: Ferhat et al., 2007;

*No data found for the "Polar Column"

As shown in Table 5, camphor was consistently among the most abundant constituents in *S. rosmarinus* VO across all canopy positions and sampling times, with values ranging from 6.71% to 15.59%. The highest camphor level was observed in the upper third at 06:00 (15.59%), closely followed by the lower third at 06:00 (15.49%), whereas the lowest value occurred in the middle third at 12:00 (6.71%). Beyond this pronounced midday minimum in the middle canopy, camphor displays a generally modest diurnal decline in the upper (15.59 → 14.67 → 13.63%) and lower thirds (15.49 → 15.00 → 13.39%), while the middle third shows a distinct midday depression followed by recovery (12.59 → 6.71 → 14.48%). This pattern may reflect diurnal changes in leaf microclimate and volatile dynamics—particularly temperature- and radiation-associated effects on volatilization/emission and within-canopy differences in exposure and boundary-layer conditions—leading to reduced detectable camphor around midday, most evident in the middle stratum. If the reported values represent relative abundances, the midday decrease may also indicate a compositional shift in which other volatiles increase at 12:00, thereby lowering camphor's proportional contribution without necessarily implying a comparable reduction in its absolute amount. Given the sampling design (one composite sample per canopy position and time), these results are best interpreted as descriptive trends that point to diurnal and vertical heterogeneity in camphor representation within the *S. rosmarinus* volatile profile.

In addition to camphor, the *S. rosmarinus* VO profile contained several other major constituents with high relative abundances, including *cis*-verbenone (10.48–17.94%), borneol (8.60–12.79%), α -pinene (4.91–11.61%), and 1,8-cineole (5.77–9.55%).

Another constituent detected at high concentration after camphor was *cis*-verbenone. *cis*-Verbenone displayed clear variation across canopy positions and sampling times, ranging from 10.48% to 17.94%. The highest level was observed in the upper third at 18:00 (17.94%), whereas the lowest value occurred in the lower third at 12:00 (10.48%). At 06:00, *cis*-verbenone was most abundant in the middle third (14.56%) compared with the lower (11.07%) and upper (11.40%) thirds, while at 12:00 the canopy gradient was reduced (10.48–11.91%). By 18:00, a strong vertical contrast emerged due to the marked increase in the upper third, indicating a time-dependent shift in the representation of *cis*-verbenone within the upper canopy.

Borneol showed a moderate abundance across all treatments, with values spanning 8.60–12.79%. The highest borneol level was recorded in the lower third at 12:00 (12.79%), whereas the lowest occurred in the upper third at 18:00 (8.60%). Diurnal trends within each canopy position differed markedly. In the lower third, borneol increased from 9.99% (06:00) to a clear midday peak at 12.79% (12:00), then declined to 10.05% (18:00), representing the strongest within-position diurnal amplitude in the table (Table 5). In contrast, the middle third was comparatively stable (10.29 → 10.19 → 10.69%), showing only a slight increase by 18:00. The upper third exhibited a gradual decrease through the day (9.55 → 9.15 → 8.60%). Vertical (within-plant) differences were also time-dependent. At 06:00, borneol was highest in the middle third (10.29%), followed by the lower (9.99%) and upper thirds (9.55%). At 12:00, a pronounced vertical gradient emerged, with the lower third (12.79%) clearly exceeding the middle (10.19%) and upper thirds (9.15%). By 18:00, the pattern shifted again, with the middle third (10.69%) highest, the lower third intermediate (10.05%), and the upper third lowest (8.60%).

α -Pinene exhibited pronounced diurnal and within-plant variation, ranging from 4.91% to 11.61% across all treatments. The lowest value occurred in the lower third at 12:00 (4.91%), whereas the highest was recorded in the middle third at 18:00 (11.61%), closely followed by the upper third at 12:00 (11.60%). Within each canopy position, the lower third showed a clear midday depression (11.53 → 4.91 → 10.88%). The middle third displayed a similar pattern with recovery by evening (11.29 → 6.84 → 11.61%). In contrast, the upper third differed by peaking at midday (7.68 → 11.60 → 9.00%). Vertically, values were comparable between the lower and middle thirds at 06:00 (11.53 and 11.29%) and 18:00 (10.88 and 11.61%),

while at 12:00 α -pinene was highest in the upper third, intermediate in the middle third, and lowest in the lower third (11.60 > 6.84 > 4.91%).

