

Article

Influence of Season and Habitat on the Essential Oils Composition, Allelopathy, and Antioxidant Activities of *Artemisia monosperma* Delile

Ahmed M. Abd-ElGawad ^{1,*} , Abdulaziz M. Assaeed ¹ , Saud L. Al-Rowaily ¹, Mohamed S. Alshahri ¹, Giuliano Bonanomi ² and Abdelsamed I. Elshamy ³ 

¹ Plant Production Department, College of Food & Agriculture Sciences, King Saud University, P.O. Box 2460, Riyadh 11451, Saudi Arabia; assaeed@ksu.edu.sa (A.M.A.); srowaily@ksu.edu.sa (S.L.A.-R.); msaaaa33@gmail.com (M.S.A.)

² Department of Agriculture, University of Naples Federico II, Portici, 80055 Naples, Italy; giuliano.bonanomi@unina.it

³ Department of Natural Compounds Chemistry, National Research Centre, 33 El Bohouth St., Dokki, Giza 12622, Egypt; elshamynrc@yahoo.com

* Correspondence: aibrahim2@ksu.edu.sa; Tel.: +966-562680864

Abstract: Plants belonging to the *Artemisia* genus (Asteraceae) are widely distributed worldwide and have many ethnopharmacological, traditional, therapeutic, and phytochemical aspects. *Artemisia monosperma* is an important aromatic plant due to its traditional and therapeutic uses and phytochemical diversity, including essential oils (EOs). The EO chemical profile of aromatic plants has been reported to be affected by exogenous and endogenous factors. Geographic and seasonal variations are crucial factors shaping the chemical composition of the EO. Herein, the variations of the yields, chemical profiles, and allelopathic and antioxidant activities of *A. monosperma* EOs collected from three regions in four seasons were assessed. A slight variation in the oil yields was observed among regions and seasons, while the chemical profile, characterized via GC-MS, exhibited significant quantitative and qualitative variation among either regions or seasons. Sesquiterpenes were the main components of all EOs, with significant variation in concentration. In most EO samples, the summer-plant samples had the highest concentration of sesquiterpenes, followed by spring, winter, and autumn. The 7-epi-trans-sesquisabinene hydrate, 6-epi-shyobunol, dehydro-cyclolongifolene oxide, isoshyobunone, diepicedrene-1-oxide, dehydro-aromadendrene, and junipene were the main compounds of all the EO samples. The extracted EOs of the *A. monosperma* samples showed considerable allelopathic activity against the weed *Dactyloctenium aegyptium* and the crop *Lactuca sativa*. A significant variation in allelopathic activity was observed among samples collected during different seasons, while the samples of the autumn and summer seasons had more potential. Also, *L. sativa* was more affected by the EO compared to *D. aegyptium*, reflecting that weeds are more resistant to allelochemicals. In this context, the EOs of *A. monosperma* samples exhibited substantial antioxidant activity with the same pattern of allelopathic activity, whereas the samples of the autumn and summer seasons showed higher antioxidant activity. These biological activities of the EOs could be ascribed to the higher content of oxygenated compounds. The present study revealed that seasons have a substantial effect on EO production as well as composition. In consequence, the biological activities varied with the variation of the chemical profile of the EO. These results show the importance of season/timing for sampling aromatic plants.

Keywords: desert shrubs; volatile organic compounds; phytotoxicity; seasonality; environmental factors



Citation: Abd-ElGawad, A.M.; Assaeed, A.M.; Al-Rowaily, S.L.; Alshahri, M.S.; Bonanomi, G.; Elshamy, A.I. Influence of Season and Habitat on the Essential Oils Composition, Allelopathy, and Antioxidant Activities of *Artemisia monosperma* Delile. *Separations* **2023**, *10*, 263. <https://doi.org/10.3390/separations10040263>

Academic Editor: Paraskevas D. Tzanavaras

Received: 24 March 2023

Revised: 13 April 2023

Accepted: 15 April 2023

Published: 17 April 2023



Copyright: © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Essential oils (EOs) are a class of volatile constituents that are produced via the secondary metabolism of aromatic plants [1]. In nature, EOs play significant functions in plants

via increasing plant resistance and/or protection against herbivores and pathogens [2]. The EOs play a main role in plant chemical communications via their dispersion in the atmosphere and thus activate the protector genes of other surrounding plants [3].

The chemical profiles and biological activities of the EOs derived from plants are broadly performed and documented worldwide [4]. Most of the documented data focused mainly on the significant applications of these EOs in the pharmaceutical, medicine, industry, and agriculture [5,6]. In medicinal applications, EOs have been strong biological and pharmaceutical agents for the treatment of several diseases such as inflammations [7], microbial and viral diseases [8], cancers [9], and others. In the field of agriculture, many research efforts have been established for limitations on using chemically synthetic products for plant protection [5]. Therefore, the researchers and scientists tried to use the EOs as a significant alternative protection way for plant disease management due to their promising potentialities and safety [5] as well as to control weeds as ecofriendly bioherbicides [10].

Asteraceae is one of the biggest families of the plant kingdom and is considered one of the most important resources for the production of fixed oils as well as EOs [6]. *Artemisia monosperma* Delile is a common medicinal aromatic plant in the Arabian Peninsula and the Mediterranean area. It is a perennial shrub (50–70 cm in height) growing on a wide scale in sandy habitats in different regions of Saudi Arabia. Many traditional uses were documented for this plant around the world via numerous applications such as antihypertensive, anthelmintic, and antispasmodic [11]. This plant was also characterized by varieties of chemical compounds including EOs [12]. Studies on EOs from different parts of *A. monosperma* collected from different places around the world revealed the abundance of (α and/or β)-pinene, α -terpinolene, limonene, β -maaliene, shyobunone, β -vinyl naphthalene, β -eudesmol, sabinene, and ocimene [12–14]. The chemical constituents of the plant are directly influenced by the different external factors comprising the geographical, environmental, and climate conditions as well as plant parts, genetic factors, and physiological variations [15]. In consequence, the qualitative and quantitative variations in the EOs components, and thus their biological potentialities, are strongly affected by habitat characteristics, soil conditions, climatic factors, and seasons [15,16].

The present work aimed to (i) assess the variations in the EOs chemical compositions of *A. monosperma* collected from three regions in Saudi Arabia (Ghat, Thumamah, and Giham), during the four seasons, (ii) evaluate the allelopathic potentialities of the extracted EOs on the seed germination, seedling radicle growth, and seedling shoot growth of the weed, *Dactyloctenium aegyptium* and the crop, *Lactuca sativa*, and (iii) determine the antioxidant of the EOs.

2. Materials and Methods

2.1. Study Area

Artemisia monosperma shrubs grow in a wide range of sandy habitats in different regions of Saudi Arabia. In the present study, the studied locations are located in the vicinity of the Riyadh Region, in the central part of the country of Saudi Arabia. The region is characterized by long and hot summers and short very cold winters [17]. The average high temperature is 43.4 °C and the highest temperature is up to 49.8 °C in August. The average low temperature is 9.0 °C and the lowest temperature is down to −2.3 °C. The region has low precipitation (112.3 mm/year), which mainly falls during March and April. The average number of rainy days is 44.8 and the region suffers from frequent dust storms.

The sampling locations were the (1) Ghat region, Ghat Governorate (26°04′11.9″ N 44°42′49.3″ E), (2) Thumamah, Riyadh Governorate (25°15′04.0″ N 46°37′45.0″ E), and (3) Giham, Rumah Governorate (25°51′55.4″ N 47°32′40.2″ E) regions (Figure 1). These locations were selected to be at least 100 km distant and they are different in their climate, while they are different in the elevation and topography of the habitat. The geographic distance between Giham and Thumamah is 115 km, while the Giham location is far from Ghat by 285 km. The geographic distance between Thumamah and Ghat is 213 km. The

elevations of the three locations are 703, 580, and 437 m a.s.l., for Ghat, Thumamah, and Giham, respectively.

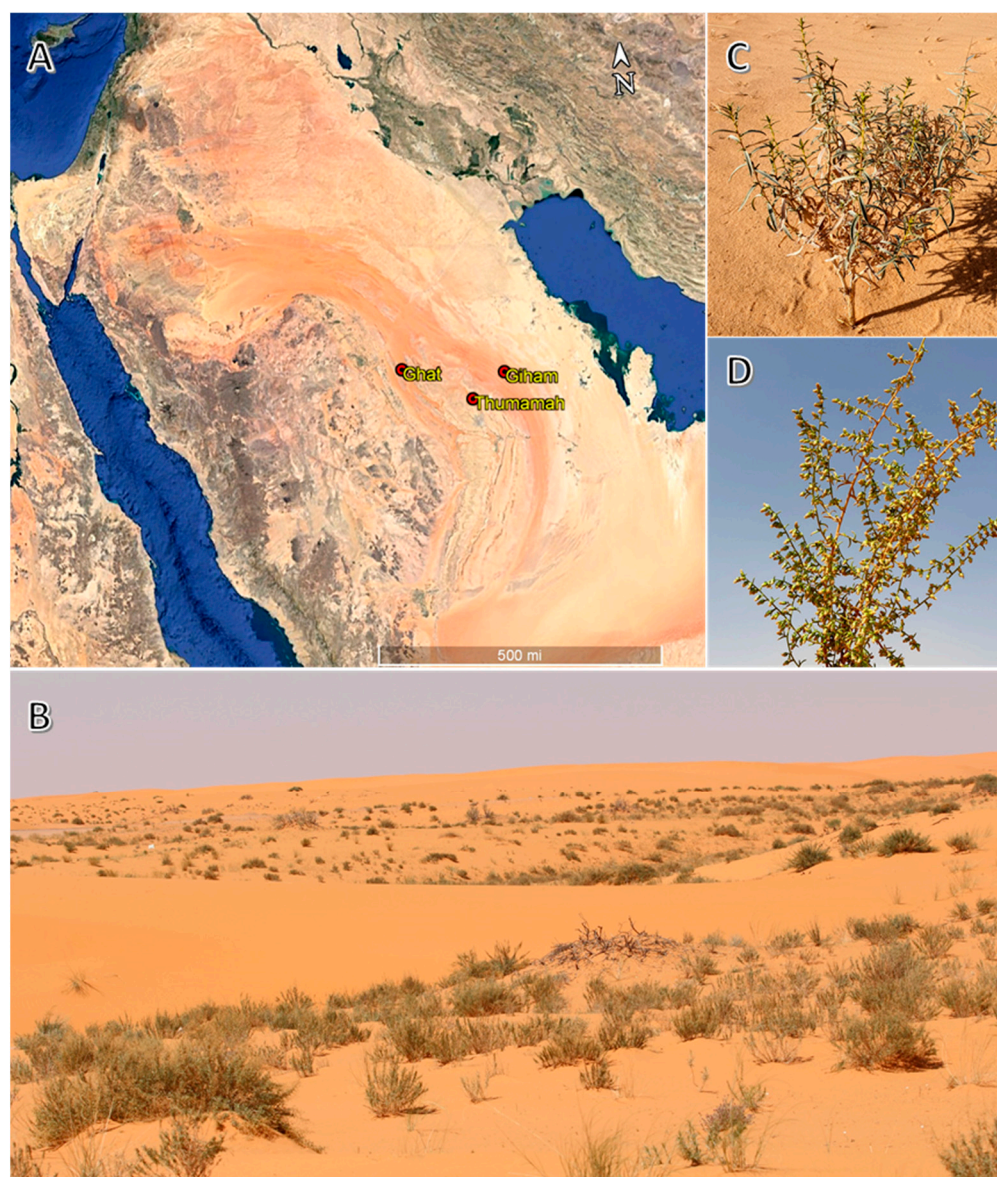


Figure 1. (A) a map of Saudi Arabia showing the three regions of the sampled *A. monosperma* populations, (B) an overview of the *A. monosperma* population in a sand dune habitat, (C) an overview of the *A. monosperma* shrub, and (D) a close view *A. monosperma* flowering branches.

