EEET ECOLOGICAL ENGINEERING & ENVIRONMENTAL TECHNOLOGY

Ecological Engineering & Environmental Technology 2024, 25(11), 1–17
https://doi.org/10.12912/27197050/191265
ISSN 2299–8993, License CC-BY 4.0

Received: 2024.07.02
Accepted: 2024.09.20
Published: 2024.10.01

Influence of Harvest Time and Environmental Factors on the Yield and Chemical Composition of Rosemary (*Rosmarinus officinalis* L.) Essential Oil in Northeast Morocco

Faouzi Farhani[1,3*], Ahmed El Aboudi[1], Amal Boujraf[2], Youssef Dallahi[3]

[1] Botany Team and Valorization of Plant and Fungal Resources (BOVAREF), Research Centre Biotechnology Vegetal and Microbial, Biodiversity and Environment, Faculty of Science, Mohammed V University in Rabat, Morocco

[2] Laboratoire des Productions Végétale, Animales et Agro-industrie, Equipe de Botanique, Biotechnologie et Protection des Plantes, Faculté des Sciences, Université Ibn Tofail, Kénitra, Morocco

[3] Laboratoire de Biotechnologie et Physiologie Végétales, Centre de Biotechnologie Végétale et Microbienne Biodiversité et Environnement, Faculté des Sciences, Université Mohammed V de Rabat, Rabat 10000, Morocco

* Corresponding author's e-mail: faouzi_farhani@um5.ac.ma

ABSTRACT

Assessment of the dynamics of rosemary (*Rosmarinus officinalis* L) is essential in the production of essential oils (EOs) in Morocco, considering the country is one of the main producers of rosemary EO. In this study, the authors aimed to examine the influence of harvesting period and environmental factors on the dynamics of rosemary EO, mainly its composition. Samples were collected from four sites in northeastern Morocco on a monthly basis between July 2021 and June 2022. Subsequently, quantitative and qualitative analyses by hydrodistillation and gas chromatography were performed to determine the yield and composition of EOs. On average, EO yields ranged from 2.3 to 3% across the four sites; they were highest in summer and lowest in autumn. A moderate negative correlation ($r = -0.59$, $p < 0.05$) was observed between precipitation and EO yield, while temperature had a moderately positive influence. A total of 17 chemical compounds, representing 88.9–99.1% of the EO extracts, were identified and consisted mainly of 1,8-cineole (44.2–46.6%), camphor (14.8–16.8%), borneol (7.5–9.1%), and α-pinene (5.2–5.9%). Harvesting period strongly influenced EO composition, with the highest concentrations of 1,8-cineole and α-pinene were recorded during the summer period (July and August), while the concentrations of borneol, camphor, and terpineol were highest in winter (December and January) and late spring. The findings of the study highlight the importance of monitoring the factors that influence the chemical composition of rosemary EO, thus providing a knowledge base that would help improve the quality and economic value of rosemary EO production in the region.

Keywords: essential oils, Morocco, *Rosmarinus officinalis* L, medicinal and aromatic plants.

INTRODUCTION

Medicinal and aromatic plants (MAP) are plants widely appreciated for their aroma, taste, and medicinal properties. They have been used for millennia on account of their healing properties, especially for the treatment of common diseases and illnesses (Houghton, 1995; Calixto, 2005; Kunle et al., 2012; El-Seedi et al., 2013; Maroyi, 2017). They are also used in the cosmetics and food industry for their flavor and fragrance. MAPs include a wide variety of plants, ranging from common culinary herbs like thyme, rosemary, and mint, to more exotic medicinal plants like ginseng and turmeric. Today, MAPs are becoming increasingly popular due to the growing interest in alternative and natural medicines, as well as herbal cosmetics and personal care products. Depending on the region, between 50–80% of the global population relies on medicinal plants to treat diseases, thus maintaining and improving their generational living conditions (Okoye et al., 2014; Wanjohi et al., 2020; Ssenku et al., 2022). In particular, rural populations in developing countries rely on traditional herbal remedies due to their easy access, cultural acceptability, and unfavorable economic conditions.

In Morocco, the varied climate and heterogeneous ecological conditions characterized by a full range of Mediterranean bioclimates have favored a rich and varied flora with a highly marked endemism resulting in more than 4 200 plant species, of which more than 400 are categorized as MAPs (Fennane and Ibn Tattou, 1998; Benabid, 2000; El-Hilaly et al., 2003; Alnamer et al., 2011), which play an important role in the socioeconomic development of the country. Indeed, Morocco is a traditional supplier to the world market, whose exports include dried plants, essential oils, and aromatic extracts, which contribute to the agricultural export balance (Zrira, 2003; Hakkou et al., 2023). However, MAPs face significant exploitation, posing a genuine threat to their diversity, in part due to the anarchic and abusive practices of the users (Ghanmi et al., 2011). Nevertheless, the national potential of MAPs remains strong and constitutes a particular asset for the socio-economic development of the country's rural areas. The contributions of scientific research, both basic and applied, to the knowledge of these MAPs are essential to developing, exploiting, and preserving them in the best conditions, especially for the well-being of the current and future generations (Fennane et al., 2016).

Rosemary (*Rosmarinus officinalis* L.), a species belonging to the Lamiaceae family, is among the most important MAPs in Morocco. It is a perennial shrub, native to the Mediterranean region that typically reaches a height of 1 to 2 meters and is characterized by needle-like leaves of 2 to 4 cm in length, dark green color, as well as a strong and distinct aroma. The components of this species can be used as a spice in cooking, as a natural preservative in the food industry, and as an ornamental and medicinal plant (Pérez-Fons et al., 2010; Rašković et al., 2014; de Oliveira et al., 2019). In Morocco, rosemary is used in traditional cooking to flavor dishes, especially meats and vegetables. It is also used in traditional medicine to treat a variety of ailments, including joint pain, headaches and digestive disorders (Btissam et al., 2015; Masure, 2018). Rosemary is predominantly found naturally in sub-humid and semi-arid environments, particularly in the Middle Atlas, High Atlas, and Oriental regions. Although it is often seen in degraded Barbary thuja, juniper, and holm oak forests in these regions, it can be found cultivated, especially for the production of essential oils (EOs) for local and commercial purposes (HCEFLCD, 2014; Karimi, 2014).

Several factors, such as geographic location, plant growth stage, environmental conditions, and extraction method, affect both the yield and chemical composition of EOs as well as the derived extracts (Jordan et al., 2013; Ben Jemia et al., 2014). For instance, the plants grown in limestone-rich soils in the Mediterranean region have been observed to contain higher amounts of camphor, while those grown in more acidic soils are associated with higher amounts of 1,8-cineole (Hendawy et al., 2017). The variations in chemical composition point to rosemary EOs chemotypes depending on the dominant chemical constituent, which are the result of the confluence of the aforementioned factors (Napoli et al., 2010; Jordan et al., 2013). Nevertheless, among the various studies in the literature, rosemary EOs are generally characterized by a predominance of camphor, 1,8-cineole (eucalyptol), verbenone, α-pinene, or borneol (Elamrani et al., 2000; Hussain et al., 2010; Rašković et al., 2014; Varna et al., 2020; Elyemni et al., 2022), the concentrations of which vary considerably depending on the region. Another important factor is the harvesting period of the plants, the influence of which on yield and composition can have an impact on the properties and uses of the extracted oils. Therefore, research on the seasonal variations in rosemary EOs and the role of harvest time on compositional variability is essential to optimize the production of EOs. Specifically, identifying the best time for extraction of specific compounds would ensure consistent quality of EOs, thus meeting the regulatory requirements of various industries, both locally in Morocco and for exports of derived products.

In this context, the objective of this study was to investigate the variability in the chemical composition of rosemary EOs based on different harvesting periods and environmental factors within three forests in Morocco. Specifically, the study involved: (i) collecting rosemary samples at four sites within the three forests at the beginning of each month for a period of one year between July 2021 and June 2022; (ii) extracting EOs from the collected samples using hydrodistillation, with gas chromatography used to analyze the chemical constituents of the extracted EOs; and (iii) conducting data analyses to determine the influence of site characteristics as well as harvest period on EOs yield and composition.

MATERIALS AND METHODS

Study area

The study area (Figure 1) is located in the province of Taourirt, in the Oriental region of Morocco. It comprises three forests, namely: Nergouchoum, Debdou, and El Atteuf. The characteristic climate is semi-continental and includes an arid to semi-arid bioclimatic zone with a temperate to cool variant. Mean annual precipitation is erratic and ranges between 200 and 300 mm, on average, while the mean temperature ranges from 15.1 to 16.6 °C, with minima and maxima of 1.4 and 34.1 °C, respectively. From a geological point of view, the study area forms part of the Horsts range, dominated by limestone, shale, clay, and silt, while the predominant soil types include weakly developed soils, calcimagnesic soils and raw mineral soils. It is characterized by a partially uneven relief, with a substantial portion featuring medium slopes and flat terrain, especially in its southern part. The vegetation cover is mainly composed of cedar (*Cedrus atlantica*), holm oak (*Quercus rotundifolia*), juniper (*Juniperus* sp.) and pine (*Pinus* sp.) forest stands. The flora associated with these formations is based on rosemary (*Rosmarinus officinalis*) and alfa grass (*Stipa tenacissima*).

Methods

Site selection and sampling

In order to establish a relationship between the nature, chemical composition, and yield of rosemary EO with the plant cycle and ecological descriptors, a year-long analysis of extracted EO was conducted at the beginning of each month between July 2021 and June 2022. To obtain a thorough inventory of rosemary in the study area, a probabilistic approach was employed using the stratified random sampling method. Accordingly, the chosen study area was subdivided into four sites (Table 1) based on ecological parameters including terrain type, soil type, bioclimatic zone, and composition of the rosemary facies. The samples were taken at the beginning of each month using the destructive technique over the whole year in order to evaluate the evolution of the essential EO according to the phenological cycle of the plant. The cut portion of the rosemary plant was at a height of 5–10 cm from the ground and was categorized into small, medium and large clumps.