1,8-Cineole showed clear diurnal and within-plant variation, ranging from 5.77% to 9.55%. The lowest level was observed in the middle third at 12:00 (5.77%), whereas the highest occurred in the upper third at 12:00 (9.55%). In the lower third, 1,8-cineole decreased from 9.44% (06:00) to 6.85% (12:00) and partially recovered by 18:00 (7.52%). The middle third showed a similar midday minimum (7.26 $\rightarrow$ 5.77%) followed by a marked increase at 18:00 (9.00%). In contrast, the upper third remained comparatively high and stable (9.23 $\rightarrow$ 9.55 $\rightarrow$ 8.27%). Vertically, the upper third was highest at 12:00, while by 18:00 the middle third became the highest (9.00%), indicating time-dependent shifts in the relative representation of 1,8-cineole across canopy strata.

Other constituents with moderate relative abundances were identified, including linalool (4.28–5.83%), limonene (2.79–3.66%), caryophyllene (2.20–6.51%), α -terpineol (2.18–2.74%), geraniol (1.55–2.98%), pinocamphone (1.57–2.46%), homomyrtenol (1.58–2.36%), camphene (0.92–2.25%), δ 3-carene (1.22–1.80%), o-cymol (0.81–2.12%), bornyl acetate (0.22–3.53%), *trans*-verbenol (0.80–1.80%), caryophyllene oxide (0.59–3.15%), and ξ -fenchene (0.79–1.72%).

Across the unified dataset (Table 5), the major constituents did not vary independently; rather, several consistent co-variation patterns suggested time- and canopy-dependent compositional redistribution (noting that values were reported as relative abundances). Among the nine-canopy position $\times$ sampling time combinations, the middle third at 12:00 showed the lowest observed relative abundances of camphor (6.71%) and 1,8-cineole (5.77%), coinciding with the highest observed values of caryophyllene (6.51%) and caryophyllene oxide (3.15%), consistent with a relative shift toward greater representation of sesquiterpene-associated constituents at that stratum and time. A second trade-off was evident in the lower third at 12:00, where borneol peaked (12.79%) concurrently with the minimum of α -pinene (4.91%), consistent with a relative displacement between this oxygenated monoterpene and the monoterpene hydrocarbon fraction in that treatment. In the upper third at 18:00, *cis*-verbenone peaked (17.94%) while borneol reached its minimum (8.60%), pointing to a vertical- and time-specific rebalancing among oxygenated monoterpenes within the upper canopy. By contrast, linalool (4.28–5.83%) and limonene (2.79–3.66%) varied within comparatively narrow ranges, suggesting greater stability relative to the more dynamic major constituents. These relationships should be interpreted as descriptive co-occurrence patterns that may reflect relative-profile shifts, rather than definitive biochemical interactions, given the single composite chromatogram per treatment.

Some of the identified minor constituents in the *S. rosmarinus* VO profile were detected at trace to low relative abundances (approximately 0.10–0.60%) and were not consistently detected across all canopy positions and sampling times, indicating pronounced spatio-temporal heterogeneity within the minor fraction of *S. rosmarinus* VO. Several minor constituents were confined to the lower and/or middle thirds and were not detected in the upper third, including filifolone (predominantly lower; only sporadic detection in the middle), γ -eudesmol, thymol, carvacrol, *p*-isopropylanisole, and 1,3,5-cycloheptatriene, 3,7,7-trimethyl-, with additional time-specific absences consistent with levels fluctuating near the reporting threshold. In contrast, some constituents showed an upper-canopy association, notably *trans*- β -terpineol, which was detected only in the upper third at all timepoints, and *cis*-sabinene hydrate, which appeared only in the upper third and only from 12:00 onward. Limonen-4-ol exhibited a distinct vertical pattern, being detected in the middle and upper thirds across times while remaining undetected in the lower third. Isopiperitenone was detected intermittently in the lower and middle thirds and appeared in the upper third only at 18:00, further illustrating time-dependent detectability among low-abundance constituents. Overall, the repeated non-detection of these minor constituents, together with their very low percentages, was consistent with a minor fraction that varied around the analytical detection/reporting limit and displayed strong canopy- and time-related shifts in detectable constituents.