The climate of these regions was acquired from the nearest station to the location of sampling according to <https://www.meteoblue.com> (accessed on 25 January 2023). The climate data (average maximum temperature, average minimum temperature, and precipitation) of Ghat was represented by Buraidah station, while the climate data of Thumamah was taken from the Riyadh Airport station. The climate of Giham was acquired from Khurais station (Figure S1). The three studied regions are climatically comparable. For precipitation, the Thumamah and Giham regions had the highest rainfall (67 and 66 mm/year, respectively), while the Ghat region received 57 mm/year. The highest amount of rain fell during March and April within the three regions, while no rainfall occurred during the period from June to September (Figure S1). The temperature of the three regions was comparable. The highest average maximum temperature was from June

to August, while the lowest was during December and February. The average minimum temperature showed the same pattern.

2.2. Soil Sampling and Analysis

Since the soil properties do not change in the short term [18], we analyzed the soil samples collected during the spring season. Moreover, we selected the spring season due to its moderate weather and its vegetation flourishing. However, the soil moisture was determined for all three regions in all seasons. From each studied location, three quadrants (10×10 m) were plotted, and three soil samples were collected beneath the *A. monosperma* shrubs at depths of 15–40 cm during the spring season. The three soil samples were merged as one composite sample in plastic bags and transferred to the laboratory for further analysis. The soil samples were dried in an oven at 65°C until complete dryness and then sieved via a 2 mm sieve to remove any debris. The soil fractions (sand, silt, and clay) were determined according to the methodology of Bouyoucos [19]. Soil calcium carbonates were measured according to Jackson [20], while soil organic matter (OM) was determined by wet combustion with dichromate at 450°C [21]. Soil water paste (1:5) was prepared with distilled water, and the soil electrical conductivity (EC) and pH were measured immediately [21]. Sodium (Na) and potassium (K) were measured using flame photometry (PHF 80B Biologie Spectrophotometer) according to Allen, et al. [22].

For the determination of soil moisture content, within each quadrat, three soil samples were collected in a moisture tin for measurements during the four seasons (spring, winter, summer, and autumn), where moisture content was determined immediately by the weight loss method at 105°C .

2.3. Plant Materials Collection and Preparation

The aboveground parts of *A. monosperma* were collected from three populations growing within the three regions (Ghat, Thumamah, and Giham) during the four seasons (winter, spring, summer, and autumn), i.e., a total of 36 samples ($3 \text{ regions} \times 4 \text{ seasons} \times 3 \text{ populations}$) were collected (Figure 1). The samples were collected in plastic bags and transferred to the Range Science Laboratory, College of Food and Agriculture Sciences, King Saud University, Riyadh, Saudi Arabia. The samples were dried in the air under shaded conditions at room temperature ($25 \pm 3^\circ\text{C}$) for 10 days and then crushed via a $\varnothing 2.0$ mm mesh using a grinder (IKA® MF 10 Basic Microfine Grinder Drive, Breisgau, Germany). Then, the samples are packed in plastic bags in the refrigerator at 4°C till further analysis.

The plant specimen was identified and authenticated according to the flora books [23,24]. In addition, a voucher specimen was organized and placed in the herbarium of the Plant Production Department, College of Food and Agricultural Sciences, King Saud University with code: KSU-AGRIC-0010113002.

2.4. EOs Extraction, Yielding, and Analysis via GC–MS

The EOs of the collected plant samples (150 g) of *A. monosperma* were extracted by hydrodistillation for 3 h over a Clevenger apparatus. Then, the oil layer was separated using 1 mL of *n*-hexane (HPLC grade, Sigma–Aldrich Chemicals Private Limited, Bangalore, India) and dried by 0.5 g of sodium sulphate anhydrous. The extraction process was performed for three replicas of each sample. The EO yield, in percentage, was determined by weight in g/g. The 36 extracted EO samples were collected and stored in glass vials at 4°C in the refrigerator till further analyses. Overall, the extracted oil samples were analyzed by gas chromatography coupled with mass spectrometry (GC–MS) according to the same previously described protocol [25]. The GC–MS used for analysis included TRACE Ultra-Gas Chromatography (THERMO Scientific™ Corporate, Waltham, MA, USA) alongside of the Thermo Scientific ISQ™ EC single quadrupole mass spectrometer. The system of GC–MS was equipped with a TR-5 MS column that was characterized by a film thickness of $0.25 \mu\text{m}$ and an internal diameter of $30 \text{ m} \times 0.32 \text{ mm}$. The carrier gas (Helium) was used with a (1.0 mL min^{-1}) flow rate and a (1:10) divided ratio. The program of temperature

was adjusted for one minute at 60 °C and then increased to 240 °C within 4.0 °C min^{−1} for 1 min. The injection of each oil sample in an aliquot (1 µL in hexane) was carried out at a ratio of 1:10 (v/v) within the injector and the detector at 210 °C. The mass spectral data were measured at 70 eV via electron ionization (EI) using a spectral range at *m/z* 40–450. Identification of the chemical composition was performed via AMDIS (automated mass spectral deconvolution and identification) software in addition to the collection of the Wiley Spectral Library and database of the NIST Library (Gaithersburg, MD, USA; Wiley, Hoboken, NJ, USA), that was used for the determination of the retention indices relative to *n*-alkanes (C₈–C₂₂), or the evaluation of the mass spectral data of authentic compounds. The retention indices (KI) were calculated according to the following equation:

$$KI = 100 \times \left[n + \frac{t(\text{comp.}) - t(n)}{t(n+1) - t(n)} \right]$$

2.5. Allelopathic Activity Bioassay

The EOs of *A. monosperma* were tested for their allelopathic activity against the weed *D. aegyptium* and the crop *L. sativa*. The ripe seeds of *D. aegyptium* were collected from an infested field, while the seeds of *L. sativa* were purchased. The weed, *D. aegyptium*, was selected since it is a noxious weed infesting many crops [26], while *L. sativa* was selected as a standard plant for allelopathic bioassay [26,27]. Upon the bioassay, the seeds were surface sterilized with 0.3% sodium hypochlorite, followed by rinsing with distilled and sterilized water three times. To test the allelopathy efficacy of the EOs, a series of concentrations of 250, 500, 750, and 1000 mg L^{−1} were prepared using 1% Tween 80® (Sigma–Aldrich, Darmstadt, Germany) as an emulsifier agent. In Petri plates with a diameter of 90 mm, 20 sterilized seeds were placed over filter paper (Whatman Grade 1) moistened with 4 mL of each concentration of the EO [25]. The seeds were distributed within the plate and the plates were sealed with Parafilm® (Sigma, St. Louis, MO, USA) to avoid leakage of the EO. In addition, a control treatment with Tween 80® was prepared with the same procedures as the EO. For each concentration of the EO and control, three replicas were performed, and the experiments were repeated three times. The Petri plates were incubated in a growth chamber adjusted with a light–dark cycle of 12 h–12 h and a temperature of 25 ± 2 °C. The germinated seeds were counted daily and after 10 days of incubation, the seedling root and shoot lengths were measured in mm. The inhibition percentage of germination, root, and shoot growth were calculated as follows:

$$\text{Inhibition (\%)} = 100 \times \left(\frac{G \text{ or } L_{\text{control}} - G \text{ or } L_{\text{treatment}}}{G \text{ or } L_{\text{control}}} \right)$$

where, “G” is germination and “L” is the length of seedling root or shoot.

2.6. Antioxidant Activity of *A. monosperma* EOs

The antioxidant activity of the extracted EOs from *A. monosperma* was evaluated by testing their ability to scavenge the 2,2-diphenyl-1-picrylhydrazyl (DPPH) according to Miguel [28]. In brief, a series of concentrations of 5, 10, 20, 30, 40, and 50 mg L^{−1} of the EO were prepared with ethanol as solvent. In test tubes, a reaction mixture of equal volumes of EO and freshly prepared DPPH (0.3 mM) was prepared, vigorously mixed, and incubated in dark conditions at room temperature (25 ± 2 °C) for 20 min. Then, the absorbance was measured at 517 nm using a spectrophotometer (Analytik Jena, Jena, Germany). In addition, positive control of catechol as a reference antioxidant was prepared with a concentration range of 5–10 mg L^{−1} and treated similarly to the EO as previously described. The scavenging activity percentage was calculated based on the following equation:

$$\text{Scavenging activity (\%)} = 100 \times \left(1 - \frac{\text{Absorbance}_{\text{sample}}}{\text{Absorbance}_{\text{control}}} \right)$$

The amount of EO or catechol required for the 50% (IC₅₀) reduction in the DPPH color was calculated by plotting the curve between the concentration and scavenging percentage using MS Excel 2016.

2.7. Statistical Analysis

The data of the experiment of allelopathic activity were expressed as the average out of three replicas \pm standard error. In addition, the raw data were subjected to variation significance by application of a one-way ANOVA test, followed by Tukey's HSD post hoc test using the CoStat software program, version 6.311 (CoHort Software, Monterey, CA, USA). Similarly, the data of the antioxidant activity experiment were expressed as the average \pm standard error and the data were also subjected to a one-way ANOVA test. In addition, the IC₅₀ was calculated as the concentration of the EO or catechol (standard antioxidant) that is required for 50% inhibition/scavenging of the DPPH. The soil data were subjected to a one-way ANOVA test to assess the significant variation among regions. However, for soil moisture content, the data were subjected to two-way ANOVA with the region as the first factor and season as the second factor. All ANOVA tests were performed using the CoStat software program.

On the other side, to assess the correlation among studied *A. monosperma* samples, from the three different regions within the four seasons (winter, spring, autumn, and summer), a datasheet of the EOs compounds expressed as the concentration percentage was subjected to principal component analysis (PCA) using JMP[®] Pro 16.0.0, SAS Institute Inc., Cary, NC, USA.

3. Results and Discussion

3.1. Soil Characteristics of the Studied Regions

The analysis of the soil samples from the three studied regions revealed no significant variation for all studied parameters, except for the calcium carbonate content which showed a highly significant difference ($p = 0.003$) among the three regions (Table 1). The soil of the Ghat region attained 23.01% of calcium carbonates, while the soils of the Thumamah and Giham regions showed calcium carbonate values of 19.825 and 18.62%, respectively. In detail, the organic matter content was higher in Giham (1.47 g/kg), compared to the Ghat and Thumamah regions. The soil of Thumamah showed the highest content (97.88%) of sand fraction, while the soil of the Giham region revealed the lowest sand content (93.49%) compared to the other studied regions (Table 1).

Table 1. Physical and chemical properties of the soil supporting the growth of *A. monosperma* within the three studied regions.