Quantitative and qualitative analyses of EOs

Isolation of EOs – hydrodistillation was the technique chosen to extract the EOs in this study.

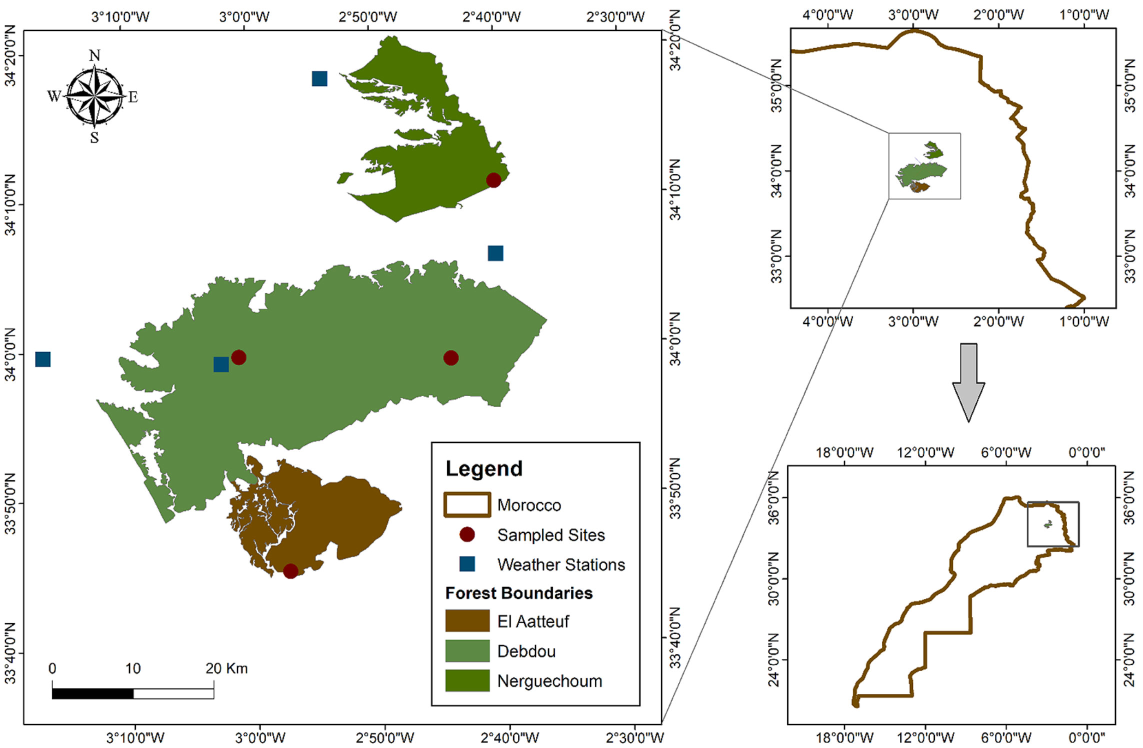

Figure 1. Map of the geographical location of the study sites

Table 1. Characteristics of the sampled sites

Site	Forest	Altitude (m)	Aspect	Substrate	Precipitation (mm/an)	Temperature (°C)
1	El Atteuf	1316	Flat	Silty-clay	278	15.3
2	Nerguechoum	784	Northeast	Limestone	366	15.1
3	Debdou	1252	Southwest	Shale	290	16.6
4	Debdou	1314	Northwest	Shale	383	16.6

The extraction process is one of the most commonly used and involves boiling a mixture of water and extractant, then liquefying the vapor in a cooler, decanting the hydrosol and collecting the EO (Xavier et al., 2011; Jeyaratnam et al., 2016). Correspondingly, the distillation of EO from samples collected from fresh rosemary clumps at the beginning of each month was conducted in a laboratory environment. The leafy portion of the collected clumps was cut into pieces and then introduced into a distillation flask. For each sample, 100 g was boiled for 3 hours under atmospheric pressure in approximately 1 l of water. The Clevenger system was used to recycle the distilled water back into the boiler via cohobation and to maintain a constant amount of water throughout the extraction process. After the steam was cooled, the water-EO mixture was condensed and separated by decantation to collect the resulting EO, which was then stored in the dark at 4 °C.

Detection and identification of EO constituents - the detection and subsequent identification of the different compounds of the extracted EO were performed using the gas chromatography (GC) technique. GC is an analytical technique first developed by Martin and Synge (1941) for separating the compounds present in a given mixture in order to identify and quantify them. It is mainly applied to gaseous compounds or those that can be vaporized by heating (Aparicio-Ruiz et al., 2018). Once the sample has been prepared, it is first vaporized at the inlet of a column containing a solid or liquid active substance called the stationary phase, and then transported through the column using a carrier gas. The different compounds of the sample are then separated and released from the column one after the other, depending on their affinity with the stationary phase and the carrier gas. The GC-detectors used in this study were: (i) gas chromatography-mass spectrometry (GC-MS); and (ii) gas chromatography-flame ionization detection (GC-FID).

GS-MS

Analysis of EO constituents was performed with a GS-MS detection system using a Shimadzu QP 5050 device, fitted with a polyethylene glycol + 2-nitroterephthalate (FFAP) capillary column (50 × 0.32 mm; film thickness: 0.25 μm). The detector and injector temperatures were set at 240 °C. The FFAP column temperature program ranged from 60 °C (1 min) to 220 °C at a rate of 5 °C min^{-1}, and then was held at 220 °C for 35 min. Helium was used as the carrier gas at a flow rate of 14 psi (1:20 split), and the injection volume for each sample was 5 μl. The ionization energy was set at 70 eV. Correspondingly, the identification of EO constituents was based on the comparison of their mass spectra with those of the Wiley/Nist 2020 mass spectra library (https://sciencesolutions.wiley.com/solutions/technique/gc-ms/nist-epa-nih-mass-spectral-library-2020-2/).

GS-FID

In contrast, using GS-FID, the constituents of the extracted EO were separated, and their retention indices (RIs) were quantified using a Hewlett-Packard (HP 6890 series) electronically controlled pressure gas chromatograph. Subsequently, 1 μl of the EO was mixed with methanol in an injector heated to 250 °C. The volatile constituents of the EO were entrained by an inert carrier gas (nitrogen), with a flow rate of 2 μl/min, into an HP-5 capillary column (30 × 0.25 mm; film thickness: 0.25 μm). The sample was then placed in a temperature-programmed oven at a temperature ranging from 50 to 250 °C with a 4 °C step. At the exit of the column, the EO constituents were analyzed by a flame ionization detector (FID) fed with a hydrogen/air mixture. Finally, the chromatogram, which showed a Gaussian distribution of the molecules, allowed quantitative analysis (relative percentage of each signal to the total signals of the analyzed mixture).

Data analyses

Pearson correlation analysis was performed on the data to identify the relationships between the major chemical compounds identified in the EO extracted from the rosemary samples. In addition, regression analysis was used to examine the relationships between rosemary EO (dependent variable) and site characteristics (independent variables). The correlation and regression analyses were performed in an R environment, with the significance level set at $p < 0.05$. As for the relationship between the harvesting period of rosemary and the composition of the EO extracts, it was investigated using hierarchical clustering analysis (HCA) and principal component analysis (PCA). Accordingly, the data on samples collected each month between July 2021 and June 2022 were preprocessed by scaling the variables using the "scale" function in R, and then PCA was performed using the "prcomp" function.

RESULTS

Overview of the yield and chemical composition of EO

The results of the yield analysis of the EOs isolated from the rosemary samples collected from the four study sites, along with the chemical compounds identified are presented in Tables 2–5. On average, the EO yield was highest for the samples collected at site 1 (El Atteuf) at 3% and lowest at site 4 (Debdou) at 2.3%. Across the four sites, EO yield averaged highest during the summer period, ranging from 2.5% (site 4) to 3.2% (site 1), while being lowest during the autumn months (2.1–2.8%). A total of 17 chemical compounds, representing 88.9–99.1% of the total EO extracted from rosemary samples, were identified for the four study sites. The results showed that EO contained mainly 1,8-cineole (44.2–46.6%), camphor (14.4–16.8%), borneol (7.5–9.1%) and α-pinene (5.2–5.9%). Other constituents representing relatively important proportions (> 2% of the total EO composition) are β-pinene, terpineol, and camphene. The extracted EO were largely composed of oxygenated monoterpenes and monoterpene hydrocarbons, accounting for 77.6–80.1% and 15.2–17.0%, respectively.