The VOs were consistently dominated by oxygenated monoterpenes (54.93–68.44%) and monoterpene hydrocarbons (15.96–26.51%), indicating a broadly monoterpene-rich profile across the nine treatments (Table 6). When combined, total monoterpenes (oxygenated monoterpenes + monoterpene hydrocarbons) ranged from 75.81% to 92.09%. In contrast, sesquiterpene classes contributed a smaller but more variable fraction: sesquiterpene hydrocarbons ranged from 3.28% to 10.28%, and oxygenated sesquiterpenes from 1.16% to 9.47%, yielding total sesquiterpenes (sesquiterpene hydrocarbons + oxygenated sesquiterpenes) of 4.80–19.75%. Minor classes remained low overall, with esters ranging from 0.33% to 3.67%, ethers from 0.15% to 0.59%, ketones from 0.48% to 1.51%, and alcohols from 0.16% to 0.72%.

Table 6. Chemical classification of *S. rosmarinus* volatile oil constituents under diurnal and intra-plant variation

Chemical groups (%)	Lower third			Middle third			Upper third		
	06:00	12:00	18:00	06:00	12:00	18:00	06:00	12:00	18:00
Monoterpene hydrocarbon (15)	26.45	15.96	25.59	26.51	20.88	26.04	19.92	26.09	21.89
Oxygenated monoterpene (33)	64.86	68.44	62.61	64.27	54.93	66.05	67.88	63.88	67.36
Sesquiterpene hydrocarbon (7)	3.28	6.36	4.94	3.41	10.28	3.51	4.09	3.64	4.41
Oxygenated sesquiterpene (9)	2.92	6.09	4.12	3.03	9.47	1.76	2.1	1.16	1.66
Ester (2)	0.34	0.41	0.33	0.76	1.31	0.7	3.67	3.23	2.87
Ether (1)	0.25	0.34	0.33	0.35	0.59	0.15	0.3	0.24	0.29
Ketone (3)	0.68	1.1	0.81	0.66	1.51	0.58	0.63	0.48	0.53
Alcohol (1)	0.4	0.4	0.36	0.71	0.16	0.56	0.72	0.72	0.66

Clear diurnal- and canopy-dependent redistributions were evident when the nine treatments were compared directly. In the lower third, 12:00 was characterized by the lowest monoterpene hydrocarbons (15.96%) alongside the highest oxygenated monoterpenes (68.44%) for that canopy position, while both sesquiterpene classes increased relative to 06:00 (sesquiterpene hydrocarbons 6.36%; oxygenated sesquiterpenes 6.09%), giving a higher total sesquiterpene contribution (12.45%) than at 06:00 (6.20%) and 18:00 (9.06%). In the middle third, the strongest shift occurred at 12:00, where oxygenated monoterpenes dropped to 54.93% (the minimum observed across the entire Table 6), concurrent with peak values for sesquiterpene hydrocarbons (10.28%) and oxygenated sesquiterpenes (9.47%), producing the highest total sesquiterpenes (19.75%) and, correspondingly, the lowest total monoterpenes (75.81%) among all nine treatments. This same middle-third 12:00 treatment also showed the highest values recorded for ether (0.59%) and ketone (1.51%) and the lowest for alcohol (0.16%). By 18:00 in the middle third, the profile returned to a strongly monoterpene-dominant pattern, with total monoterpenes reaching 92.09% (the maximum total monoterpenes across the Table 6) and total sesquiterpenes decreasing to 5.27%.