Parameters	Regions			<i>p</i> -Value	F-Value
	Ghat	Thumamah	Giham		
Sand (%)	96.86 \pm 0.76 ^{ab}	97.88 \pm 0.26 ^a	93.49 \pm 5.12 ^b	0.0826 ^{ns}	3.89
Silt (%)	2.35 \pm 0.66 ^a	1.58 \pm 0.36 ^a	3.73 \pm 2.96 ^a	0.1636 ^{ns}	2.49
Clay (%)	0.79 \pm 0.58 ^a	0.54 \pm 0.11 ^a	2.78 \pm 2.16 ^a	0.1255 ^{ns}	2.99
CaCO ₃ (%)	23.01 \pm 0.78 ^a	19.82 \pm 0.72 ^b	18.62 \pm 0.65 ^c	0.0003 ^{***}	44.40
pH	8.08 \pm 0.11 ^a	8.02 \pm 0.06 ^a	8.07 \pm 0.12 ^a	0.3788 ^{ns}	1.15
EC (dS/m)	0.14 \pm 0.01 ^a	0.14 \pm 0.02 ^a	0.13 \pm 0.02 ^a	0.7703 ^{ns}	0.27
Na (mg/kg)	6.53 \pm 1.15 ^a	6.13 \pm 1.01 ^a	5.00 \pm 2.71 ^a	0.7303 ^{ns}	0.33
K (mg/kg)	7.33 \pm 1.81 ^a	8.10 \pm 2.74 ^a	8.68 \pm 2.74 ^a	0.7475 ^{ns}	0.31
OM (g/kg)	0.96 \pm 0.71 ^a	0.39 \pm 0.38 ^a	1.47 \pm 1.87 ^a	0.1782 ^{ns}	2.33

Similar superscript letters within each parameter (row) revealed significant variation at $p < 0.05$. OM: organic matter, ns: nonsignificant, *** $p < 0.001$.

Regarding soil moisture content, highly significant variations were observed among the soil samples collected beneath *A. monosperma* from the three regions as well as during different seasons (Table 2). In addition, the two-way ANOVA showed that the interaction between regions and seasons revealed significant variation in the soil moisture content as

well. The Giham region showed the highest soil moisture content, compared to Thumamah and Ghat regions (Figure S1). Moreover, during the winter season, the soil moisture content in the Giham region was highest in winter (0.33%) and lowest in summer (0.15%). In the Thumamah region, the soil under *A. monosperma* was higher in winter (0.11) and lower in spring (0.06%).

Table 2. The two-way ANOVA table of the soil moisture content under the *A. monosperma* during the four seasons from the three regions (Ghat, Thumamah, and Giham).

Source	Sum of Squares	df	Mean Square	F-Value	p-Value
Regions (R)	0.079	2	0.049	66.38	<0.001 ***
Seasons (S)	0.026	3	0.008	11.64	0.001 ***
R × S	0.043	6	0.007	9.71	<0.001 ***

df: degree of freedom, *** $p < 0.001$.

3.2. Yielding and Composition of *A. monosperma* EOs

The hydrodistillation of *A. monosperma* above-ground parts collected from three different regions (Ghat, Thumamah, and Giham), during the four seasons, showed a significant variation ($p < 0.001$) in the EO quantity among both regions and seasons (Figure 2). The color of the extracted EO was golden-yellow and its content varied from 0.43 to 0.83% (w/v). The autumn season attained the lowest values of the EO, while the spring season showed the highest production of the EO. During the spring season, *A. monosperma* growing in Ghat produced 0.83% of the EO, while Giham and Thumamah samples attained 0.80% and 0.77%, respectively (Figure 2). In harmony with our results, the EO yield of *Solidago canadensis* was higher in the summer season, compared to other seasons [29].

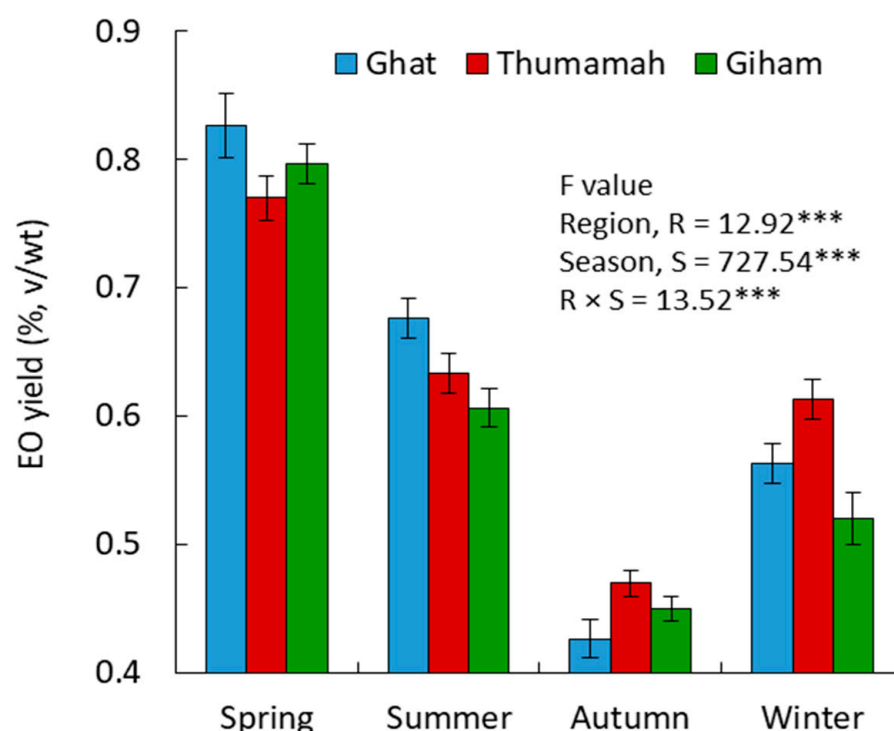


Figure 2. Yielded EOs of *A. monosperma* during the four seasons from the three regions. *** $p < 0.001$.

The EO yield of *M. piperita* has been reported in higher amounts during the summer season and for the hottest year [30]. The EO yield of the present work is comparable with those described for the *A. monosperma* sample collected from Al-Hair, central Saudi Arabia (38 km south of Riyadh) which produced 0.3–1.3% EO [14], while our samples produced more Eos than those reported for other Egyptian (0.16%) [31] and Libyan (0.16–0.26%)

ecospecies [13]. The variation among ecospecies could be ascribed to the effect of climate, soil factors, and genetic characteristics [15,32].

Although soil properties have been reported to have a crucial role in the EO of aromatic plants [33], our study showed no significant variation in all the soil parameters among regions, except for calcium carbonates and moisture content. Therefore, the significant variation in the EO yield among regions could be ascribed to the variation in the soil moisture content (Table S2). Soil moisture content has been reported to affect significantly the EO yield and composition of various plants such as *Origanum vulgare* [34], *Thymbra spicata* [35], and *Mentha piperita* [33].

3.2.1. EOs Profile of *A. monosperma* Collected from the Ghat Region

The extracted EOs from plant samples collected from Ghat during spring, winter, summer, and autumn were characterized via GC-MS. The data of Table 3 and Figure S3 presented all the identified compounds along with the retention time and Kovats index of each compound, in addition to its relative concentration in each season, represented 97.93, 89.76, 99.42, and 92.89%, respectively. In total, sixty-four chemical constituents were identified from the afforded four EOs. All these components were categorized into seven classes comprising monoterpenes (oxygenated and hydrocarbons), sesquiterpenes (oxygenated and hydrocarbons), diterpenes (oxygenated and hydrocarbons), and other nonterpenoids. The sesquiterpenes were determined as the main components of the EOs during the four seasons with respective relative concentrations of 90.41, 88.14, 91.58, and 84.75%. The monoterpenes, diterpenes, and other nonterpenoid constituents were characterized as traces in all seasons.

Table 3. Essential-oil constituents of *A. monosperma* collected from the Ghat region during the four seasons.

No	Rt. ¹	KI ²	Component Name	Concentration % ³			
				Autumn	Winter	Spring	Summer
Monoterpene hydrocarbons							
1	4.63	973	β-Pinene	0.08 ± 0.01	-	-	0.17 ± 0.01
2	7.01	1062	γ-Terpinene	-	-	-	0.31 ± 0.01
3	5.6	1026	p-Cymenene	0.05 ± 0.00	-	-	-
Oxygenated Monoterpenes							
4	7.05	1098	L-Linalool	-	-	-	-
5	7.19	1101	Hotrienol	0.17 ± 0.00	-	3.25 ± 0.07	0.17 ± 0.00
6	9.1	1174	1,8-menthadien-4-ol	0.88 ± 0.03	-	0.24 ± 0.01	0.88 ± 0.03
7	9.41	1177	Myrtenal	0.98 ± 0.04	-	0	0.98 ± 0.04
8	9.47	1183	p-Cymen-8-ol	0.14 ± 0.00	-	1.39 ± 0.06	0.14 ± 0.00
9	9.53	1189	α-Terpineol	0.15 ± 0.00	-	0	0.15 ± 0.00
10	10.1	1211	α-Citronellol	0.11 ± 0.00	-	0.08 ± 0.00	0.11 ± 0.00
11	10.2	1217	1-p-Menthen-9-al	0.38 ± 0.02	-	0.16 ± 0.00	0.38 ± 0.02
12	10.4	1142	cis-Verbenol	-	-	-	-
13	10.8	1249	trans-Geraniol	-	-	0.33 ± 0.01	-
14	12.5	1282	Piperitone	1.21 ± 0.08	0.14 ± 0.00	0.33 ± 0.01	1.21 ± 0.08
15	13.1	1298	Carvacrol	0.52 ± 0.03	-	0.19 ± 0.00	0.52 ± 0.03
Sesquiterpene hydrocarbons							
16	13.4	1335	α-Elemene	2.59 ± 0.15	0.43 ± 0.02	0.78 ± 0.03	2.16 ± 0.09
17	13.9	1351	α-Cubebene	2.25 ± 0.06	0.33 ± 0.01	0.70 ± 0.02	1.63 ± 0.07
18	14.1	1352	α-Longipinene	2.76 ± 0.08	0.21 ± 0.01	0.72 ± 0.01	1.92 ± 0.07
19	14.2	1373	Isoledene	-	-	0.06 ± 0.00	-
20	14.3	1375	α-Ylangene	0.14 ± 0.00	-	0.20 ± 0.01	-
21	14.5	1376	α-Copaene	0.71 ± 0.04	-	0.06 ± 0.00	0.25 ± 0.01
22	14.8	1409	α-Gurjunene	0.11 ± 0.00	0.18 ± 0.00	0.25 ± 0.01	0.51 ± 0.02
23	15	1410	α-Cedrene	-	0.19 ± 0.00	0.37 ± 0.01	-
24	15.2	1427	trans-Caryophyllene	2.52 ± 0.12	0.28 ± 0.01	1.81 ± 0.05	1.58 ± 0.04

Table 3. Cont.