Table 6 presents the results of Pearson's correlation analysis between the major constituents of the EO extracted from rosemary in the study. The analysis revealed a significantly strong positive correlation between the concentration of 1,8-cineole and α-pinene ($r = 0.69$, $p < 0.05$), and a moderate

Table 2. Yield (%) assessment of chemical compounds derived from EO extracted from rosemary samples for site 1

Compound	2021						2022					
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Overall	3.6	3.0	3.0	2.7	2.7	2.9	2.7	3.0	2.6	2.6	3.5	3.1
α-Thujene	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.2	0.2
α-Pinene	7.6	7.8	7.2	6.6	6.8	4.0	4.2	5.8	6.4	4.3	4.3	6.0
Camphene	3.5	3.3	3.4	2.9	3.2	2.0	2.4	3.0	3.4	2.4	2.2	3.0
β-Pinene	5.0	3.9	3.1	2.9	3.3	2.9	3.7	5.0	6.8	6.1	5.3	5.8
Myrcene	1.2	1.1	1.0	1.0	1.1	0.9	0.9	1.0	1.1	1.0	1.2	1.3
α-Terpinene	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.5	0.5
p-Cymene	0.7	1.3	1.4	1.4	1.3	1.3	1.2	1.3	1.1	1.8	0.6	0.7
1,8-Cineole	49.5	51.5	47.5	51.7	45.0	43.9	43.7	45.4	43.8	46.6	44.2	45.9
γ-Terpinene	0.7	0.6	0.5	0.5	0.6	0.6	0.8	0.7	1.0	1.0	1.1	0.8
Terpinolene	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.4
Linalool	1.2	1.2	1.1	1.4	1.2	1.1	1.3	1.0	0.9	1.0	1.1	1.3
Camphor	15.8	14.7	17.6	17.3	16.7	20.4	18.5	14.3	15.2	16.9	16.8	17.0
Borneol	7.6	7.9	9.6	7.3	9.5	10.0	10.2	10.0	8.7	8.2	12.3	8.2
Terpinen-4-ol	1.3	1.3	1.4	1.5	1.4	1.6	1.6	1.6	1.7	2.2	1.8	1.9
P-Cymen-8-ol	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.2	0.2	0.2
Terpineol	4.3	4.7	4.8	5.0	4.9	5.5	4.9	4.7	4.8	5.0	5.2	5.0
Bornyl acetate	0.5	0.6	0.7	0.7	1.1	1.3	1.6	1.4	2.3	2.5	1.7	0.9
Monoterpenes hydrocarbons	19.9	18.8	17.5	16.0	17.1	12.3	13.6	17.4	20.3	16.5	15.3	18.7
Oxygenated monoterpenes	78.8	80.4	81.4	83.3	78.7	82.8	80.5	77.3	76.0	80.6	81.9	78.9
Sesquiterpenes hydrocarbons	2.1	1.3	1.1	0.9	1.9	2.6	3.3	3.1	2.9	2.5	2.3	1.7
Oxygenated sesquiterpenes	0.6	0.7	1.0	0.9	1.7	1.9	1.9	1.6	1.2	1.1	1.2	1.0

Table 3. Yield (%) assessment of chemical compounds derived from EO extracted from rosemary samples for site 2

Compound	2021						2022					
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Overall	3.2	2.6	2.6	2.3	2.3	2.5	2.3	2.6	2.2	2.2	3.1	2.7
α-Thujene	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.1	0.2
α-Pinene	7.3	7.5	6.9	6.3	6.5	3.7	3.9	5.5	6.1	4.0	4.0	5.7
Camphene	3.4	3.2	3.3	2.8	3.1	1.9	2.3	2.9	3.3	2.3	2.1	2.9
β-Pinene	4.9	3.8	3.0	2.8	3.2	2.8	3.6	4.9	6.7	6.0	5.2	5.7
Myrcene	1.1	1.0	0.9	0.9	1.0	0.8	0.8	0.9	1.0	0.9	1.1	1.2
α-Terpinene	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.5
p-Cymene	0.7	1.2	1.3	1.4	1.3	1.3	1.1	1.2	1.0	0.6	0.5	0.6
1,8-Cineole	49.1	51.1	47.1	51.3	44.6	43.5	43.3	45.0	43.4	46.2	43.8	45.5
γ-Terpinene	0.7	0.6	0.5	0.5	0.6	0.6	0.8	0.7	1.0	1.0	1.1	0.8
Terpinolene	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.4
Linalool	1.2	1.2	1.1	1.4	1.2	1.1	1.3	1.0	0.9	1.0	1.1	1.3
Camphor	15.5	14.4	17.3	17.0	16.4	20.1	18.2	14.0	14.9	16.6	16.5	16.7
Borneol	7.5	7.8	9.5	7.2	9.4	9.9	10.1	9.9	8.6	8.1	12.2	8.1
Terpinen-4-ol	1.3	1.3	1.3	1.5	1.4	1.6	1.6	1.5	1.7	2.1	1.8	1.8
P-Cymen-8-ol	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.1
Terpineol	4.0	4.4	4.5	4.7	4.6	5.2	4.6	4.4	4.5	4.7	4.9	4.7
Bornyl acetate	0.5	0.6	0.7	0.5	1.1	1.3	1.5	1.4	2.3	2.4	1.6	0.9
Monoterpenes hydrocarbons	19.6	18.5	17.2	15.7	16.8	12.0	13.3	17.1	20.0	16.2	15.0	18.4
Oxygenated monoterpenes	77.8	79.4	80.4	82.3	77.7	81.8	79.5	76.3	75.0	79.6	80.9	77.9
Sesquiterpenes hydrocarbons	2.0	1.2	1.0	0.8	1.8	2.5	3.2	3.0	2.8	2.4	2.2	1.6
Oxygenated sesquiterpenes	0.5	0.6	1.0	0.8	1.7	1.9	1.8	1.6	1.2	1.1	1.1	1.0

positive correlation with camphene ($r = 0.51$, $p < 0.05$). In contrast, it shows a significantly moderate negative correlation with borneol ($r = -0.49$, $p < 0.05$). A very strong positive correlation is observed between camphor and terpineol ($r = 0.77$, $p < 0.05$), whereas camphor and borneol are moderately correlated ($r = 0.41$, $p < 0.05$). Conversely, camphor had significant moderate negative correlations with α-pinene and camphene. Except for β-pinene, camphene showed significant correlations with the other constituents, the strongest being with α-pinene ($r = 0.94$, $p < 0.05$). β-pinene had weak negative correlations with all other constituents except camphene, with which it had a weak positive correlation. Overall, the major constituents of rosemary EO were moderately correlated with each other, with some negative and positive correlations observed.

Relationship between site characteristics and EO yield

Effect of bioclimatic factors

Bioclimatic parameters were found to influence EO yields, as indicated by the results of the study of their relationships presented in Figure 2. Indeed, regression analysis showed a generally moderate correlation between EO yield and mean precipitation (P_{mean}), mean (T_{mean}), maximum (T_{max}), and minimum (T_{min}) temperatures at the sites during the harvest period. Notably, EO yield was significantly negatively correlated with monthly precipitation ($r = -0.59$, $p < 0.05$), implying the inconsequential effect of a generally inadequate and erratic precipitation regime associated with the study sites on EO synthesis in rosemary. Conversely, EO yield was positively correlated with temperature parameters ($r = 0.57$, $r = 0.57$, and $r = 0.56$ for T_{mean}, T_{max}, and T_{min}, respectively).

Effect of geographic, orographic, and soil factors

The results of the regression analysis to investigate the relationship between elevation, latitude, and longitude on EO yield are presented in Figure 3. They revealed a weak non-significant correlation between yield and either longitude or elevation, implying a largely inconsequential influence of geographic position on EO variation. Notably, though not significant, a

Table 4. Yield (%) assessment of chemical compounds derived from EO extracted from rosemary samples for site 3

Compound	2021						2022					
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Overall	3.0	2.4	2.4	2.1	2.1	2.3	2.1	2.4	2.0	2.0	2.9	2.5
α-Thujene	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.2	0.2	0.1
α-Pinene	6.9	7.1	6.5	5.9	6.1	3.3	3.5	5.1	5.7	3.6	3.6	5.3
Camphene	3.2	3.0	3.1	2.6	2.9	1.7	2.1	2.7	3.1	2.1	1.9	2.7
β-Pinene	3.9	2.8	2.0	1.8	2.2	1.8	2.6	3.9	5.7	5.0	4.2	4.7
Myrcene	1.0	0.9	0.8	0.8	0.9	0.7	0.7	0.8	0.9	0.8	1.0	1.1
α-Terpinene	0.4	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.4
p-Cymene	0.6	1.2	1.2	1.3	1.3	1.2	1.1	1.2	1.0	0.6	0.5	0.5
1,8-Cineole	47.5	49.5	45.5	49.7	43.0	41.9	41.7	43.4	41.8	44.6	42.2	43.9
γ-Terpinene	0.6	0.5	0.4	0.4	0.5	0.5	0.7	0.6	0.9	0.9	1.0	0.7
Terpinolene	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.3	0.3	0.4	0.4	0.3
Linalool	1.0	1.0	0.9	1.2	1.0	0.9	1.1	0.8	0.7	0.8	0.9	1.1
Camphor	13.4	12.3	15.2	14.9	14.3	18.0	16.1	11.9	12.8	14.5	14.4	14.6
Borneol	6.0	6.3	8.0	5.7	7.9	8.4	8.6	8.4	7.1	6.6	10.7	6.6
Terpinen-4-ol	1.1	1.1	1.1	1.3	1.2	1.4	1.4	1.3	1.5	1.9	1.6	1.6
P-Cymen-8-ol	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1
Terpineol	3.8	4.2	4.3	4.5	4.4	5.0	4.4	4.2	4.3	4.5	4.7	4.5
Bornyl acetate	0.3	0.4	0.5	0.3	0.9	1.1	1.3	1.2	2.1	2.2	1.4	0.7
Monoterpenes hydrocarbons	18.5	17.4	16.1	14.6	15.7	10.9	12.2	16.0	18.9	15.1	13.9	17.3
Oxygenated monoterpenes	76.3	77.9	78.9	80.8	76.2	80.3	78.0	74.8	73.5	78.1	79.4	76.4
Sesquiterpenes hydrocarbons	1.7	0.9	0.7	0.5	1.5	2.2	2.9	2.7	2.5	2.1	1.9	1.3
Oxygenated sesquiterpenes	0.3	0.4	0.8	0.6	1.5	1.7	1.6	1.4	1.0	0.9	0.9	0.8