In the upper third, oxygenated monoterpenes remained high and comparatively stable across the day (67.88% at 06:00, 63.88% at 12:00, 67.36% at 18:00), while monoterpene hydrocarbons peaked at 12:00 (26.09%) and were lower at 06:00 (19.92%) and 18:00 (21.89%). Sesquiterpenes in the upper third reached their minimum at 12:00 (sesquiterpene hydrocarbons 3.64%; oxygenated sesquiterpenes 1.16%; total sesquiterpenes 4.80%, the lowest total sesquiterpenes among all treatments). A pronounced within-plant (vertical) pattern was most evident for esters, which were consistently enriched in the upper third at all times (3.67%, 3.23%, 2.87% at 06:00, 12:00, and 18:00, respectively), compared with the middle third (0.76%, 1.31%, 0.70%) and lower third (0.34%, 0.41%, 0.33%). Collectively, these class-level data show that while the oils were monoterpene-dominated across all conditions, the magnitude and direction of class redistribution across the day depended on canopy position, with the most conspicuous departure from monoterpene dominance occurring at 12:00 in the middle third, and a consistent vertical enrichment of esters toward the upper canopy.

DISCUSSION

Volatile oil content (%)

Although pharmacopeial/monograph specifications for *S. rosmarinus* leaf report a minimum VO content of not less than 1.2% (v/w) (WHO, 2009; EMA, 2024), the VO values obtained in the present study were lower. *S. rosmarinus* is a perennial shrub of Mediterranean origin and is predominantly cultivated under Mediterranean-type climates characterized by high solar radiation, irregular rainfall, and a pronounced summer drought (May–September), during which maximum daily temperatures frequently exceed 30 °C (González-Minero et al., 2020). Moreover, across medicinal and aromatic plants, both the yield and the profile of secondary metabolites are shaped by a combination of determinants—most notably genotype, prevailing climate, soil attributes, crop management, harvest timing, the harvested organ, and post-harvest handling (Soltanbeigi and Sakartepe, 2020). Therefore, VO values below pharmacopeial/monograph thresholds may plausibly reflect combined bioclimatic and post-harvest effects on oil accumulation and/or recovery rather than methodological inconsistency.

The VO content showed a clear canopy-position effect, with the upper third presenting higher yields than the middle and lower thirds at all sampling times (Table 4). This positional pattern is consistent with the anatomical localization of *S. rosmarinus* volatiles in glandular trichomes: *S. rosmarinus* leaves are densely covered with capitate and peltate glandular trichomes, where volatile biosynthesis predominantly occurs (Boix et al., 2011). In Lamiaceae, volatile terpenoids are closely associated with glandular trichomes—particularly peltate and capitate types—which are recognized as key secretory structures involved in VO production and storage (Gostin, 2024). Moreover, within-plant heterogeneity of volatile terpenoids has been directly documented in *S. rosmarinus*: total concentrations of terpenes vary with tissue source and age, and the highest total terpene concentration occurs in young leaves, with total terpene concentration reported as up to three times higher in young leaves than in mature leaves (Bellumori et al., 2021). In this context, the higher VO yields measured in the upper plant section (Table 4)—where the maximum VO (0.75%) occurred in the upper third at 12:00 and 18:00, while the minimum (0.43%) occurred in the lower third at 06:00—are consistent with published evidence that *S. rosmarinus* exhibits strong within-plant variability in volatile terpenoid pools and that leaf tissues can differ markedly in total terpene abundance (Barton et al., 2019; Mahdavi et al., 2020; Bellumori et al., 2021).

On the other hand, terpenoid synthesis in plants occurs largely through the MEP pathway, and this pathway is strongly influenced by light. Higher light availability is associated with enhanced terpenoid biosynthesis (Cheng et al., 2025). Both photosynthetically active radiation (PAR) and leaf temperature increase during midday (12:00 PM) and afternoon (6:00 PM). While this increases biosynthetic capacity and the volatilization tendency of terpenoids stored in tissues, the higher amount synthesized contributes to the higher measured VO yield. The increase in VO observed at midday can be explained by the fact that maximum light intensity increases the activity of key enzymes in the MEP pathway, supports substrate flow for photosynthesis, and activates intratrachome synthesis. Studies have reported maximum yields at similar times to diurnal measurements (Ramezani et al., 2009). Most VO constituents (volatile terpenoids) are synthesized and stored in peltate and capitate glandular trichomes of *Rosmarinus* leaves. Because photosynthesis ceases during the night, the substrate flux of the MEP pathway decreases, reducing new terpenoid synthesis. Lower trichome density in lower leaves and lower morning light and temperature conditions limit both new terpenoid synthesis and the volatilization of stored volatile constituents, resulting in minimum VO content. This pattern is consistent with other diurnal studies (Ramezani et al., 2009; Khademi Doozakhdarreh et al., 2022). Accordingly, canopy position (via leaf ontogeny and glandular trichome abundance) establishes

the baseline VO content, whereas diurnal fluctuations in light and temperature modulate this baseline through MEP-pathway-driven terpenoid biosynthesis, thereby shaping the observed diurnal pattern in VO yield.