No	Rt. ¹	KI ²	Component Name	Concentration % ³			
				Autumn	Winter	Spring	Summer
25	15.4	1439	Aromandendrene	4.85 ± 0.15	1.25 ± 0.05	3.19 ± 0.09	3.94 ± 0.09
26	15.4	1442	α-Guaiene	0.69 ± 0.03	-	0.26 ± 0.01	0.53 ± 0.03
27	15.8	1462	α-Humulene	0.31 ± 0.01	0.26 ± 0.01	0.09 ± 0.00	0.38 ± 0.01
28	16.1	1466	Dehydro-aromadendrene	11.42 ± 0.25	0.85 ± 0.02	13.46 ± 0.23	12.30 ± 0.21
29	16.4	1481	ar-Curcumene	2.46 ± 0.09	0.36 ± 0.01	2.05 ± 0.11	3.28 ± 0.09
30	16.5	1486	Germacrene D	0.35 ± 0.01	-	0.20 ± 0.01	0.17 ± 0.00
31	16.5	1492	Berkheyaradulene	0.16 ± 0.00	-	0.23 ± 0.01	-
32	16.8	1499	α-Muurolene	0.69 ± 0.03	1.91 ± 0.07	0.65 ± 0.02	1.48 ± 0.07
33	17	1505	α-Bulnesene	3.87 ± 0.19	5.86 ± 0.10	3.21 ± 0.09	4.10 ± 0.14
34	18	1557	Junipene	5.94 ± 0.27	3.52 ± 0.09	6.32 ± 0.16	4.49 ± 0.18
Oxygenated Sesquiterpenes							
35	13.3	1317	Dehydro-cyclolongifolene oxide	0.38 ± 0.01	1.56 ± 0.03	3.01 ± 0.06	6.57 ± 0.16
36	15.7	1456	Aromadendrene oxide-(1)	1.48 ± 0.06	4.69 ± 0.12	1.11 ± 0.05	1.81 ± 0.08
37	16.2	1480	6-Epishyobunone	0.32 ± 0.01	0.39 ± 0.01	0.18 ± 0.00	0.17 ± 0.00
38	17.1	1517	6-epi-shyobunol	10.28 ± 0.28	1.91 ± 0.05	9.70 ± 0.14	10.42 ± 0.23
39	17.3	1533	trans-Nerolidol	0.90 ± 0.03	-	0.12 ± 0.00	0.63 ± 0.01
40	17.5	1538	7-epi-trans-Sesquisabinene hydrate	8.31 ± 0.12	25.07 ± 0.41	17.18 ± 0.28	12.76 ± 0.23
41	17.8	1554	Diepicedrene-1-oxide	3.28 ± 0.07	2.06 ± 0.04	2.11 ± 0.07	5.11 ± 0.10
42	18.3	1571	Isoshyobunone	4.08 ± 0.11	3.55 ± 0.09	3.60 ± 0.09	2.96 ± 0.05
43	18.4	1579	(-)-Spathulenol	0.81 ± 0.02	2.04 ± 0.05	1.56 ± 0.06	0.93 ± 0.02
44	18.5	1581	Caryophyllene oxide	1.00 ± 0.02	2.51 ± 0.07	2.31 ± 0.06	1.54 ± 0.04
45	18.7	1582	Geranyl isovalerate	1.81 ± 0.06	2.92 ± 0.09	1.66 ± 0.03	1.17 ± 0.05
46	18.7	1594	Carotol	0.85 ± 0.03	3.47 ± 0.09	2.33 ± 0.06	2.32 ± 0.08
47	19	1631	Ledene oxide-(II)	1.19 ± 0.06	8.95 ± 0.15	4.28 ± 0.09	0.69 ± 0.02
48	19.1	1632	Alloaromadendrene oxide-(2)	1.39 ± 0.04	1.30 ± 0.05	1.22 ± 0.04	0.80 ± 0.02
49	19.2	1634	Longipinocarveol, trans-	0.94 ± 0.03	0.64 ± 0.02	-	0.27 ± 0.01
50	19.4	1640	.tau.-Cadinol	0.10 ± 0.00	0.66 ± 0.02	0.32 ± 0.01	0.91 ± 0.02
51	19.5	1641	Alloaromadendrene epoxide	1.60 ± 0.05	0.71 ± 0.02	0.27 ± 0.01	0.28 ± 0.01
52	19.7	1646	.tau.-Muurolol	1.02 ± 0.04	1.48 ± 0.03	2.85 ± 0.06	1.15 ± 0.06
53	19.7	1654	α-Cadinol	0.35 ± 0.01	1.92 ± 0.04	-	0.93 ± 0.03
54	20	1668	Ledene oxide-(I)	0.21 ± 0.01	1.07 ± 0.03	0.33 ± 0.02	0.32 ± 0.01
55	20.2	1685	α-Bisabolol	0.75 ± 0.03	0.79 ± 0.02	0.33 ± 0.02	0.28 ± 0.01
56	20.8	1722	Farnesol	0.40 ± 0.02	2.09 ± 0.06	1.10 ± 0.04	0.62 ± 0.02
57	22	1725	Isocalamendiol	0.99 ± 0.02	1.80 ± 0.05	0.23 ± 0.01	0.22 ± 0.00
58	22.1	1845	Hexahydrofarnesyl acetone	0.49 ± 0.01	0.93 ± 0.03	-	-
Diterpene hydrocarbons							
59	23.4	1931	Stachene	0.41 ± 0.02	0.55 ± 0.02	-	-
Oxygenated Diterpenes							
60	24.3	2203	trans-Geranylgeraniol	0.17 ± 0.00	0.22 ± 0.01	0.07 ± 0.00	
Others							
61	8.02	1137	Nopinone	-	-	-	0.35 ± 0.01
62	12	1253	Chavicol	-	-	0.11 ± 0.00	-
63	15.6	1444	Citronellyl propionate	2.61 ± 0.05	0.52 ± 0.01	1.37 ± 0.03	2.81 ± 0.05
64	23.1	1857	Hexadeca-7,11 -dien-1-ol	0.28 ± 0.01	0.19 ± 0.00	-	-
Monoterpene hydrocarbons (MH)				0.13	-	-	0.48
Oxygenated Monoterpene (OM)				4.54	0.14	5.97	4.2
Sesquiterpene hydrocarbons (SH)				41.82	15.63	34.61	38.72
Oxygenated Sesquiterpenes (OS)				42.93	72.51	55.8	52.86
Diterpene hydrocarbons (DH)				0.41	0.55	-	-
Oxygenated Diterpene OD				0.17	0.22	0.07	-
Others				2.89	0.71	1.48	3.16
Total				92.89	89.76	97.93	99.42

¹ Rt: Retention time; ² KIexp: experimental Kovats retention index; ³ values are average ± SE. “-” showed that it was below the limit of detection (LOD).

From all the assigned compounds, 7-epi-trans-sesquisabinene hydrate, dehydro-aromadendrene, junipene, 6-epi-shyobunol, and α -bulnesene were identified as the main EOs components (Figure 3). Although the dehydro-aromadendrene was represented in a high concentration in the EOs of the plant collected in spring, summer, and autumn, it was identified in trace amounts in the winter sample. Ledene oxide-(II) was determined as a major compound in EOs extracted from spring and winter samples (4.28% and 8.95%, respectively), though it was assessed as a minor compound in the EOs of the samples collected in the summer and autumn samples. In addition, aromandendrene was assigned with considerable concentrations in the EOs of spring (3.19%), summer (3.94%), and autumn (4.85%) samples but low in the winter sample. The dehydro-cyclolongifolene oxide was identified with a high concentration in the EO of the summer sample only, with a concentration of 6.57%. The diterpenes were totally absent in the EOs collected during the summer from the Ghat region. Furthermore, the monoterpene hydrocarbons were totally missed in the EOs of the plant samples collected during the spring and winter seasons.

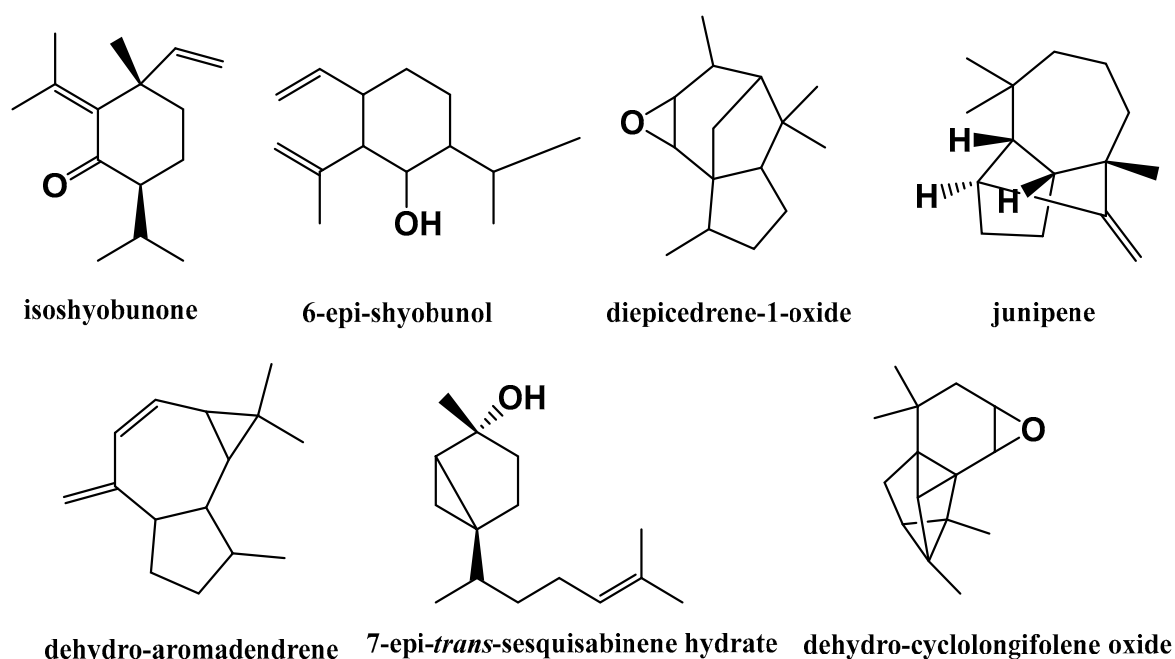


Figure 3. The structures of the major chemical compounds identified in the EOs of *A. monosperma*.

3.2.2. EOs Profile of *A. monosperma* Collected from the Thumamah Region

All the identified compounds alongside the retention time and Kovats index of each compound, in addition to its relative concentration of the *A. monosperma* samples collected from the Thumamah region during the four seasons, were presented in Table 4 and Figure S4. Sixty-seven components were assigned from the EOs samples of the plant samples collected from the four seasons with relative concentrations of 99.52, 99.83, 98.84, and 99.30%, for spring, winter, summer, and autumn, respectively.

Five classes of compounds were found, including monoterpenes (oxygenated and hydrocarbons), sesquiterpenes (oxygenated and hydrocarbons), and other nonterpenoids, while no diterpenes were detected. The sesquiterpenes represented the major constituents of the EOs of the plant samples collected during the four seasons with relative concentrations of 92.61, 86.79, 81.21, and 82.57% for spring, winter, summer, and autumn, respectively. The other constituents were characterized by low concentrations in all the EO samples, including monoterpenes and other nonterpenoid constituents.

Table 4. Essential-oil constituents of *A. monosperma* collected from the Thumamah region during the four seasons.