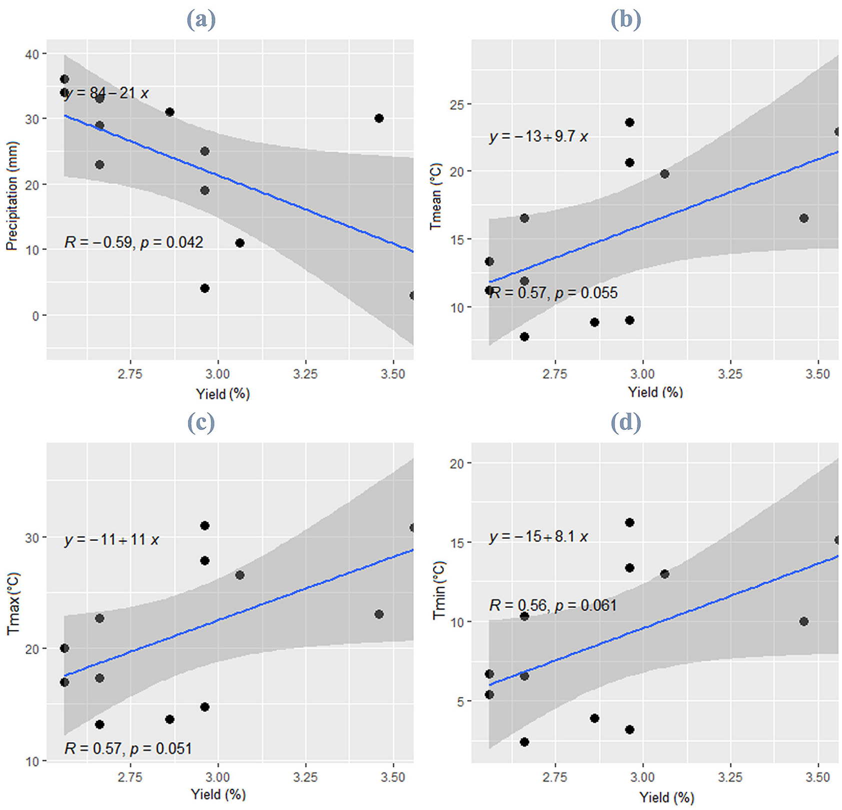

Figure 2. Scatterplots showing Pearson correlation between site bioclimatic parameters and EO yield

Table 5. Yield (%) assessment of chemical compounds derived from EO extracted from rosemary samples for site 4

Compound	2021						2022					
	Jul	Aug	Sep	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun
Overall	2.9	2.3	2.3	2.0	2.0	2.2	2.0	2.3	1.9	1.9	2.8	2.4
α-Thujene	0.1	0.1	0.0	0.0	0.0	0.0	0.1	0.1	0.1	0.1	0.1	0.1
α-Pinene	7.0	7.2	6.6	6.0	6.2	3.4	3.6	5.2	5.8	3.7	3.7	5.4
Camphene	3.2	3.0	3.1	2.6	2.9	1.7	2.1	2.7	3.1	2.1	1.9	2.7
β-Pinene	4.5	3.4	2.6	2.4	2.8	2.4	3.2	4.5	6.3	5.6	4.8	5.3
Myrcene	1.0	0.9	0.8	0.8	0.9	0.7	0.7	0.8	0.9	0.8	1.0	1.1
α-Terpinene	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.3	0.3	0.4	0.5	0.5
p-Cymene	0.6	1.1	1.2	1.3	1.2	1.2	1.0	1.1	0.9	0.5	0.4	0.5
1,8-Cineole	47.1	49.1	45.1	49.3	42.6	41.5	41.3	43.0	41.4	44.2	41.8	43.5
γ-Terpinene	0.6	0.5	0.4	0.4	0.5	0.5	0.7	0.6	0.9	0.9	1.0	0.7
Terpinolene	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.4	0.3	0.4	0.4	0.3
Linalool	1.0	1.0	0.9	1.2	1.0	0.9	1.1	0.8	0.7	0.8	0.9	1.1
Camphor	13.8	12.7	15.6	15.3	14.7	18.4	16.5	12.3	13.2	14.9	14.8	15.0
Borneol	6.9	7.2	8.9	6.6	8.8	9.3	9.5	9.3	8.0	7.5	11.6	7.5
Terpinen-4-ol	1.2	1.2	1.2	1.4	1.3	1.5	1.5	1.4	1.6	2.0	1.7	1.7
P-Cymen-8-ol	0.1	0.1	0.1	0.1	0.2	0.2	0.1	0.1	0.1	0.1	0.2	0.2
Terpineol	3.8	4.2	4.3	4.5	4.4	5.0	4.4	4.2	4.3	4.5	4.7	4.5
Bornyl acetate	0.4	0.5	0.6	0.4	1.0	1.2	1.4	1.3	2.2	2.3	1.5	0.8
Monoterpenes hydrocarbons	18.1	17.0	15.7	14.2	15.3	10.5	11.8	15.6	18.5	14.7	13.5	16.9
Oxygenated monoterpenes	76.5	78.1	79.1	81.0	76.4	80.5	78.2	75.0	73.7	78.3	79.6	76.6
Sesquiterpenes hydrocarbons	1.8	1.0	0.8	0.6	1.6	2.3	3.0	2.8	2.6	2.2	2.0	1.4
Oxygenated sesquiterpenes	0.4	0.5	0.9	0.7	1.6	1.8	1.7	1.5	1.1	1.0	1.0	0.9

Table 6. Correlations between the main EO constituents (yield > 2.0%)

Specification	1,8-cineole	Camphor	Borneol	α-pinene	Terpineol	β-pinene	Camphene
1,8-cineole	1.00						
Camphor	-0.07	1.00					
Borneol	-0.49*	0.41*	1.00				
α-pinene	0.69*	-0.37*	-0.44*	1.00			
Terpineol	-0.15	0.77*	0.53*	-0.46*	1.00		
β-pinene	-0.17	-0.24	0.09	-0.06	-0.02	1.00	
Camphene	0.51*	-0.40*	-0.38*	0.94*	-0.49*	0.17	1.00

Note: *correlation significant at $p < 0.05$.

moderate negative correlation was observed between EO yield and latitude ($r = 0.56$, $p > 0.05$), indicating that EO yield and composition might differ by site. In contrast, EO yield appeared to be influenced by both the aspect and substrate nature of the study sites (Figure 4). Indeed, the lowest EO yields were correlated with shale substrates on northwest- and southwest-facing slopes where temperatures were generally warm. These substrate types were dominant at sites 3 and 4 in Debdou Forest where the lowest EO yields were recorded. Conversely, the silty-clay and especially limestone substrates that dominate El-Atteuf (site 1) and Nerguechoum (site 2) forests, along with gentle to flat slopes characterized by cool temperatures, would be most likely to favor the conditions conducive to rosemary establishment and development, as evidenced by the highest EO yields recorded during the study.

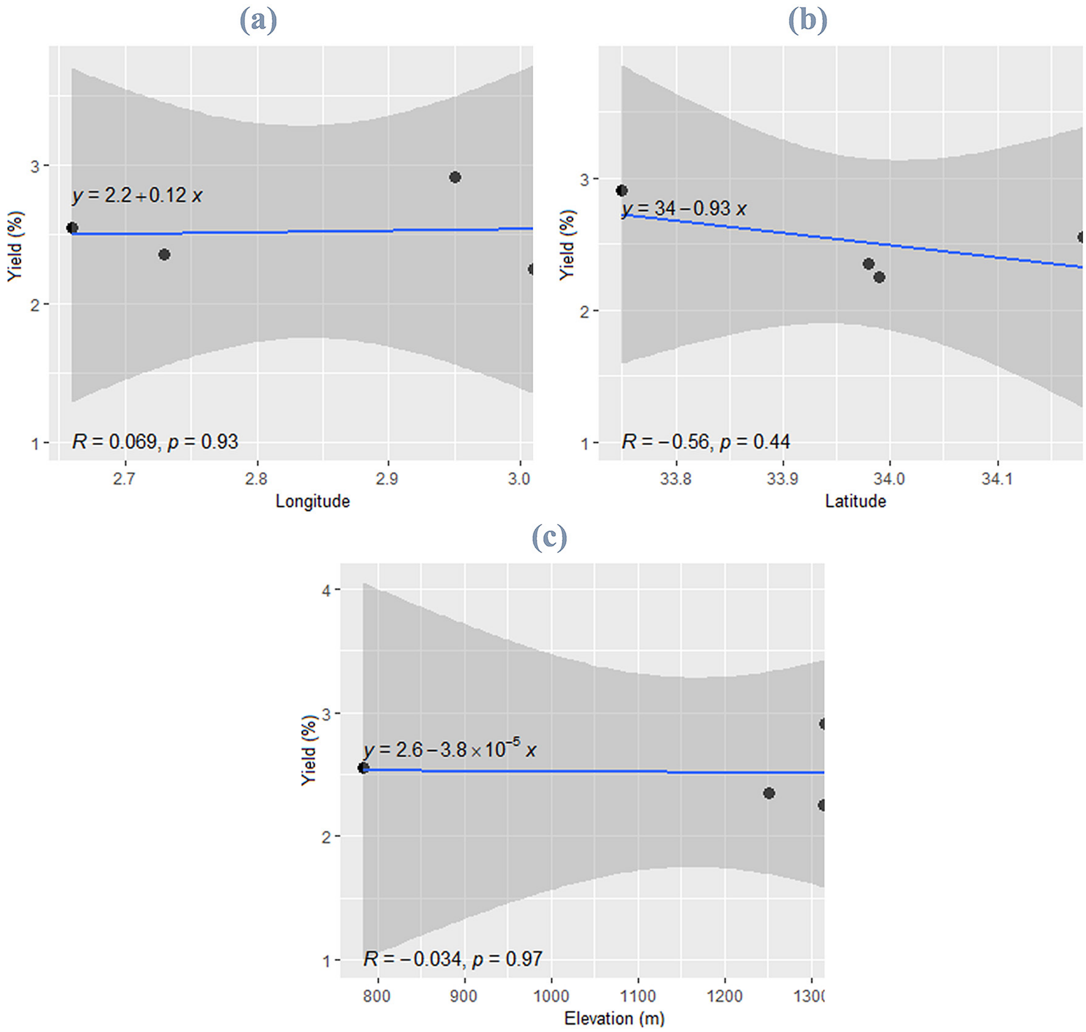

Figure 3. Scatterplots showing Pearson correlation between site orographic parameters and EO yield