Volatile oil constituents

Overall, the *S. rosmarinus* VO profile exhibited clear diurnal and within-plant heterogeneity. While several constituents remained comparatively stable, others showed pronounced shifts in their relative abundances across sampling times and canopy positions. In addition, the intermittent non-detection of a number of low-abundance constituents indicates that the minor fraction was highly sensitive to spatio-temporal changes in detectability. These patterns are consistent with time- and canopy-dependent differences in leaf microenvironment and volatile dynamics (e.g., emission/volatilization and related compositional redistribution), and they emphasize that both harvest time and canopy position can influence the resulting VO chemical profile. Given the use of single composite samples per treatment, the observed trends should be interpreted descriptively as evidence of potential spatio-temporal variability in *S. rosmarinus* VO constituents. Accordingly, careful definition of sampling/harvest conditions may improve the consistency and comparability of *S. rosmarinus* VO profiles when a specific compositional target is desired.

According to the European Pharmacopoeia monograph for *S. rosmarinus* VO (Rosmarini aetheroleum; Ph. Eur. 01/2008:1846), as summarized in the EMA/HMPC assessment report (EMA/HMPC/681037/2021), compliance with the pharmacopoeial chromatographic profile is assessed against two chemotypic profiles (Spanish type vs Moroccan & Tunisian type) defined by acceptance ranges for 12 marker constituents (reported as %). Spanish type: α -pinene 18–26%; camphene 8.0–12.0%; β -pinene 2.0–6.0%; β -myrcene 1.5–5.0%; limonene 2.5–5.0%; 1,8-cineole 16.0–25.0%; *p*-cymene 1.0–2.2%; camphor 13.0–21.0%; bornyl acetate 0.5–2.5%; α -terpineol 1.0–3.5%; borneol 2.0–4.5%; verbenone 0.7–2.5%. Moroccan & Tunisian type: α -pinene 9.0–14.0%; camphene 2.5–6.0%; β -pinene 4.0–9.0%; β -myrcene 1.0–2.0%; limonene 1.5–4.0%; 1,8-cineole 38.0–55.0%; *p*-cymene 0.8–2.5%; camphor 5.0–15.0%; bornyl acetate 0.1–1.5%; α -terpineol 1.0–2.6%; borneol 1.5–5.0%; verbenone max 0.4% (EMA, 2024).