No	Rt. ¹	KI ²	Component Name	Concentrations (%) ³			
				Autumn	Winter	Spring	Summer
Monoterpene hydrocarbons							
1	3.83	920	α-Pinene	0.31 ± 0.02	-	-	0.23 ± 0.01
2	4.63	973	β-Pinene	1.60 ± 0.05	0.70 ± 0.02	0.86 ± 0.02	1.36 ± 0.03
3	5.93	1033	dl-Limonene	-	-	0.17 ± 0.01	-
4	6.77	1044	E-β-Ocimene	0.48 ± 0.03	0.18 ± 0.01	0.53 ± 0.03	0.37 ± 0.02
5	7.01	1062	γ-Terpinene	-	-	-	0.41 ± 0.01
6	5.6	1026	p-Cymenene	0.72 ± 0.05	0.32 ± 0.02	0.25 ± 0.01	0.78 ± 0.03
Oxygenated Monoterpene							
7	4.89	991	2,3-Dehydro-1,8-cineole	-	-	0.09 ± 0.00	0.25 ± 0.02
8	7.05	1098	L-Linalool	0.38 ± 0.02	0.19 ± 0.01	-	0.49 ± 0.02
9	7.19	1101	Hotrienol	0.49 ± 0.03	4.69 ± 0.11	1.55 ± 0.06	1.43 ± 0.05
10	8.25	1139	trans-Pinocarveol	0.37 ± 0.01	0.31 ± 0.02	0.08 ± 0.00	0.48 ± 0.02
11	8.34	1143	2-Bornanone	-	0.14 ± 0.00	-	-
12	8.76	1162	Pinocarvone	-	-	-	0.22 ± 0.01
13	9.1	1174	1,8-menthadien-4-ol	3.58 ± 0.14	0.86 ± 0.03	0.32 ± 0.02	2.57 ± 0.10
14	9.41	1177	Myrtenal	1.53 ± 0.07	0.63 ± 0.02	-	1.38 ± 0.06
15	9.47	1183	p-Cymen-8-ol	-	1.72 ± 0.08	0.73 ± 0.03	-
16	9.53	1189	α-Terpineol	1.12 ± 0.05	0.24 ± 0.01	-	1.81 ± 0.07
17	10.1	1211	α-Citronellol	0.40 ± 0.02	-	-	0.30 ± 0.01
18	10.2	1217	1-p-Menthen-9-al	0.26 ± 0.01	0.22 ± 0.00	0.08 ± 0.00	0.36 ± 0.01
19	10.4	1142	cis-Verbenol	0.17 ± 0.01	-	-	0.21 ± 0.01
20	10.8	1249	trans-Geraniol	-	0.17 ± 0.00	-	-
21	12.5	1282	Piperitone	1.67 ± 0.09	0.53 ± 0.02	0.45 ± 0.02	1.26 ± 0.06
22	13.1	1298	Carvacrol	0.79 ± 0.02	0.26 ± 0.01	0.28 ± 0.01	0.68 ± 0.03
Sesquiterpene hydrocarbons							
23	13.4	1335	α-Elemene	3.15 ± 0.08	1.49 ± 0.02	1.21 ± 0.05	2.46 ± 0.06
24	13.9	1351	α-Cubebene	2.62 ± 0.06	1.15 ± 0.03	0.92 ± 0.03	1.98 ± 0.05
25	14.1	1352	α-Longipinene	3.02 ± 0.03	0.62 ± 0.01	0.82 ± 0.05	2.13 ± 0.07
26	14.2	1373	Isoledene	0.46 ± 0.03	-	-	0.34 ± 0.01
27	14.3	1375	α-Ylangene	-	-	0.36 ± 0.01	-
28	14.5	1376	α-Copaene	-	-	0.11 ± 0.00	-
29	14.6	1407	Isocaryophyllene	-	-	0.20 ± 0.01	-
30	14.8	1409	α-Gurjunene	0.55 ± 0.02	0.19 ± 0.01	0.27 ± 0.01	0.46 ± 0.01
31	15	1410	α-Cedrene	3.28 ± 0.07	0.43 ± 0.02	0.61 ± 0.02	-
32	15.2	1427	trans-Caryophyllene	1.84 ± 0.08	1.26 ± 0.02	2.52 ± 0.07	1.62 ± 0.05
33	15.4	1439	Aromandendrene	2.68 ± 0.09	4.38 ± 0.19	3.25 ± 0.13	3.89 ± 0.08
34	15.4	1442	α-Guaiene	0.80 ± 0.03	0.37 ± 0.02	0.44 ± 0.02	0.50 ± 0.01
35	15.8	1462	α-Humulene	0.31 ± 0.01	0.18 ± 0.01	-	0.30 ± 0.01
36	16.1	1466	Dehydro-aromadendrene	10.85 ± 0.28	9.48 ± 0.26	14.96 ± 0.31	10.11 ± 0.20
37	16.4	1481	ar-Curcumene	2.05 ± 0.05	3.09 ± 0.10	2.28 ± 0.09	4.63 ± 0.14
38	16.5	1486	Germacrene D	0.34 ± 0.01	0.40 ± 0.02	0.32 ± 0.01	0.28 ± 0.01
39	16.5	1492	Berkheyaradulene	-	0.15 ± 0.00	-	-
40	16.8	1499	α-Muurolene	0.66 ± 0.02	1.68 ± 0.03	0.46 ± 0.02	1.44 ± 0.06
41	17	1505	α-Bulnesene	2.77 ± 0.06	2.27 ± 0.05	2.66 ± 0.12	3.45 ± 0.07
42	18	1557	Junipene	5.58 ± 0.11	7.53 ± 0.32	13.12 ± 0.26	4.47 ± 0.09
Oxygenated Sesquiterpenes							
43	13.3	1317	Dehydro-cyclolongifolene oxide	8.99 ± 0.21	5.54 ± 0.10	4.14 ± 0.12	7.70 ± 0.15
44	15.7	1456	Aromadendrene oxide-(1)	1.48 ± 0.04	0.57 ± 0.01	1.92 ± 0.07	0.92 ± 0.03
45	16.2	1480	6-Epishyobunone	0.29 ± 0.00	0.18 ± 0.00	-	-
46	17.1	1517	6-epi-shyobunol	9.14 ± 0.11	9.82 ± 0.17	13.43 ± 0.21	9.80 ± 0.13
47	17.3	1533	trans-Nerolidol	0.91 ± 0.02	0.17 ± 0.00	0.26 ± 0.00	0.67 ± 0.01
48	17.5	1538	7-epi-trans-Sesquisabinene hydrate	6.12 ± 0.13	20.37 ± 0.29	10.30 ± 0.26	10.34 ± 0.21
49	17.8	1554	Diepicedrene-1-oxide	2.32 ± 0.03	2.53 ± 0.05	3.21 ± 0.08	4.16 ± 0.07
50	18.3	1571	Isoshyobunone	4.15 ± 0.09	2.87 ± 0.08	2.35 ± 0.09	0.42 ± 0.02

Table 4. Cont.

No	Rt. ¹	KI ²	Component Name	Concentrations (%) ³			
				Autumn	Winter	Spring	Summer
51	18.4	1579	(-)-Spathulenol	1.25 ± 0.06	0.81 ± 0.02	-	2.04 ± 0.05
52	18.5	1581	Caryophyllene oxide	0.51 ± 0.01	2.04 ± 0.04	1.08 ± 0.02	2.00 ± 0.06
53	18.7	1582	Geranyl isovalerate	1.74 ± 0.04	0.83 ± 0.02	0.78 ± 0.01	0.61 ± 0.01
54	18.7	1594	Carotol	0.42 ± 0.01	0.58 ± 0.01	1.07 ± 0.04	0.39 ± 0.01
55	19	1631	Ledene oxide-(II)	1.47 ± 0.03	0.83 ± 0.03	1.77 ± 0.05	0.61 ± 0.02
56	19.1	1632	Alloaromadendrene oxide-(2)	0.77 ± 0.02	0.29 ± 0.01	0.27 ± 0.01	0.21 ± 0.00
57	19.2	1634	Longipinocarveol, trans-	0	0.19 ± 0.00	0.15 ± 0.00	0.57 ± 0.02
58	19.4	1640	.tau.-Cadinol	0.29 ± 0.01	0.36 ± 0.01	0.09 ± 0.00	0.59 ± 0.02
59	19.5	1641	alloaromadendrene epoxide	0.26 ± 0.01	0.54 ± 0.01	0.18 ± 0.00	0.37 ± 0.01
60	19.7	1646	.tau.-Muurolol	0.85 ± 0.03	0.83 ± 0.02	5.41 ± 0.09	0.24 ± 0.01
61	19.7	1654	α-Cadinol	0.28 ± 0.01	1.22 ± 0.03	1.11 ± 0.03	0.69 ± 0.01
62	20	1668	Ledene oxide-(I)	0.17 ± 0.00	0.63 ± 0.02	0.07 ± 0.00	0.29 ± 0.01
63	20.2	1685	α-Bisabolol	0	0.26 ± 0.01	0.10 ± 0.00	0.21 ± 0.01
64	20.8	1722	Farnesol	0.20 ± 0.00	0.45 ± 0.02	0.41 ± 0.01	0.32 ± 0.01
65	22	1725	Isocalamendiol	-	0.2 ± 0.01	-	-
Others							
66	12	1253	Chavicol	0.26 ± 0.01	0.15 ± 0.01	0.11 ± 0.00	-
67	15.6	1444	Citronellyl propionate	2.60 ± 0.07	1.73 ± 0.03	1.41 ± 0.04	3.04 ± 0.06
Monoterpene hydrocarbons (MH)				3.11	1.2	1.81	3.15
Oxygenated Monoterpene (OM)				10.76	9.96	3.58	11.44
Sesquiterpene hydrocarbons (SH)				40.96	34.67	44.51	38.06
Oxygenated Sesquiterpenes (OS)				41.61	52.12	48.1	43.15
Others				2.86	1.88	1.52	3.04
Total				99.3	99.83	99.52	98.84

¹ Rt: Retention time; ² KIexp: experimental Kovats retention index; ³ values are average ± SE. “-” showed that it was below the limit of detection (LOD).

The EOs derived from plant samples collected from Thumamah in the four seasons were characterized by the abundance of dehydro-aromadendrene, junipene, 6-epi-shyobunol, 7-epi-trans-sesquisabinene hydrate, and dehydro-cyclolongifolene oxide. Tau-muurolol was assigned in a significant concentration (5.41%) in the EO of the spring sample out of all the EOs of the plant samples. In addition, hotrienol was identified with a significantly higher concentration in the EO of the winter sample (4.69%) compared to the other seasons. The 1,8-menthadien-4-ol represented a major compound in the EO of the only sample collected in autumn (3.58%). The main remarkable results were summarized by the total disappearance of diterpenes in both forms, hydrocarbons and oxygenated, in all EO samples. This result was very near to the results of samples collected from Ghat.

3.2.3. EOs Profile of *A. monosperma* Collected from the Giham Region

The chemical characterization of the EOs extracted from *A. monosperma* collected from the Giham region during the four seasons is tabulated in Table 5 and Figure S5. The identified compounds represented 99.30, 99.51, 99.85, and 99.91%, for the spring, winter, summer, and autumn, respectively. Overall, 62 compounds were characterized from all the EO samples in the four seasons. All the constituents of EOs of Giham plant samples were classified into five classes encompassing monoterpenes (oxygenated and hydrocarbons), sesquiterpenes (oxygenated and hydrocarbons), and other nonterpenoids with a complete absence of the diterpenes. The sesquiterpenes were found as fundamental constituents of the EOs of the Giham plants samples during the four seasons by relative concentrations of 89.50, 87.49, 88.79, and 84.48%, respectively, for spring, winter, summer, and autumn, respectively. However, monoterpenes and nonterpenoid constituents were determined in low amounts.

Table 5. Essential-oil constituents of *A. monosperma* collected from the Giham region during the four seasons.