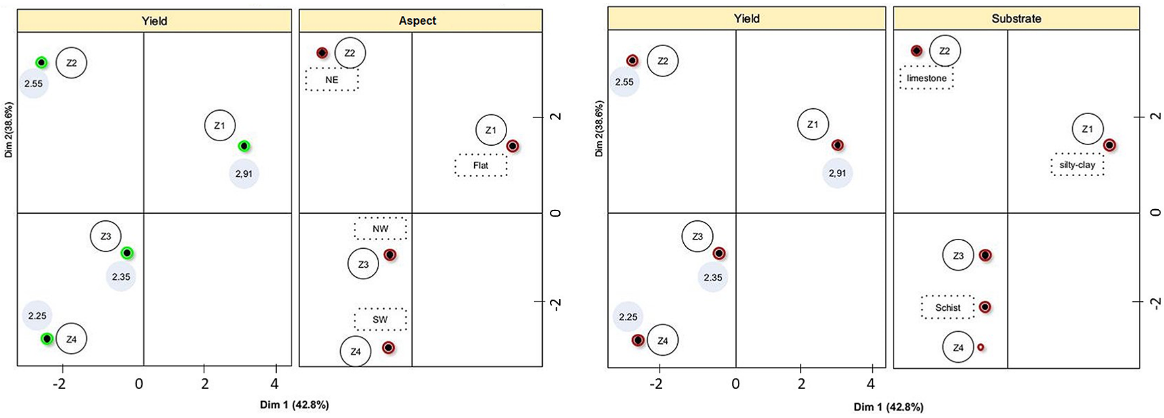

Figure 4. Factor plots for investigating the influence of aspect and substrate on EO yield

Assessment of variability in EO composition by harvest month

The results of the PCA are presented in the variable plots (Figure 5). The first two axes of the analysis represented 70.5%, 79.6% and 68.7% of the total variance in the data under sites 1, 2, and (3 and 4) respectively, indicating that a large part of the variability is captured. Generally, the analysis revealed that only the first principal component contained significant information, separating individuals harvested in August and July from

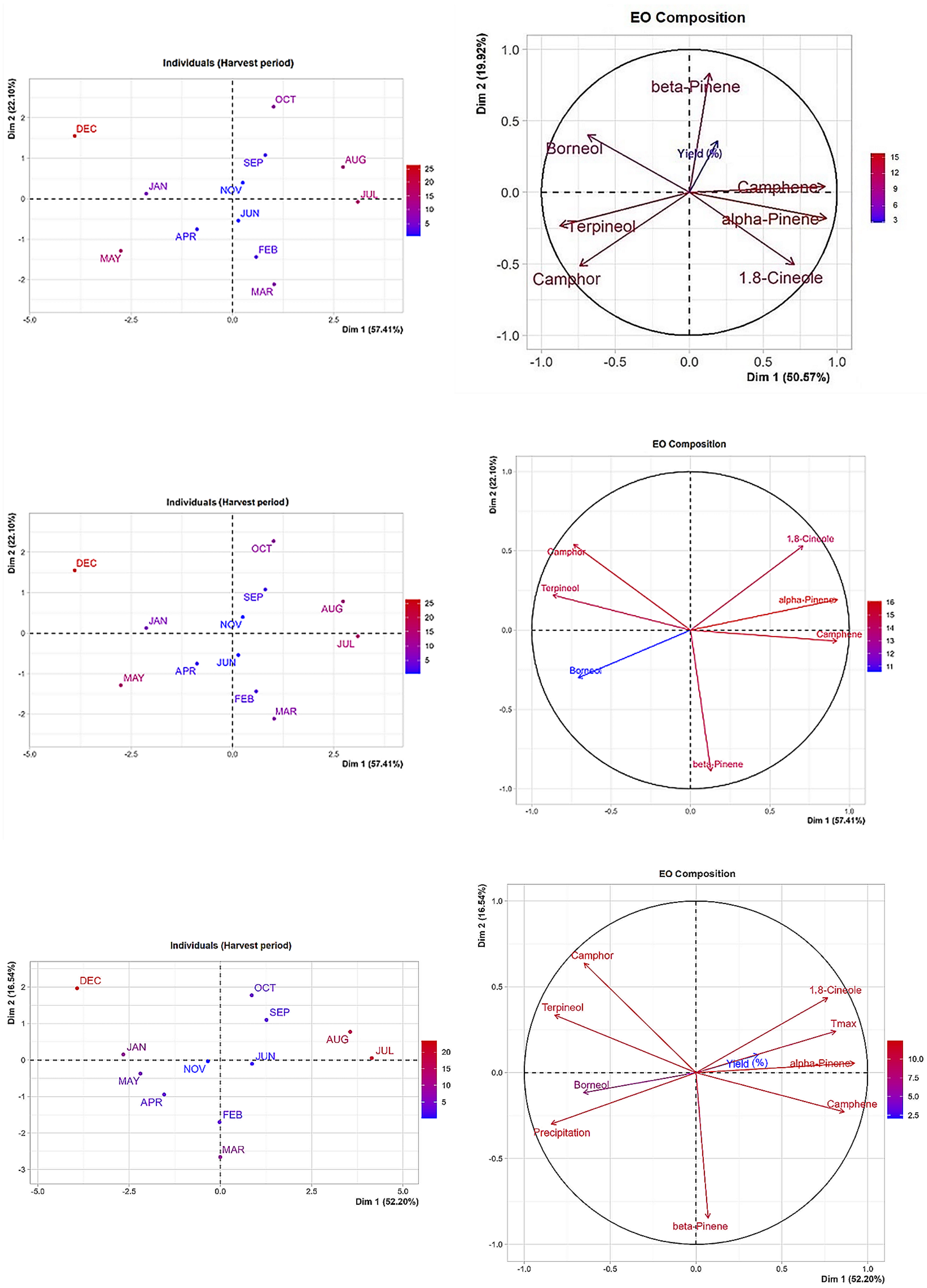

Figure 5. Variable correlation plots of variability in EO composition by harvest period for sites 1 (top) and 2 (bottom) located in the El Atteuf and Nerguechoum forests, respectively; PCA plots of variability in EO composition by harvest period for sites 3 and 4 located in the Debdou forest

those harvested in December, January, and May. The group of individuals harvested in August and July exhibited high concentrations of 1,8-cineole and α-pinene and low concentrations of terpineol; they were associated with periods of high temperatures and low precipitation. On the other hand, the group of individuals harvested in December, January, and May exhibited high concentrations of borneol, camphor, and terpineol and low concentrations of camphene and α-pinene.

1-8 cineole, α-pinene, and camphene were highly correlated in the first principal component and could summarize the existing variability. Overall, the PCA results strongly suggested that the time of harvest significantly affects the EO composition of rosemary samples. The study also revealed a strong correlation between EO composition, temperature and precipitation, indicating that they may play an important role in determining the composition of EO in rosemary samples.

The results of HCA performed to investigate the variation in EO are summarized in Table 7. The analysis revealed four main clusters corresponding to optimal harvest periods. In sites 1 and 2, the first cluster, corresponding to the pre-flowering stage in early winter and late spring, was associated with peak production of borneol, terpineol, and camphor, and low concentrations of 1,8-cineole, camphene, and α-pinene (site 1). In contrast, the second cluster (flowering) in late winter, early and mid-spring, and early summer was characterized by high β-pinene production. The third cluster, at the height of summer, was characterized by high 1,8-cineole and α-pinene resulting from high temperatures and low precipitation that stimulated the plant's metabolism, while the fourth cluster differed from the third due to low β-pinene values. In sites 3 and 4, the first cluster consisted of samples harvested in December, January, and May, characterized by high concentrations of borneol, camphor, and terpineol, and low concentrations of camphene and α-pinene. The second cluster was composed of samples harvested in February, March, April, and June, which had high concentrations of β-pinene. In contrast, the third cluster, consisting of samples harvested in September, October, and November, had low concentrations of β-pinene. In comparison, the fourth, consisting of samples harvested in July and August, had high concentrations of 1,8-cineole and α-pinene when the temperature was high and precipitation was low, as well as terpineol compounds.

Analysis of seasonal variability in EO composition

Table 8 presents the variation in yield of extracted rosemary EO and its main constituents across four sites during different seasons of harvest. The summer season had the highest concentration of essential oils, dominated by 1,8-cineole, while lowest concentrations were observed in autumn. Two distinct groups were identified based on the similarity of the variability of essential oils. Winter and spring, typically associated with high precipitation, had lower concentrations of 1,8-cineole, camphene, and α-pinene, but higher concentrations of borneol, terpineol, and camphor. On the other hand, summer and autumn, when temperatures are highest, had the highest concentrations of 1,8-cineole, camphene, and α-pinene, but low concentrations of borneol, terpineol, and camphor. The highest peaks of borneol, camphor, and terpineol were observed during the winter and spring harvests, while the highest peaks of 1,8-cinéole were observed during the summer and autumn harvests.