When the Ph. Eur. marker constituents (Spanish type vs Moroccan & Tunisian type) were compared against current GC–MS dataset (nine composite samples: 3 times $\times$ 3 canopy positions), the observed ranges in Table 5 were: α -pinene 4.91–11.61%, camphene 0.92–2.25%, β -pinene 0.22–0.89%, β -myrcene 0.65–1.07%, limonene 2.79–3.66%, 1,8-cineole 5.77–9.55%, cymene (reported in Table 5 as “o-Cymol”) 0.81–2.12%, camphor 6.71–15.59%, bornyl acetate 0.22–3.53%, α -terpineol 2.18–2.74%, borneol 8.60–12.79%, and verbenone (reported as *cis*-verbenone) 10.48–17.94%. Overall, this profile shows only partial overlap with the pharmacopoeial chemotypic ranges and does not align cleanly with either Ph. Eur. type definition. The strongest mismatch is 1,8-cineole, which remained <10% across all treatments (5.77–9.55%)—well below the minimum required for the Spanish type (16–25%) and far below the Moroccan & Tunisian type (38–55%). In parallel, camphene (0.92–2.25%) and β -pinene (0.22–0.89%) were consistently below the lower bounds of both types, and β -myrcene (0.65–1.07%) was below the Spanish range and mostly below (or only marginally reaching) the Moroccan & Tunisian lower bound. By contrast, limonene (2.79–3.66%) and cymene (0.81–2.12%) fell largely within the acceptance windows reported for both types, while camphor (6.71–15.59%) overlapped both standards only incompletely (including values compatible with the Moroccan & Tunisian window, and several within the Spanish window, but also a maximum slightly above the Moroccan & Tunisian upper limit). Most decisively, borneol (8.60–12.79%) and verbenone (*cis*-verbenone) (10.48–17.94%) were far above the pharmacopoeial ranges for both types, and bornyl acetate reached values (up to 3.53%) exceeding the stated upper limits as well—patterns that, taken together with the low cineole, indicate that the studied *S. rosmarinus* VO does not conform to either of the two Ph. Eur. chromatographic “type” profiles as written. Deviations from the Ph. Eur. chemotypic ranges are not unexpected because those limits refer to pharmacopoeial *S. rosmarinus* VO obtained by steam distillation of flowering aerial parts and defined for specific chemotypic profiles, whereas the present dataset derives from leaf material sampled across canopy strata and diurnal timepoints, conditions that can yield distinct relative profiles and may reflect chemotypes beyond the two pharmacopoeial types.

Rofouei et al. (2021) examined the diurnal variation of *S. rosmarinus* VO composition by sampling at three timepoints (before sunrise, noon, and after sunset). Compared with that work, the present study shows broad agreement in the identity of key marker constituents (e.g., α -pinene, camphene, limonene, 1,8-cineole, camphor, borneol, verbenone, bornyl acetate, and selected sesquiterpenes). However, the diurnal trajectories are not fully concordant: in the aforementioned study several markers exhibited largely unidirectional daytime shifts, whereas the present results indicate that diurnal patterns can be modulated by canopy position, with some constituents showing different directional responses across strata. Moreover, the relative major-component balance differed between studies, most notably in the higher contribution of *cis*-verbenone and the lower relative abundance of 1,8-cineole in the present samples. Compared with Flamini et al. (2002), the present study showed a broadly similar marker set but a different major-component balance. 1,8-Cineole remained low (5.77–9.55%), aligning in magnitude with the low-cineole profile rather than the cineole-rich ecotype reported in that work, whereas α -pinene was also markedly lower (4.91–11.61%) than in the α -pinene-rich ecotype. In contrast, the present samples were strongly weighted toward oxygenated constituents, particularly camphor (6.71–15.59%), borneol (8.60–12.79%), and *cis*-verbenone (10.48–17.94%), which were not comparably prominent in the tabulated leaf profiles. A positional tendency was nevertheless consistent, with bornyl acetate being relatively enriched toward the upper canopy in the present work. Overall, the divergence in dominant markers plausibly reflects ecotype/harvest-context differences and the present stratified (diurnal $\times$ canopy) sampling design. A within-plant study on *S. rosmarinus* quantified volatile terpenoids across young vs. mature leaves and different branch positions, showing that *S. rosmarinus* terpene profiles can vary within a single plant as a function

of tissue age and tissue source (Bellumori et al., 2021). In that work, total volatile terpenoids were reported to be up to ~3-fold higher in young leaves than in mature leaves, whereas mature leaves collected from the top vs. base of the branch did not differ significantly in the relative percentage of each compound when age was controlled; in contrast, comparisons across samples differing in tissue source/age yielded significant differences in relative terpene composition. Notably, the leaf volatile profile included several constituents that also occur among the key markers in the present study (e.g., α -pinene, verbenone, 1,8-cineole, and β -caryophyllene), providing direct precedent that within-plant leaf position/age-related heterogeneity can be reflected in both abundance patterns and relative composition. More generally, monoterpene emission is strongly temperature-dependent, while broader abiotic forcing—including light—has been identified as an important modulator of emission dynamics (Tingey et al., 1980; Malik et al., 2023). Volatile isoprenoids have also been discussed in the context of plant responses to abiotic/oxidative stress (Vickers et al., 2009).