No	Rt. ¹	KI ²	Component Name	Concentration (%) ³			
				Autumn	Winter	Spring	Summer
Monoterpene hydrocarbons							
1	3.83	920	α-Pinene	-	0.30 ± 0.01	0.29 ± 0.01	-
2	4.63	973	β-Pinene	0.32 ± 0.01	1.47 ± 0.04	1.39 ± 0.04	0.68 ± 0.02
3	6.77	1044	E-β-Ocimene	0.17 ± 0.01	0.31 ± 0.02	0.25	0.28 ± 0.01
4	7.01	1062	γ-Terpinene	0.21 ± 0.01	-	-	0.38 ± 0.02
5	5.6	1026	p-Cymenene	0.27 ± 0.02	0.60 ± 0.04	0.56 ± 0.03	0.32 ± 0.02
Oxygenated Monoterpene							
6	7.05	1098	L-Linalool	0.30 ± 0.01	0.28 ± 0.01	0.17 ± 0.01	0.14 ± 0.00
7	7.19	1101	Hotrienol	0.91 ± 0.01	1.72 ± 0.05	1.56 ± 0.04	1.52 ± 0.03
8	8.25	1139	trans-Pinocarveol	0.34 ± 0.02	0.35 ± 0.02	0.34 ± 0.01	-
9	8.34	1143	2-Bornanone	-	0.14 ± 0.00	0.14 ± 0.01	-
10	8.76	1162	Pinocarvone	-	0.15 ± 0.00	0.17 ± 0.01	-
11	9.1	1174	1,8-menthadien-4-ol	3.99 ± 0.13	0.79 ± 0.01	0.69 ± 0.03	1.43 ± 0.09
12	9.41	1177	Myrtenal	2.36 ± 0.09	0.67 ± 0.03	0.40 ± 0.01	0.70 ± 0.05
13	9.47	1183	p-Cymen-8-ol	1.41 ± 0.04	1.59 ± 0.06	0.67 ± 0.02	0.91 ± 0.02
14	9.53	1189	α-Terpineol	0.42 ± 0.02	0.31 ± 0.01	0.66 ± 0.02	0.14 ± 0.00
15	10.1	1211	α-Citronellol	-	0.16 ± 0.01	-	-
16	10.2	1217	1-p-Menthen-9-al	-	-	-	0.12 ± 0.00
17	10.4	1142	cis-Verbenol	-	-	-	0.14 ± 0.00
18	10.8	1249	trans-Geraniol	0.18 ± 0.00	-	-	0.21 ± 0.01
19	12.5	1282	Piperitone	1.69 ± 0.08	0.89 ± 0.03	0.73 ± 0.03	1.07 ± 0.04
20	13.1	1298	Carvacrol	0.48 ± 0.03	0.35	0.32 ± 0.01	0.77 ± 0.02
Sesquiterpene hydrocarbons							
21	13.4	1335	α-Elemene	2.64 ± 0.06	2.21 ± 0.05	1.68 ± 0.04	2.33 ± 0.03
22	13.9	1351	α-Cubebene	2.39 ± 0.06	1.50 ± 0.04	1.31 ± 0.05	1.93 ± 0.08
23	14.1	1352	α-Longipinene	2.92 ± 0.08	0.79 ± 0.02	0.90 ± 0.02	2.85 ± 0.07
24	14.2	1373	Isoledene	0.42 ± 0.02	-	-	-
25	14.3	1375	α-Ylangene	0.47 ± 0.02	-	-	0.14 ± 0.00
26	14.8	1409	α-Gurjunene	-	0.19 ± 0.00	0.25 ± 0.01	0.51 ± 0.01
27	15	1410	α-Cedrene	3.74 ± 0.09	0.70 ± 0.01	0.49 ± 0.02	0.39 ± 0.02
28	15.2	1427	trans-Caryophyllene	1.62 ± 0.05	1.08 ± 0.02	1.83 ± 0.05	1.74 ± 0.03
29	15.4	1439	Aromandendrene	2.77 ± 0.07	3.34 ± 0.06	4.00 ± 0.11	5.05 ± 0.09
30	15.4	1442	α-Guaiene	0.81 ± 0.02	0.50 ± 0.03	0.46 ± 0.02	0.55 ± 0.02
31	15.8	1462	α-Humulene	0.41 ± 0.01	-	-	0.37 ± 0.01
32	16.1	1466	Dehydro-aromadendrene	9.77 ± 0.18	11.19 ± 0.16	10.64 ± 0.24	11.27 ± 0.21
33	16.4	1481	ar-Curcumene	2.05 ± 0.05	3.11 ± 0.09	2.49 ± 0.07	3.17 ± 0.08
34	16.5	1486	Germacrene D	0.28 ± 0.02	0.30 ± 0.02	0.16 ± 0.01	0.47 ± 0.02
35	16.5	1492	Berkheyaradulene	0.84 ± 0.02	0.23 ± 0.01	0.15 ± 0.00	0.12 ± 0.00
36	16.8	1499	α-Muurolene	-	1.26 ± 0.07	1.19 ± 0.03	1.47 ± 0.08
37	17	1505	α-Bulnesene	2.43 ± 0.07	2.36 ± 0.06	2.66 ± 0.08	3.27 ± 0.09
38	18	1557	Junipene	5.23 ± 0.06	8.76 ± 0.17	7.20 ± 0.19	4.48 ± 0.07
Oxygenated Sesquiterpenes							
39	13.3	1317	Dehydro-cyclolongifolene oxide	7.87 ± 0.13	8.27 ± 0.14	6.77 ± 0.11	6.92 ± 0.09
40	15.7	1456	Aromadendrene oxide-(1)	1.46 ± 0.08	0.75 ± 0.02	0.75 ± 0.01	1.89 ± 0.19
41	16.2	1480	6-Epishyobunone	0.15 ± 0.00	0.30 ± 0.01	0.18 ± 0.00	-
42	17.1	1517	6-epi-shyobunol	10.07 ± 0.17	9.58 ± 0.19	9.59 ± 0.15	9.88 ± 0.12
43	17.3	1533	trans-Nerolidol	0.67 ± 0.02	0.22 ± 0.01	0.20 ± 0.00	0.44 ± 0.02
44	17.5	1538	7-epi-trans-Sesquisabinene hydrate	7.89 ± 0.11	13.87 ± 0.32	19.01 ± 0.36	10.45 ± 0.25
45	17.8	1554	Diepicedrene-1-oxide	3.38 ± 0.06	3.18 ± 0.08	3.03 ± 0.06	3.96 ± 0.06
46	18.3	1571	Isoshyobunone	3.78 ± 0.10	3.08 ± 0.09	2.79 ± 0.07	0.49 ± 0.01
47	18.4	1579	(-)-Spathulenol	0.88 ± 0.01	1.11 ± 0.06	0.99 ± 0.02	1.86 ± 0.08
48	18.5	1581	Caryophyllene oxide	0.70 ± 0.01	1.77 ± 0.07	1.63 ± 0.06	0.94 ± 0.03
49	18.7	1582	Geranyl isovalerate	1.85 ± 0.05	2.33 ± 0.04	0.98 ± 0.02	1.41 ± 0.06
50	18.7	1594	Carotol	0.82 ± 0.02	1.50 ± 0.05	0.99 ± 0.07	1.52 ± 0.04

Table 5. Cont.

No	Rt. ¹	KI ²	Component Name	Concentration (%) ³			
				Autumn	Winter	Spring	Summer
51	19	1631	Ledene oxide-(II)	1.92 ± 0.07	-	1.85 ± 0.06	1.39 ± 0.05
52	19.1	1632	Alloaromadendrene oxide-(2)	0.57 ± 0.02	0.65 ± 0.03	0.59 ± 0.02	2.12 ± 0.07
53	19.2	1634	Longipinocarveol, trans-	0.33 ± 0.01	0.17 ± 0.00	0.25 ± 0.01	0.36 ± 0.01
54	19.4	1640	.tau.-Cadinol	0.19 ± 0.01	0.17 ± 0.00	0.22 ± 0.01	0.16 ± 0.00
55	19.5	1641	alloaromadendrene epoxide	0.94 ± 0.02	-	0.32 ± 0.01	1.14 ± 0.03
56	19.7	1646	/ .tau.-Muurolol	1.22 ± 0.04	0.73 ± 0.01	1.44 ± 0.03	1.68 ± 0.04
57	19.7	1654	α-Cadinol	0.43 ± 0.01	1.01 ± 0.02	1.07 ± 0.03	0.91 ± 0.02
58	20	1668	Ledene oxide-(I)	0.27 ± 0.01	0.42 ± 0.01	0.47 ± 0.02	0.35 ± 0.01
59	20.2	1685	α-Bisabolol	-	0.18 ± 0.00	0.26 ± 0.02	0.34 ± 0.01
60	20.8	1722	Farnesol	0.30 ± 0.02	0.38 ± 0.02	0.51 ± 0.02	0.35 ± 0.02
61	22	1725	Isocalamendiol	-	0.30 ± 0.01	0.20 ± 0.00	0.12 ± 0.00
Others							
62	12	1253	Chavicol	0.21 ± 0.01	-	-	-
63	15.6	1444	Citronellyl propionate	2.17 ± 0.05	1.94 ± 0.04	1.46 ± 0.04	2.25 ± 0.06
Monoterpene hydrocarbons (MH)				0.97	2.68	2.49	1.66
Oxygenated Monoterpene (OM)				12.08	7.4	5.85	7.15
Sesquiterpene hydrocarbons (SH)				38.79	37.52	35.41	40.11
Oxygenated Sesquiterpenes (OS)				45.69	49.97	54.09	48.68
Others				2.38	1.94	1.46	2.25
Total				99.91	99.51	99.3	99.85

¹ Rt: Retention time; ² KIexp: experimental Kovats retention index; ³ values are average ± SE. “-” showed that it was below the limit of detection (LOD).

The essential components of the EOs derived from plants collected in Giham during the overall year seasons were 7-epi-trans-sesquisabinene hydrate and dehydro-aromadendrene, 6-epi-shyobunol. There was no significant variation in the quantity of almost all other identified compounds, with some minor exceptions such as isoshyobunone and 1,8-menthadien-4-ol. Isoshyobunone was identified as a major compound in all seasons except in EO derived from the summer sample. However, 1,8-menthadien-4-ol was characterized as traces in all EOs except the autumn sample (3.99%).

The present data showed that the EOs derived from the plant samples have the sesquiterpenes as the main constituents and monoterpenes and other nonterpenoids as traces. The EOs of plant samples collected from Ghat were characterized by the presence of minor concentrations of diterpenes in spring, winter, and autumn with a complete absence in the EO of the summer sample. However, the chemical profiles of *A. monosperma* EOs collected from the Giham region were in harmony with the EOs constituents of the plant samples collected from the Thumamah region.

The EO samples collected in the summer had the highest relative concentration of the sesquiterpenes among the four seasonal EO samples, followed by spring, winter, and finally autumn. The abundance of the sesquiterpenes in the EOs of *A. monosperma* was already reported in EOs derived from the Egypt ecoplant [31]. While sesquiterpenes were reported as minors in the EOs of the ecospecies collected from Saudi Arabia [14] and Libya [13]. The oxygenated sesquiterpenes in the EOs of winter samples were assigned as the highest, followed by spring, summer, and autumn. Sesquiterpene hydrocarbons in autumn EOs were the highest among the four seasons, followed by summer, spring, and winter. The other constituents, including monoterpenes, diterpenes, and other nonterpenoids, were characterized as traces in the four seasonal samples.

From all the tabulated data in Tables 3–5, the abundance of the sesquiterpenes, especially the oxygenated compounds, in all our findings was in harmony with the reported data [36,37]. The previously described data revealed that the oxygenated sesquiterpenes are the main constituents of *c. A. scoparia*, *A. judaica*, and *A. sieberi* collected from Saudi

Arabia [36,37]. Furthermore, the findings revealed that these EOs contain nearly the same major compounds, such as trans-sesquisabinene hydrate, dehydro-aromadendrene, dehydro-cyclolongifolene oxide, and junipene, with slight variations in concentrations.

The variation of the EOs was directly attributed to the variation in climatic conditions such as temperature and humidity [38]. According to Kaul, et al. [39], the terpene hydrocarbons were the most affected compounds by humidity, while the oxygenated terpenes and their esters are directly correlated with the temperature [39,40].

In the present study, the concentrations of the major compounds showed considerable variation among the four seasons. The dehydro-aromadendrene and junipene were determined in high concentrations for the samples of *A. monosperma* collected during the spring season, compared to the other seasons. Also, the major 7-epi-trans-sesquisabinene hydrate has been determined in higher content during the winter season. Based on data from EOs extracted from *Alpinia zerumbet* during five years, the compounds have been reported in low concentration during the high-temperature season [41].