DISCUSSION

Rosemary *(Rosmarinus officinalis* L.) is an important MAP species in Morocco, which has been used for its various benefits. The rationale for this study was to investigate the variability in yield and composition of rosemary EO extracted from the samples collected from four different sites in three forests in northern Morocco. Specifically, this study was conducted to provide

Table 7. Overview of the hierarchical clustering of rosemary characteristics including the optimal harvesting period in the study area

Site	EO constituents	Phenological stage	Harvest period	Optimal conditions
1, 3 and 4	Borneol, camphor, terpineol	Pre-flowering	DEC-JAN; MAY	Rosemary whose main constituent is borneol; optimize harvest
2			DEC-JAN	
1	β-pinene	Flowering	FEB-APR; JUN	Rosemary with low 1,8-cineole and borneol content; promote beekeeping activities
2–4			FEB-JUN	
1–4	1,8-cineol, α-pinene	Post-flowering	JUL-AUG	Rosemary whose main constituent is 1,8-cineole; optimize harvest
1–4	1,8-cineol, α-pinene	Post-flowering	SEP-NOV	Rosemary whose main constituent is 1,8-cineole; possibility of harvest in November if T < 40 °C

Table 8. Seasonal variation of EO yield and chemical composition across the four sites

Site	Constituent	Winter	Spring	Summer	Autumn
Site 1	Yield	2.87	2.90	3.23	2.80
	1,8-Cineole	44.33	44.87	48.97	48.07
	Camphor	17.73	16.30	15.83	17.20
	Borneol	10.07	9.73	7.90	8.80
	α-Pinene	4.67	5.00	7.13	6.87
	Terpineol	5.03	5.00	4.67	4.90
	β-Pinene	3.87	6.07	4.90	3.10
	Camphene	2.47	2.67	3.27	3.17
Site 2	Yield	2.47	2.50	2.83	2.40
	1,8-Cineole	43.93	44.47	48.57	47.67
	Camphor	17.43	16.00	15.53	16.90
	Borneol	9.97	9.63	7.80	8.70
	α-Pinene	4.37	4.70	6.83	6.57
	Terpineol	4.73	4.70	4.37	4.60
	β-Pinene	3.77	5.97	4.80	3.00
	Camphene	2.37	2.57	3.17	3.07
Site 3	Yield	2.27	2.30	2.63	2.20
	1,8-Cineole	42.33	42.87	46.97	46.07
	Camphor	15.33	13.90	13.43	14.80
	Borneol	8.47	8.13	6.30	7.20
	α-Pinene	3.97	4.30	6.43	6.17
	Terpineol	4.53	4.50	4.17	4.40
	β-Pinene	2.77	4.97	3.80	2.00
	Camphene	2.17	2.37	2.97	2.87
Site 4	Yield	2.17	2.20	2.53	2.10
	1,8-Cineole	41.93	42.47	46.57	45.67
	Camphor	15.73	14.30	13.83	15.20
	Borneol	9.37	9.03	7.20	8.10
	α-Pinene	4.07	4.40	6.53	6.27
	Terpineol	4.53	4.50	4.17	4.40
	β-Pinene	3.37	5.57	4.40	2.60
	Camphene	2.17	2.37	2.97	2.87

insight into how the properties of the plant's EO may vary depending on the time of harvest, as this knowledge base may have implications for the cultivation and harvesting of rosemary.

The average yield of EO obtained by hydrodistillation was found to be about 2.3–3.0%, which was similar to the yield obtained by Bouyahya et al. (2017) from the rosemary samples collected in northwest Morocco. Studies conducted in other regions of the country have reported slightly lower yields (El Asbahani et al., 2015; Sabbahi et al., 2020), indicating that the variation in yield is influenced by factors such as site conditions, plant origin, genetics, and maturity. The main chemical constituents identified by GC-FID and GC-MS were 1,8-cineole, camphor, borneol, and α-pinene. This finding is consistent with the reports in several studies (Ait-Ouazzou et al., 2011; El Asbahani et al., 2015; Satyal et al., 2017; Yeddes et al., 2018) regarding the chemical composition of EO isolated from rosemary collected from various origins both in Morocco and in the Mediterranean region.

Notably, 1,8-cineole was by far the most common constituent identified in the EO extracts across the four study sites, accounting for more than 40% of their composition, which corroborates the results of other studies conducted in the Mediterranean region (Chalchat et al., 1993; Jordán et al., 2013; Laamari et al., 2020;

Boukhobza et al., 2021; Al-Maharik et al., 2022). 1,8-cineole, also known as eucalyptol, is an oxygenated monoterpene known to be the dominant constituent of essential oil extracts of plants native to the Mediterranean region. In rosemary, it is produced by the glands of the leaves and is responsible for its characteristic aroma (Bakkali et al., 2008). It has been shown to have anti-inflammatory and antioxidant properties, as well as beneficial effects on the respiratory system (Silva et al., 2003). The prevalence of this rosemary chemotype in Morocco and throughout the region could be attributed to a combination of factors ranging from environmental conditions (climate, soils) to management practices (Ghasemi Pirbalouti et al., 2013). However, it should be noted that this abundance of 1,8-cineole is not always the case in the country and the region. In a study conducted in the Rabat region, Elamrani et al. (2000) noted that the main component was α-Pinene (37–40%), as did Elyemni et al. (2022) in their study analyzing wild rosemary samples in central Morocco, where it accounted for just over half of the EO composition.

Various factors affect the yield and composition of EOs in MAPs, among which temperature, intensity and duration of sunlight, as well as relative humidity are identified as the main external parameters (Fatthi Siahkamari et al., 2017; Paulus et al., 2018). In particular, temperature has a substantial effect on plant physiological processes, especially the photosynthetic pathway and the biochemical compounds produced in this process (Hazrati et al., 2022). In this study, temperature and precipitation had contrasting moderate influences on EO yield. Precipitation appeared to have a negative impact, with EO yield being high at the driest sites. This finding is consistent with the results of Zaouali et al. (2010), who noted that oil yield of *Rosmarinus officinalis* var. *typicus* was higher in the upper semi-arid areas than that obtained in the subhumid regions of Tunisia. However, they noted that the variation in chemical composition should be attributed almost exclusively to the difference between plant varieties rather than to bioclimatic conditions, which is a sentiment shared by several authors (Jordan et al., 2013; Ben Jemia et al., 2014). On the other hand, temperature had a positive effect on the EO yield, which could be attributed to its ability to increase the metabolic activity of the plant, leading to increased essential oil production (Solouki et al., 2023). Moreover, higher temperatures can also improve the vaporization of essential oils, thus facilitating their extraction. However, it is important to note that extremely high temperatures resulting in heat stress can be detrimental to the quality and quantity of EO (Suzuki and Mittler, 2006; Heydari et al., 2018).

Seasonality and harvesting period strongly influenced the yield and in particular the composition of rosemary oil extracts, which is consistent with several studies in the literature (Diab et al., 2002; Singh and Guleria, 2013; Sadeh et al., 2019; Ben Arfa et al., 2022). Among the main chemical constituents identified, borneol, terpineol, and camphor were in peak concentrations during the pre-flowering stage in early winter and late spring, while high concentrations of β-pinene were recorded the flowering stage in late winter, spring, and early summer. The post-flowering stage at the height of summer and early autumn was characterized by high 1,8-cineole and α-pinene. This finding of increased 1,8-cineole and α-pinene concentration with the onset of warm temperatures from late spring to autumn has also been reported by Yildrim (2018) in Turkey. Similarly, Papageorgiou et al. (2008) in Greece observed that the highest concentration of 1,8-cineole, occurred in May and August. Contrastingly, Melito et al. (2019) observed the highest concentrations of 1,8-cineole in winter and the lowest in summer in Sardinia, Italy. In addition, they noted that camphor concentrations were highest in the autumn and lowest in the spring, while borneol concentrations were highest in winter and lowest in autumn. Variation in the chemical composition of EO in rosemary plants throughout the year is influenced by the phenological stage of the plant, with accumulation of specific compounds occurring in response to environmental conditions and seasons. Furthermore, monoterpenes are synthesized and stored in secretory organs, and their emission is a temperature-induced diffusion process, with significant monoterpene emissions reported during the summer season (Nogues et al., 2015; Oliveira et al., 2016; Rathore et al., 2022), which could explain the variation of the dominant constituent (1,8-cineole) in this study.

CONCLUSIONS

The findings of the conducted study highlight the significant impact of harvesting period and environmental factors on the yield and chemical composition of rosemary EOs in northeastern Morocco. It was demonstrated that EO yields are highest in summer and lowest in autumn, with temperature positively influencing and precipitation negatively affecting yields. The chemical composition of the EOs, notably the concentrations of 1,8-cineole, camphor, borneol, and α-pinene, showed marked seasonal variations, with 1,8-cineole and α-pinene peaking in summer and camphor and borneol peaking in winter and late spring. This study bridges a critical gap by providing detailed insights into the seasonal dynamics of rosemary EOs, offering a valuable knowledge base for optimizing harvest times to enhance EO quality and economic value. Moreover, these findings have practical implications for producers and users of rosemary EOs, emphasizing the need to consider environmental factors and seasonal timing to achieve the best possible yield and composition. Nonetheless, future research should explore the influence of different distillation methods and the nature of the samples (fresh or dry) on EO yield and composition to further enhance the quality as well as efficiency of rosemary EO production.