Taken together, the present GC–MS dataset (71 identified constituents) demonstrates clear diurnal and within-plant heterogeneity, expressed both as changes in detected constituent richness (55–68 compounds across canopy position $\times$ time) and as time- and stratum-specific shifts in the relative abundances of major and minor constituents. Across the nine treatments, key constituents (camphor, *cis*-verbenone, borneol, α -pinene, and 1,8-cineole) showed non-uniform diurnal trajectories that depended on canopy position, alongside coherent co-variation patterns consistent with relative compositional redistribution (e.g., the middle third at 12:00 combining minima of camphor and 1,8-cineole with maxima of caryophyllene and caryophyllene oxide). Several minor constituents occurred intermittently at trace levels and were not consistently detected, indicating strong spatio-temporal heterogeneity around the reporting/detection threshold. Partial overlap but non-alignment with Ph. Eur. chemotypic ranges (EMA, 2024) is interpretable in light of the pharmacopoeial scope (steam-distilled essential oil from flowering aerial parts and defined chemotypic profiles) versus the present sampling frame (leaf material stratified by canopy and time). Comparisons with time-only (Rofouei et al., 2021) and organ/ecotype-focused datasets (Flamini et al., 2002), together with evidence that within-plant terpene profiles vary with tissue source and age (Bellumori et al., 2021), support the conclusion that *S. rosmarinus* VO composition can differ markedly with sampling context; accordingly, specifying harvest time and canopy position is essential for obtaining comparable and interpretable volatile profiles under a single-composite-sample design.

Differences in constituent depending on diurnal and leaf position seen in Table 6 indicate that *S. rosmarinus* VO is shaped by complex interactions between environmental conditions and intra-plant physiology. The high presence of oxygenated monoterpenes (approximately 70%) at noon in the lower canopy can be explained by microclimate effects (shading, humidity, boundary layer diffusion) and possible constituent accumulation in the lower leaves, although temperature and light increase volatility (Tingey et al., 1980; Malik et al., 2023; Bellumori et al., 2021). The reverse exchange of monoterpene hydrocarbons with oxygenated forms may be related to biosynthetic competition or shared precursor (GPP) utilization (Vickers et al., 2014). The increase in sesquiterpenes at noon in the mid-canopy is consistent with the lower volatility of these constituents and different enzymatic activity (Hellén et al., 2021). The higher volatility of esters in the morning and decreasing throughout the day can be attributed to their higher volatility (Khademi Doozakhdarreh et al., 2022; Rathore et al., 2022). Although the total volatile content remained constant, the relative distribution of constituent classes varied with time and leaf position, indicating that the chemical profile in *S. rosmarinus* is regulated by environmental microgradients and tissue characteristics and is important for harvest timing (Rofouei et al., 2021; Bellumori et al., 2021).

CONCLUSIONS

This work provides a structured, within-plant and diurnal snapshot of *S. rosmarinus* VO, showing that both canopy position and harvest time can shape oil yield and the resulting chemical profile. VO content followed a clear vertical pattern, with the upper canopy tending to concentrate more oil than lower strata, highlighting canopy position as a practical factor when sampling or harvesting for consistent output. GC–MS profiling confirmed a profile dominated by monoterpene-related fractions, while the relative contribution of sesquiterpene-associated constituents and the minor fraction showed greater sensitivity to sampling context. Across the diurnal–vertical matrix, the volatile profile was repeatedly characterized by a set of major constituents—most notably camphor, alongside *cis*-verbenone, borneol, α -pinene, and 1,8-cineole—whose relative representation shifted with time and canopy stratum, indicating meaningful within-plant heterogeneity in the major-compound balance. Taken together, the composite sampling design captures integrated, field-relevant patterns and supports a clear practical implication: defining canopy position and harvest time is essential for comparability across studies and for producing *S. rosmarinus* VO with more consistent or purpose-oriented profiles. Further replicated sampling can build on these patterns to quantify the strength of the observed trends under different environments and production settings.

Author Contributions

Emir Soltanbeigi: Design and performing of field experiments, Methodology, Data collection and curation, Software, Writing – original draft preparation and final revision of the manuscript.

Sedef Özliman: Methodology, Data collection and curation, Software, Writing – original draft preparation and final revision of the manuscript.

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