On the other hand, the major compound, diepicedrene-1-oxide was determined in a higher concentration of the samples collected during the summer season of the three regions. The content of 1,8-cineole has been determined in higher quantity in the EO of *Myrtus communis* during summer [42]. Other major compounds did not show a specific pattern with seasons such as ledene oxide-(II) and aromadendrene.

Summing up, the chemical compositions of EO samples from *A. monosperma* showed slight variation among regions due to the nonsignificant variation in the soil and environmental conditions. However, high variation was determined in the EO yield as well as the composition among seasons due to the variation in the climatic condition and soil moisture content within different seasons. Other studies have reported the same variation within regions and seasons [15,16,43].

3.3. Chemometric Analysis of *A. monosperma* EOs

A dataset of all identified compounds from the EOs extracted from *A. monosperma* collected from three different regions (Ghat, Thumamah, and Giham) during the four seasons (winter, spring, summer, and autumn) was prepared and subjected to principal components analysis (PCA) to assess the correlation among regions as well as seasons (Figure 4). The results showed that the Ghat sample of *A. monosperma* was segregated on the upper-right side of the PCA biplot which means this sample was varied from the rest of the samples (Figure 4). On the same side, the samples of Thumamah winter, Ghat spring, and Giham spring showed a close correlation to each other and that they are closely correlated with the 7-epi-trans-sesquisabinene hydrate. On the other side, the autumn samples of the three regions (Ghat, Thumamah, and Giham) were separated on the lower-right side of the PCA biplot, where they showed correlation with the junipene, 6-epi-shyobunol, and dehydro-aromadendrene. The samples of the summer season from the three regions as well as Giham winter and Thumamah spring samples were segregated on the central-right part of the PCA biplot, where they showed a close correlation regarding their chemical composition of the EOs as well as showed a close correlation with isoshyobunone (Figure 4).

3.4. Allelopathic Activity of *A. monosperma* EOs

3.4.1. Ghat Region

The allelopathic activity of the EOs extracted from *A. monosperma* collected from the Ghat regions during the four seasons (spring, winter, summer, and autumn) was tested against the seed germination, seedling radicle growth, and seedling shoot growth of *Dactyloctenium aegyptium* and *Lactuca sativa* (Figure 5). In a general statement, the seed germination, seedling radicle growth, and seedling shoot growth of both *D. aegyptium* and *L. sativa* were significantly inhibited ($p < 0.05$) in response to seasons, concentrations, and their interactions.

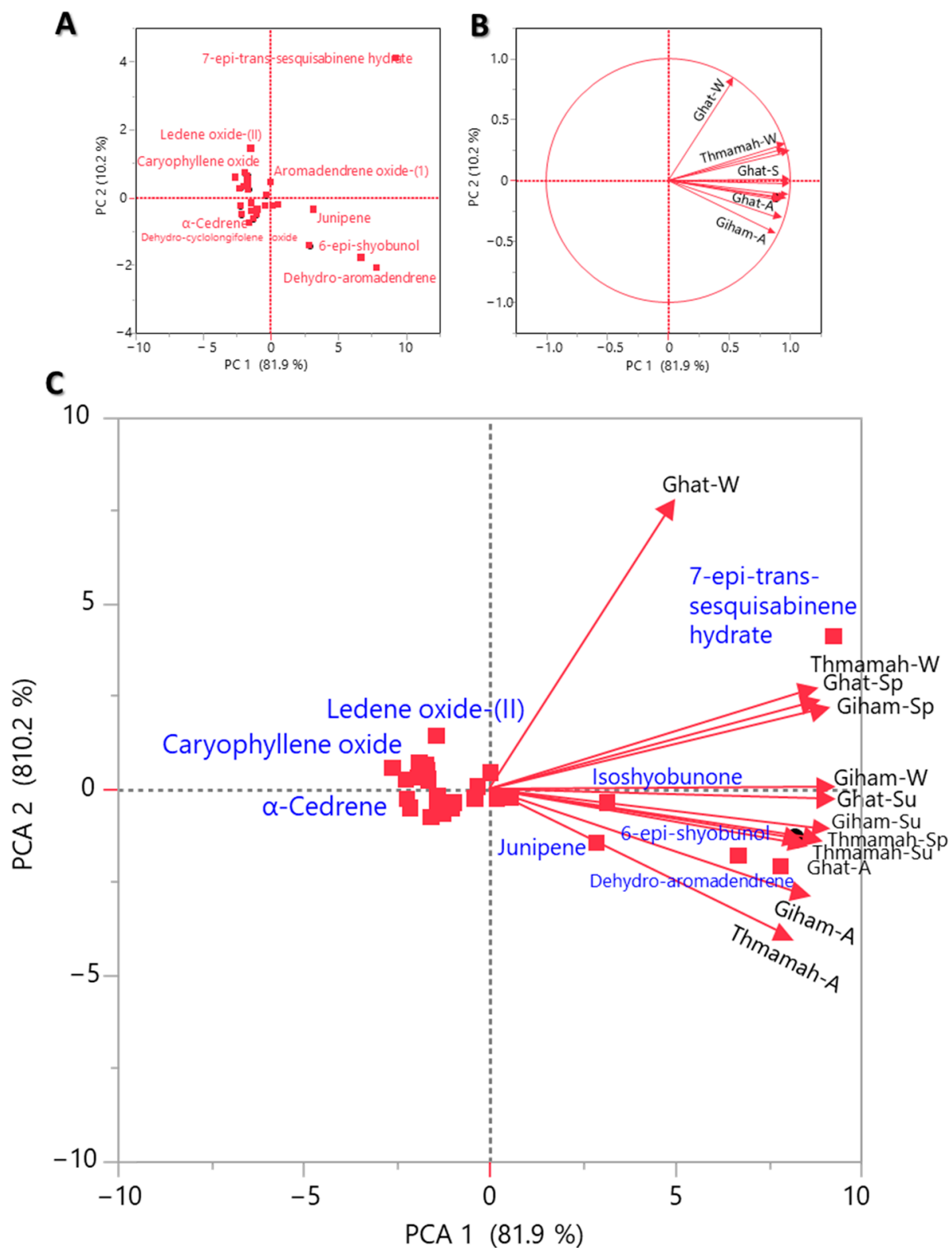


Figure 4. The principal components analysis (PCA) of various compounds identified from the samples collected from the three studied regions (Ghat, Thumamah, Giham) during the four seasons. (A) the observation in the PCA space, (B) the correlation circle (variables chart), and (C) the biplot diagram.

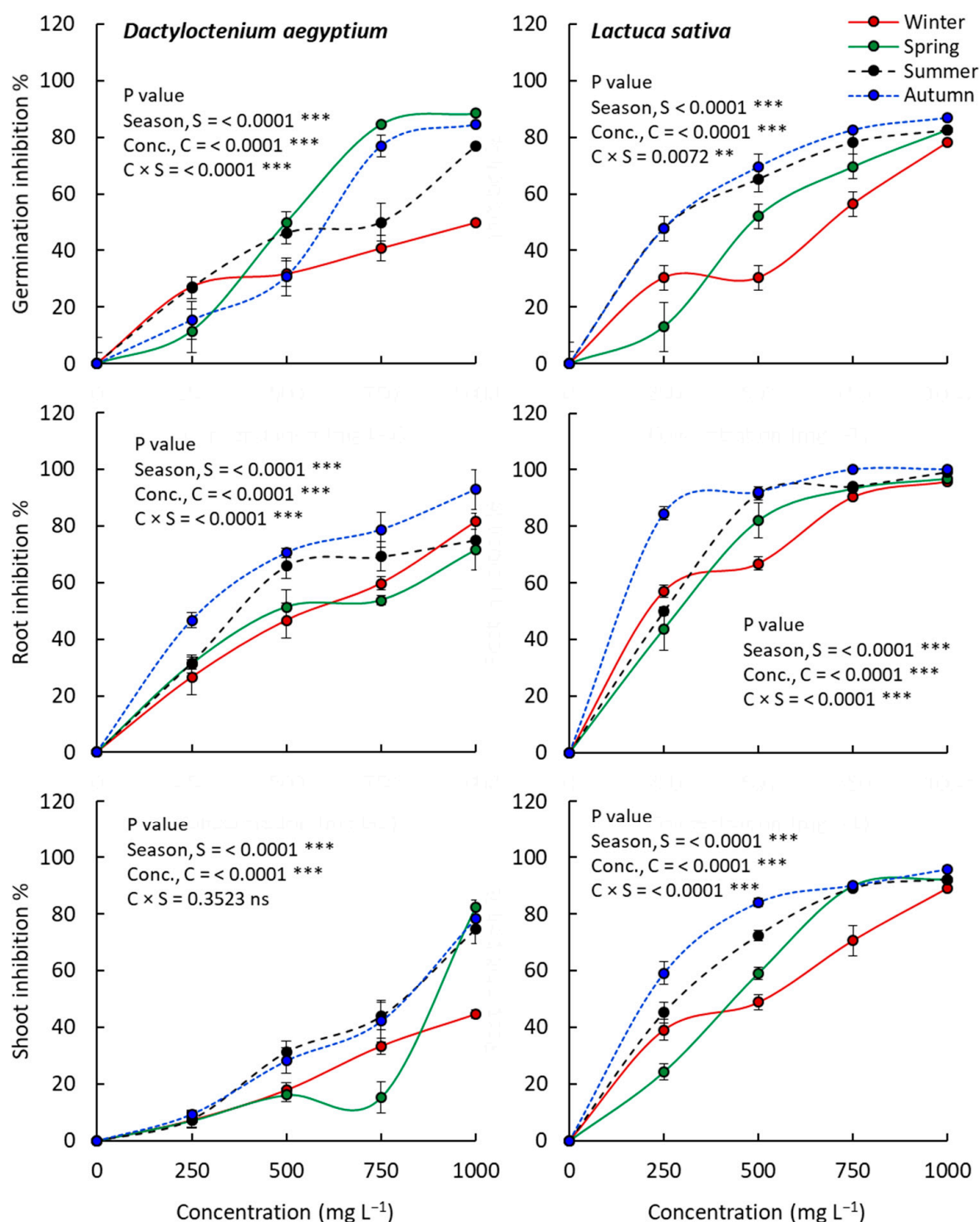


Figure 5. Allelopathic activity of the essential oils extracted from *A. monosperma* collected from the Ghat region during the four seasons (spring, winter, summer, and autumn) tested against the seed germination, seedling radicle growth, and seedling shoot growth of *Dactyloctenium aegyptium* (left) and *Lactuca sativa* (right). ns: nonsignificant, ** $p < 0.01$, *** $p < 0.001$.

At the highest concentration (1000 mg L⁻¹) of *A. monosperma* EOs collected from the Ghat region, the seed germination of *D. aegyptium* was inhibited by 88.5, 84.6, 76.9, and 50% for spring, autumn, summer, and winter, respectively. Meanwhile, the seed germination of *L. sativa* was inhibited by 82.6, 87.0, 82.6, and 78.3% for spring, autumn, summer, and winter, respectively (Figure 5). The seedling root growth of *D. aegyptium* was inhibited by

92.9, 81.5, 75.1, and 71.5% when exposed to 1000 mg L⁻¹ EO extracted from the samples collected during autumn, winter, summer, and spring, respectively. However, the seedling root growth of *L. sativa* was more sensitive to the EO, where it declined by 100.0, 99.2, 96.9, and 95.8% in autumn, summer, spring, and winter.