REFERENCES

1. Ait-Ouazzou, A., Lorán, S., Bakkali, M., Laglaoui, A., Rota, C., Herrera, A., Pagán, R., Conchello, P. 2011. Chemical composition and antimicrobial activity of essential oils of *Thymus algeriensis*, *Eucalyptus globulus* and *Rosmarinus officinalis* from Morocco. J. Sci. Food Agric., 91, 2643–2651. https://doi.org/10.1002/jsfa.4505
2. Al-Maharik, N., Jaradat, N., Hawash, M., Al-Lahham, S., Qadi, M., Shoman, I., Jaber, S., Rahem, R.A., Hussein, F., Issa, L. 2022. chemical composition, antioxidant, antimicrobial and anti-proliferative activities of essential oils of *Rosmarinus officinalis* from five different sites in Palestine. Separations, 9, 339. https://doi.org/10.3390/separations9110339
3. Alnamer, R., Alaoui, K., Bouidida, E.H., Benjouad, A., Cherrah, Y. 2011. Toxicity and psychotropic activity of essential oils of *Rosmarinus officinalis* and *Lavandula officinalis* from Morocco. Journal of Biologically Active Products from Nature, 1(4), 262–272. doi: 10.1080/22311866.2011.10719093
4. Aparicio-Ruiz, R., García-González, D.L., Morales, M.T., Lobo-Prieto, A., Romero, I. 2018. Comparison of two analytical methods validated for the determination of volatile compounds in virgin olive oil: GC-FID vs GC-MS. Talanta, 187, 133–141. https://doi.org/10.1016/j.talanta.2018.05.008
5. Bakkali, F., Averbeck, S., Averbeck, D., and Idaomar, M. 2008. Biological effects of essential oils – A review. Food and Chemical Toxicology, 46(2), 446–475. doi:10.1016/j.fct.2007.09.106
6. Ben Arfa, A., Gouja, H., Hannachi, H., Isoda, H., Neffati, M., Najjaa, H. 2022. Seasonal changes in rosemary species: A chemotaxonomic assessment of two varieties based on essential oil compounds, antioxidant and antibacterial activities. PLoS ONE, 17(8), e0273367. https://doi.org/10.1371/journal.pone.0273367
7. Ben Jemia, M., Tundis, R., Pugliese, A., Menichini, F., Senatore, F., Bruno, M., Kchouk, M.E., Loizzo, M.R. 2014. Effect of bioclimatic area on the composition and bioactivity of Tunisian *Rosmarinus officinalis* essential oils. Natural Product Research: Formerly Natural Product Letters. doi: 10.1080/14786419.2014.942305
8. Benabid, A. 2000. Flore et écosystèmes du Maroc: évaluation et préservation de la biodiversité. Paris: Ibis Press.
9. Boukhobza, Z., Boulenouar, N., Abdelkrım, C., Kadri, Z. 2021. Essential oil of *Rosmarinus officinalis* L. from West Highlands of Algeria: Chemical Composition and Antifungal Activity against *Fusarium oxysporum f.* sp. albedinis. Natural Volatiles and Essential Oils, 8(3), 44–45.
10. Bouyahya, A., Et-Touys, A., Bakri, Y., Talbaui, A., Fellah, H., Abrini, J., Dakka, N. 2017. Chemical composition of Mentha pulegium and *Rosmarinus officinalis* essential oils and their antileishmanial, antibacterial and antioxidant activities. Microbial Pathogenesis, 111, 41–49. https://doi.org/10.1016/j.micpath.2017.08.015
11. Btissam, R., Rajae, R., Amina, A., Brigitte, V., Mohamed, N. 2015. In vitro study of anti-glycation and radical scavenging activities of the essential oils of three plants from Morocco: Origanum compactum, *Rosmarinus officinalis* and *Pelargonium asperum*. Polymer Journal, 7, 124–135.
12. Calixto, J.B. 2005. Twenty-five years of research on medicinal plants in Latin America: a personal view. J Ethnopharmacol, 100, 131–134.
13. Chalchat, J.C., Garry, R.P., Michet, A., Benjilali, B., Chabart, J. 1993. Essential oils of rosemary (*Rosmarinus officinalis* L.). The chemical composition of oils of various origins (Morocco, Spain, France). Journal of Essential Oil Research, 5, 613–618.
14. de Oliveira, J.R., Camargo, S.E.A., and de Oliveira, L.D. 2019. *Rosmarinus officinalis* L. (rosemary) as therapeutic and prophylactic agent. J Biomed Sci, 26, 5. https://doi.org/10.1186/s12929-019-0499-8
15. El Asbahani, A., Jilale, A., Voisin, S.N., Aït Addi, E.H., Casabianca, H., El Mousadik, A., Hartmann, D.J., Renaud, F.N.R. 2015. Chemical composition and antimicrobial activity of nine essential oils

obtained by steam distillation of plants from the Souss-Massa Region (Morocco). Journal of Essential Oil Research, 27(1), 34–44. doi:10.1080/10412905.2014.964426

16. El Hilah, F., Ben Akka, F., Dahmani, J., Belahbib, N., Zidane, L. 2015. Étude ethnobotanique des plantes médicinales utilisées dans le traitement des infections du système respiratoire dans le plateau central marocain. Journal of Animal and Plant Sciences, 25, 3886–3897.
17. Elamrani, A., Zrira, S., Benjilali, B., Berrada, M. 2000. A study of moroccan rosemary oils. Journal of Essential Oil Research, 12(4), 487–495. doi: 10.1080/10412905.2000.9699572
18. El-Hilaly, J., Hmamouchi, M., and Lyoussi, B. 2003. Ethnobotanical studies and economic evaluation of medicinal plants in Taoutnate province. J. Ethnopharmacology, 86, 149–158.
19. El-Seedi, H.R., Burman, R., Mansour, A., Turki, Z., Boulos, L., and Gullbo, J. 2013. The traditional medical uses and cytotoxic activities of sixty-one Egyptian plants: discovery of an active cardiac glycoside from Urginea maritima. J Ethnopharmacol, 145, 746–757.
20. Elyemni, M., El Ouadrhiri, F., Lahkimi, A., Elkamli, T., Bouia, A., Eloutassi, N. 2022. Chemical composition and antimicrobial activity of essential oil of wild and cultivated *Rosmarinus officinalis* from two moroccan localities. Journal of Ecological Engineering, 23(3), 214–222. https://doi.org/10.12911/22998993/145458
21. Fatthi Siahkamari, S., Ghojehbegloo, A., Salehi Sardoei, H., and Khairullah, A. 2017. Evaluation of the effects of drought stress and salicylic acid on some growth traits, photosynthetic pigments and yield of peppermint essential oil (*Mentha piperita* L.). New Agric. Find. 11, 125–137.
22. Fennane, M., and Ibn Tattou, M. 1998. Catalogue des plantes vasculaires rares, menacées ou endémiques du Maroc. Palerme: Herbarium Mediterraneum Panormitanum.
23. Fennane, M., and Rejdali, M. 2016. Aromatic and medicinal plants of Morocco: richness, diversity and threats. Bull Inst Sci, 38.
24. Ghanmi, M., Satrani, B., Aberchane, M., Ismaili, M.R., Aafi, A., Elabid, A. 2011. Plantes aromatiques et médicinales du Maroc. In: Collection Maroc Nature. Rabat: Centre de Recherche Forestière.
25. Ghasemi Pirbalouti, A., Hashemi, M., and Ghahfarokhi, F.T. 2013. Essential oil and chemical compositions of wild and cultivated Thymus daenensis Celak and *Thymus vulgaris* L. Industrial Crops and Products, 48, 43–48.
26. Gourich, A.A., Bencheikh, N., Bouhrim, M., Regragui, M., Rhafouri, R., Drioiche, A., Asbabou, A., 2022. Comparative analysis of the chemical composition and antimicrobial activity of four Moroccan North Middle atlas medicinal plants' essential oils: *Rosmarinus officinalis* L., *Mentha pulegium* L., *Salvia officinalis* L., *Thymus zygis subsp. gracilis* (Boiss.) R. Morales. Chemistry, 4(4), 1775–1788. MDPI AG. Retrieved from http://dx.doi.org/10.3390/chemistry4040115
27. Hakkou, S., Sabir, M., Machouri, N. 2023. Principales plantes aromatiques et médicinales forestières dans la région Rabat-Salé-Kénitra, Maroc. Rev. Mar. Sci. Agron. Vét., 11(1), 1–13.
28. Hazrati, S., Beidaghi, P., Beyraghdar Kashkooli, A., Hosseini, S.J., Nicola, S. 2022. Effect of harvesting time variations on essential oil yield and composition of sage (*Salvia officinalis*). Horticulturae, 8, 149. https://doi.org/10.3390/horticulturae8020149
29. HCEFLCD. 2014. Projet PAM: intégration de la biodiversité dans la chaîne de valeurs des plantes aromatiques et médicinales méditerranéennes au Maroc — Manuel des bonnes pratiques de collecte du romarin «*Rosmarinus officinalis*». Retrieved from http://www.fellah-trade.com/ressources/pdf/MBPC_Romarin_Francais.pdf
30. Hendawy, S., Hussein, M., Amer, H., El-Gohary, A., and Soliman, W. 2017. Effect of soil type on growth, productivity, and essential oil constituents of rosemary, *Rosmarinus officinalis*. Asian Journal of Agriculture and Biology, 5, 303–311.
31. Heydari, M., Zanfardino, A., Taleei, A., Bushehri, A.A.S., Hadian, J., Maresca, V., Sorbo, S., Napoli, M.D., Varcamonti, M., Basile, A., Rigano, D. 2018. Effect of heat stress on yield, monoterpene content and antibacterial activity of essential oils of *Mentha x piperita var. Mitcham and Mentha arvensis var. piperascens*. Molecules, 23(8), 1903. https://doi.org/10.3390/molecules23081903
32. Houghton, P.J. 1995. The role of plants in traditional medicine and current therapy. J. Altern. Complementary Med., 1, 131–143. doi: 10.1089/acm.1995.1.131.
33. Hussain, A.I., Anwar, F., Chatha, S.A., Jabbar, A., Mahboob, S., Nigam, P.S. 2010. *Rosmarinus officinalis* essential oil: antiproliferative, antioxidant and antibacterial activities. Brazilian Journal of Microbiology, 41(4), 1070–1078. https://doi.org/10.1590/S1517-838220100004000027
34. Jeyaratnam, N., Nour, A.H., Kanthasamy, R., Nour, A.H., Yuvaraj, A.R., and Akindoyo, J.O. 2016. Essential oil from *Cinnamomum cassia* bark through hydrodistillation and advanced microwave assisted hydrodistillation. Industrial Crops and Products, 92, 57–66. doi:10.1016/j.indcrop.2016.07.049
35. Jordan, M.J., Lax, V., Rota, M.C., Loran, S., and Sotomayor, J.A. 2013. Effect of bioclimatic area on the essential oil composition and antibacterial activity of *Rosmarinus officinalis* L. Food Control, 30, 463–468.

36. Karimi, M. 2014. Les plantes aromatiques et médicinales du Maroc Oriental. Phytoécologie – Ethnobotanique – Modélisation – Phytochimie. [Doctoral dissertation, Université Mohammed Premier], Oujda, Morocco.

37. Kunle, O.F., Egharevba, H.O., and Ahmadu, P.O. 2012. Standardization of herbal medicines-A review. Int. J. Biodivers. Conserv., 4, 101–112. doi: 10.5897/IJBC11.163.

38. Laamari, R., Ochatt, S., and Ferchichi, A. 2020. Chemical composition, antioxidant and antibacterial activities of *Pistacia lentiscus* and *Rosmarinus officinalis* essential oils. Journal of New Sciences, 69.

39. Maroyi, A. 2017. Diversity of use and local knowledge of wild and cultivated plants in the Eastern Cape province. South Africa. J. Ethnobiol. Ethnomed., 13, 43.

40. Martin, A.J.P., and Synge, R.L.M. 1941. A new form of chromatogram employing two liquid phases. Biochem. J., 35, 1358.

41. Masure, M. 2018. Suivi du romarin dans la province de Jerada au Maroc: Cartographie à partir de données secondaires et exploration de la couverture végétale pour l'estimation de la phytomasse [Master's thesis, Université de Liège], Liège, Belgium. Retrieved from http://hdl.handle.net/2268.2/5728/

42. Melito, S., Petretto, G.L., Chahine, S., Pintore, G., and Chessa, M. 2019. Seasonal variation of essential oil in *Rosmarinus officinalis* leaves in Sardinia. Natural Product Communications, 14(7), 1934578X19864005.

43. Napoli, E.M.G., Curcuruto, G., and Ruberto, G. 2010. Screening of the essential oil composition of wild Sicilian rosemary. Biochemical Systematics and Ecology, 38, 659–670.

44. Nogues, I., Muzzini, V., Loreto, F., Bustamante, M. 2015. Drought and soil amendment effects on monoterpene emission in rosemary plants. Sci. Total Environ., 15, 768–778. doi: 10.1016/j.scitotenv.2015.08.080.

45. Okoye, T.C., Uzor, P.F., Onyeto, C.A., Okereke, E.K. 2014. Safe African Medicinal Plants for Clinical Studies. In V. Kuete (Ed.), Toxicological Survey of African Medicinal Plants (pp. 535–555). Elsevier. https://doi.org/10.1016/B978-0-12-800018-2.00018-2.

46. Oliveira, T.S., Bombo, A.B., Oliveira, A.S.D., Garcia, V.L., Appezzato-da-Glória, B. 2016. Seasonal variation of the essential oil from two Brazilian native Aldama La Llave (Asteraceae) species. Anais da Academia Brasileira de Ciências, 88, 1899-1907.

47. Papageorgiou, V., Gardeli, C., Mallouchos, A., Papaioannou, M., Komaitis, M. 2008. Variation of the chemical profile and antioxidant behavior of *Rosmarinus officinalis* L. and Salvia fruticosa Miller grown in Greece. Journal of Agricultural and Food Chemistry, 56, 7254–7264.

48. Paulus, D., Valmorbida, R., and Ramos, C.E.P. 2019. Productivity and chemical composition of the essential oil of *Ocimum x citriodorum* Vis. according to ontogenetic and diurnal variation. J. Appl. Res. Med. Aromat. Plants, 12, 59–65.

49. Pérez-Fons, L., Garzón, M.T., and Micol, V. 2010. Relationship between the antioxidant capacity and effect of rosemary (*Rosmarinus officinalis* L.) polyphenols on membrane phospholipid order. J. Agric. Food Chem., 58, 161–171.

50. Rašković, A., Milanović, I., Pavlović, N., Ćebović, T., Vukmirović, S., Mikov, M. 2014. Antioxidant activity of rosemary (*Rosmarinus officinalis* L.) essential oil and its hepatoprotective potential. BMC Complement Altern Med., 14, 225. https://doi.org/10.1186/1472-6882-14-225.

51. Rathore, S., Mukhia, S., Kapoor, S., Bhatt, V., Kumar, R., Kumar, R. 2022. Seasonal variability in essential oil composition and biological activity of *Rosmarinus officinalis* L. accessions in the western Himalaya. Scientific Reports, 12(1), 3305. https://doi.org/10.1038/s41598-022-07298-x.

52. Sabbahi, M., El-Hassouni, A., Tahani, A., El-Bachiri, A. 2020. Altitude effect on the chemical composition and antioxidant activity of rosemary in the region of Talsint (Morocco). Moroccan Journal of Chemistry, 8(4), 866–875. https://doi.org/10.48317/IMIST.PRSM/morjchem-v8i4.18311

53. Sadeh, D., Nitzan, N., Chaimovitsh, D., Shachter, A., Ghanim, M., Dudai, N. 2019. Interactive effects of genotype, seasonality and extraction method on chemical compositions and yield of essential oil from rosemary (*Rosmarinus officinalis* L.). Industrial Crops and Products, 138, 111419. https://doi.org/10.1016/j.indcrop.2019.05.068

54. Satyal, P., Jones, T.H., Lopez, E.M., McFeeters, R.L., Ali, N.A., Mansi, I., Al-Kaf, A.G., Setzer, W.N. 2017. Chemotypic characterization and biological activity of *Rosmarinus officinalis*.

Foods, 6(3), 20. https://doi.org/10.3390/foods6030020

55. Silva, J., Abebe, W., Sousa, S.M., Duarte, V.G., Machado, M.I., Matos, F.J. 2003. Analgesic and anti-inflammatory effects of essential oils of Eucalyptus. Journal of Ethnopharmacology, 89(2–3), 277–283.

56. Solouki, A., Zare Mehrjerdi, M., Aliniaeifard, S., and Azimi, R. 2023. Postharvest light and temperature elicitors improve chemical composition and level of essential oils in basil (*Ocimum basilicum* L.) through boosting antioxidant machinery. Postharvest Biology and Technology, 199, 112279. https://doi.org/10.1016/j.postharvbio.2023.112279

57. Ssenku, J.E., Okurut, S.A., Namuli, A. 2022. Medicinal plant use, conservation, and the associated traditional knowledge in rural communities in Eastern Uganda. Tropical Medicine and Health, 50, 39. https://doi.org/10.1186/s41182-022-00428-1

58. Suzuki, N., and Mittler, R. 2006. Reactive oxygen species and temperature stresses: A delicate balance between signaling and destruction. Physiologia Plantarum, 126, 45–51. doi: 10.1111/j.0031-9317.2005.00582.x

59. Taleb, M.S. 2017. Aromatic and medicinal plants in Morocco: Diversity and socio-economic role. International Journal of Agricultural and Biosystems Engineering, 11, 812–816.

60. Verma, R.S., Padalia, R.C., Chauhan, A., Upadhyay, R.K., Singh, V.R. 2020. Productivity and essential oil composition of rosemary (*Rosmarinus officinalis* L.) harvested at different growth stages under the subtropical region of north India. Journal of Essential Oil Research, 32(2), 144–149. https://doi.org/10.1080/10412905.2019.1684391

61. Wanjohi, B.K., Njenga, E.W., Sudoi, V., Kipkore, W.K., Moore, H.L., Davies, M.I.J. 2020. Ecological Knowledge of indigenous plants among the Marakwet Community (Embobut Basin), Elgeyo Marakwet County (Kenya). Ethnobotany Research and Applications, 20.

62. Xavier, V., Vargas, R., Casse, E., Lucas, A., Santos, M., Mondin, C., Santarem, E., Astarita, L., Sartor, T. 2011. Mathematical modeling for extraction of essential oil from *Baccharis* spp. by steam distillation. Industrial Crops and Products, 33, 599–604.

63. Yeddes, W., Aidi Wannes, W., Hammami, M., Smida, M., Chebbi, A., Marzouk, B., Saidani Tounsi, M. 2018. Effect of environmental conditions on the chemical composition and antioxidant activity of essential oils from *Rosmarinus officinalis* L. growing wild in Tunisia. Journal of Essential Oil Bearing Plants, 21, 972–986.

64. Yıldırım, E.D. 2018. The effect of seasonal variation on *Rosmarinus officinalis* (L.) essential oil composition. Uluslararası Tarım ve Yaban Hayatı Bilimleri Dergisi, 4(1), 33–38.

65. Zaouali, Y., Bouzaine, T., Boussaid, M. 2010. Essential oils composition in two *Rosmarinus officinalis* L. varieties and incidence for antimicrobial and antioxidant activities. Food and Chemical Toxicology, 48, 3144–3152.

66. Zrira, S. 2003. Le marché des plantes aromatiques et médicinales au Maroc. IAV Hassan II. 39 p.