Seedling shoot growth of *D. aegyptium* exposed to the EO of *A. monosperma* at a concentration of 1000 mg L⁻¹, declined by 82.3, 78.5, 74.7, and 44.5% for the samples collected during spring, autumn, summer, and winter, respectively. While the seedling shoot growth of *L. sativa* was more inhibited under the same concentration of the EO, where it declined by 95.8, 92.2, 92.2, and 89.1% for autumn, spring, summer, and winter, respectively (Figure 5).

In general, the data revealed that the root growth of the test plant, *L. sativa*, was more sensitive to the EO of *A. monosperma* compared to the weed, *D. aegyptium*. Weeds have been reported to be more resistant to chemicals than crops [44,45]. Also, monocot plants were reported to be more resistant to bioherbicides than dicot species [10,46,47]. As an average of seasons, seedling root growth was more inhibited than seedling shoot growth and/or seed germination of *D. aegyptium* and *L. sativa* (Figure 5). The root growth has been reported to be affected by the EO treatments more than the shoot in several plants [25,32]. This could be attributed to the direct contact with the chemical compounds as well as the higher permeability of the root cell membrane [48].

Regarding seasons, the *A. monosperma* samples collected during the autumn and summer seasons showed more inhibitory activity against *D. aegyptium* and *L. sativa* than those collected during the winter and spring seasons. The observed variation in the allelopathic activity of the EO among seasons could be attributed to the variation in the chemical composition of the EO profile (Table 3). The allelopathic activity of the *A. monosperma* EO collected from the Ghat region could be ascribed to their higher content of dehydro-aromadendrene, junipene, 6-epi-shyobunol, and 7-epi-trans-sesquisabinene hydrate which could act singular or in combination as allelochemicals. The EO that is rich in 6-epi-shyobunol of *Pulicaria somalensis* has been reported to exhibit significant inhibition to the weeds *D. aegyptium* and *Bidens pilosa* [32].

The major compounds were determined in all samples of the four seasons though in varied amounts. This variation was ascribed to the variation in the climatic variations among seasons (Figure S1) as well as to the variation in the soil moisture content (Table 2). This variation in the bioactive compounds among seasons could be the main cause of the determined varied allelopathic activity. Although the EOs of *A. monosperma* collected during the summer and autumns seasons revealed more allelopathic activity, we cannot exactly ascribe this effect to certain bioactive compounds since we cannot follow a specific pattern for the concentration of the major bioactive compounds with respect to seasons. Therefore, further study is recommended to test the allelopathic activity of either the singular or combined form of the identified major compounds against a wide range of weeds and crops.

3.4.2. Thumamah Region

The EO extracted from *A. monosperma* collected from the Thumamah region showed significant allelopathic activity among the different seasons, concentrations, and their interactions (Figure 6). At the highest concentration (1000 mg L⁻¹) of *A. monosperma* EOs collected during the autumn season from the Thumamah region, the seed germination of *D. aegyptium* was totally retarded, while it was reduced by 84.6% for the samples collected during the summer and winter seasons. On the other hand, the seed germination of *L. sativa* was more affected by the EO of *A. monosperma* collected from the Thumamah region, where the concentration of 1000 mg L⁻¹ completely inhibited germination for the samples collected during autumn and spring (Figure 6).

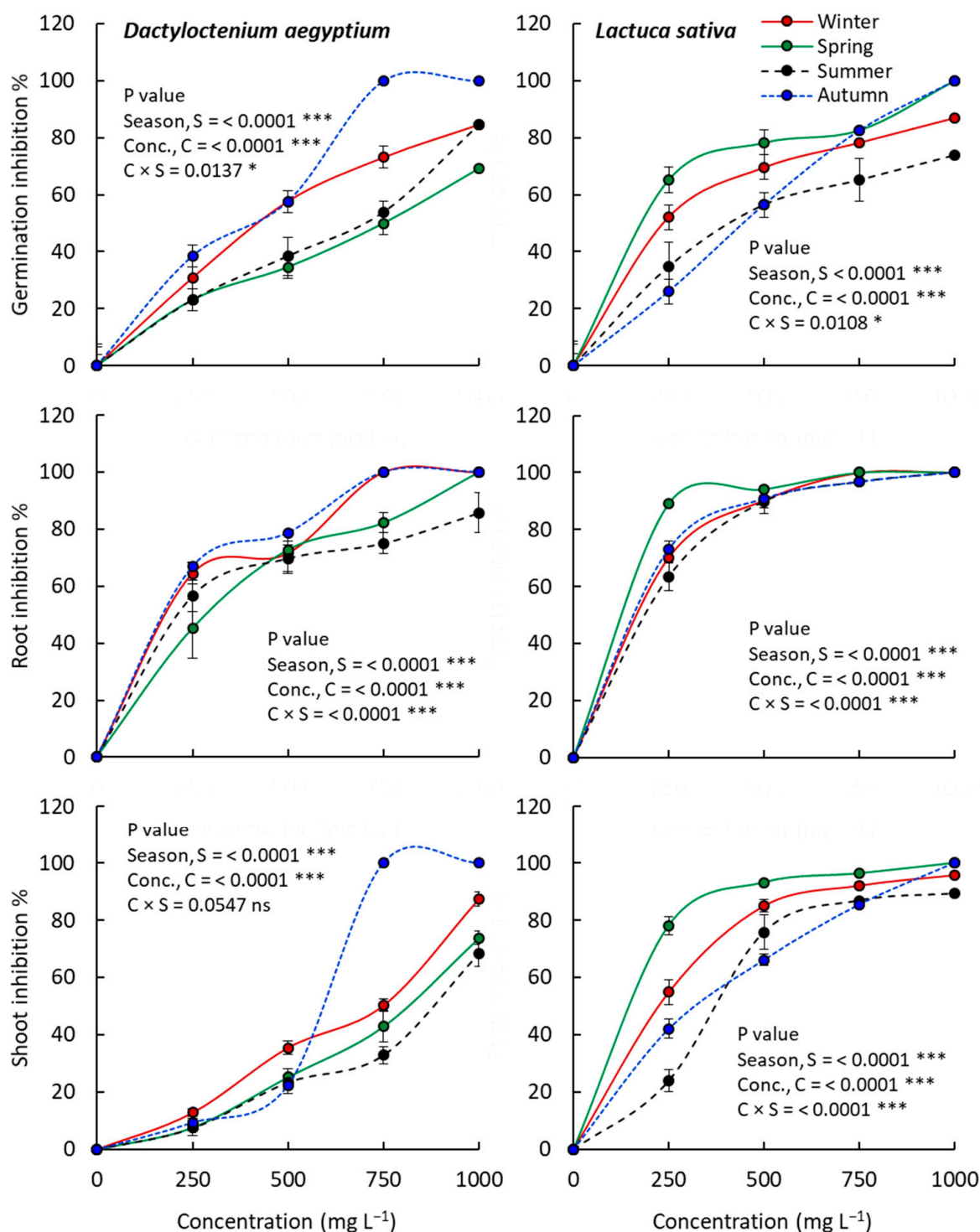


Figure 6. Allelopathic activity of the essential oils extracted from *A. monosperma* collected from the Thumamah region during the four seasons (spring, winter, summer, and autumn) tested against the seed germination, seedling radicle growth, and seedling shoot growth of *Dactyloctenium aegyptium* (left) and *Lactuca sativa* (right). ns: nonsignificant, * $p < 0.05$, *** $p < 0.001$.

Regarding the seedling growth, roots were more sensitive than shoots, where the seedling root growth of *D. aegyptium* was completely inhibited at a concentration of 750 mg L⁻¹ of *A. monosperma* EOs that were collected during autumn and winter. While the seedling root growth of *L. sativa* was totally inhibited by the EO samples collected during winter and spring. The seedling shoot growth of *D. aegyptium* was totally retarded by the

treatment of 1000 mg L⁻¹ of *A. monosperma* EOs collected during the autumn season, while it was reduced by 87.3, 73.8, and 68.4% for the samples collected during winter, spring, and summer, respectively. On the other hand, the root growth of *L. sativa* was more sensitive than *D. aegyptium*, where it was totally inhibited under a treatment of 1000 mg L⁻¹ of *A. monosperma* EO extracted from the samples collected during autumn and spring.

3.4.3. Giham Region

The allelopathic activity of the EO extracted from the *A. monosperma* collected from the Giham region on the germination of *D. aegyptium* and *L. sativa* showed a nonsignificant variation among seasons, while strong significant variation was determined for the concentration of the EO (Figure 7). At a concentration of 750 mg L⁻¹, the seed germination of *D. aegyptium* was completely inhibited for the samples collected during autumn, spring, and winter, while it was inhibited by 88.5% for the sample collected during summer. For *L. sativa*, seed germination was inhibited by 65.2, 47.8, 47.8, and 53.5% for the autumn, summer, spring, and winter samples at the concentration of 750 mg L⁻¹.

Regarding the *D. aegyptium* seedling root and shoot growth, no significant variation was observed among seasons, while a highly significant variation occurred among concentrations of the EO ($p < 0.05$). However, for *L. sativa*, a highly significant variation was observed among the seasons, concentrations, and interactions (Figure 7). The seedling root growth of *D. aegyptium* completely declined when treated with 750 mg L⁻¹ of the *A. monosperma* EO samples collected during autumn and winter, while it declined by 88.9% for the samples collected during the summer and spring seasons. On the other hand, the root growth of *L. sativa* was totally inhibited at the concentration of 750 mg L⁻¹ for the *A. monosperma* samples collected during winter and spring, while the samples of summer and autumn showed inhibition of 71.5 and 50.2%, respectively.

Concerning the shoot growth, at a concentration of 750 mg L⁻¹ of the *A. monosperma* EO, the samples collected during autumn, summer, spring, and winter inhibited the *D. aegyptium* shoot growth by 91.5, 79.9, 77.9, and 66.4%, respectively. While *L. sativa* shoot growth was totally inhibited by autumn, spring, and winter samples.

Summing up, the extracted EOs from *A. monosperma* showed considerable allelopathic activity against the weed, *D. aegyptium*, and the crop, *L. sativa*; however, the weed was more resistant to the EO, i.e., allelochemicals [49]. In addition, a significant variation was determined among the samples collected during the four seasons, which shows the importance of the sampling time of the aromatic plants that could be used as candidates for bioherbicide production [50]. The observed variation among samples collected during different seasons could be ascribed to the effect of season on the chemical composition of the EO as shown in GC-MS analysis, particularly the major compounds that could act in a single or combination manner [48].

3.5. Antioxidant Activity of *A. monosperma* EOs

The antioxidant activity of the EOs extracted from the different locations during the four seasons was tested using the DPPH method (Figure 8). In general, the samples collected during the summer and autumn seasons showed higher antioxidant activity compared to the spring and winter seasons. Based on the IC₅₀ value, a significant variation was observed among both locations and seasons ($p < 0.05$).

For samples collected from the Ghat region, the highest antioxidant activity (IC₅₀ = 34.13 mg L⁻¹) was reported for the sample collected during the autumn season, while the samples of summer, spring, and winter attained IC₅₀ values of 39.39, 97.64, and 136.96 mg L⁻¹, respectively (Figure 8). However, the EOs extracted from the samples collected during the summer season from the Thumamah region attained an IC₅₀ value of 59.34 mg L⁻¹, while that collected during the autumn, winter, and spring seasons attained IC₅₀ values of 77.00, 130.40, and 193.66 mg L⁻¹. Finally, the EOs of the samples collected during the summer season from the Giham region attained the highest antioxidant activity

($IC_{50} = 62.17 \text{ mg L}^{-1}$), compared to the autumn (IC_{50} value of 70.07 mg L^{-1}), winter (IC_{50} value of 98.44 mg L^{-1}), and spring seasons (IC_{50} value of 177.41 mg L^{-1}).

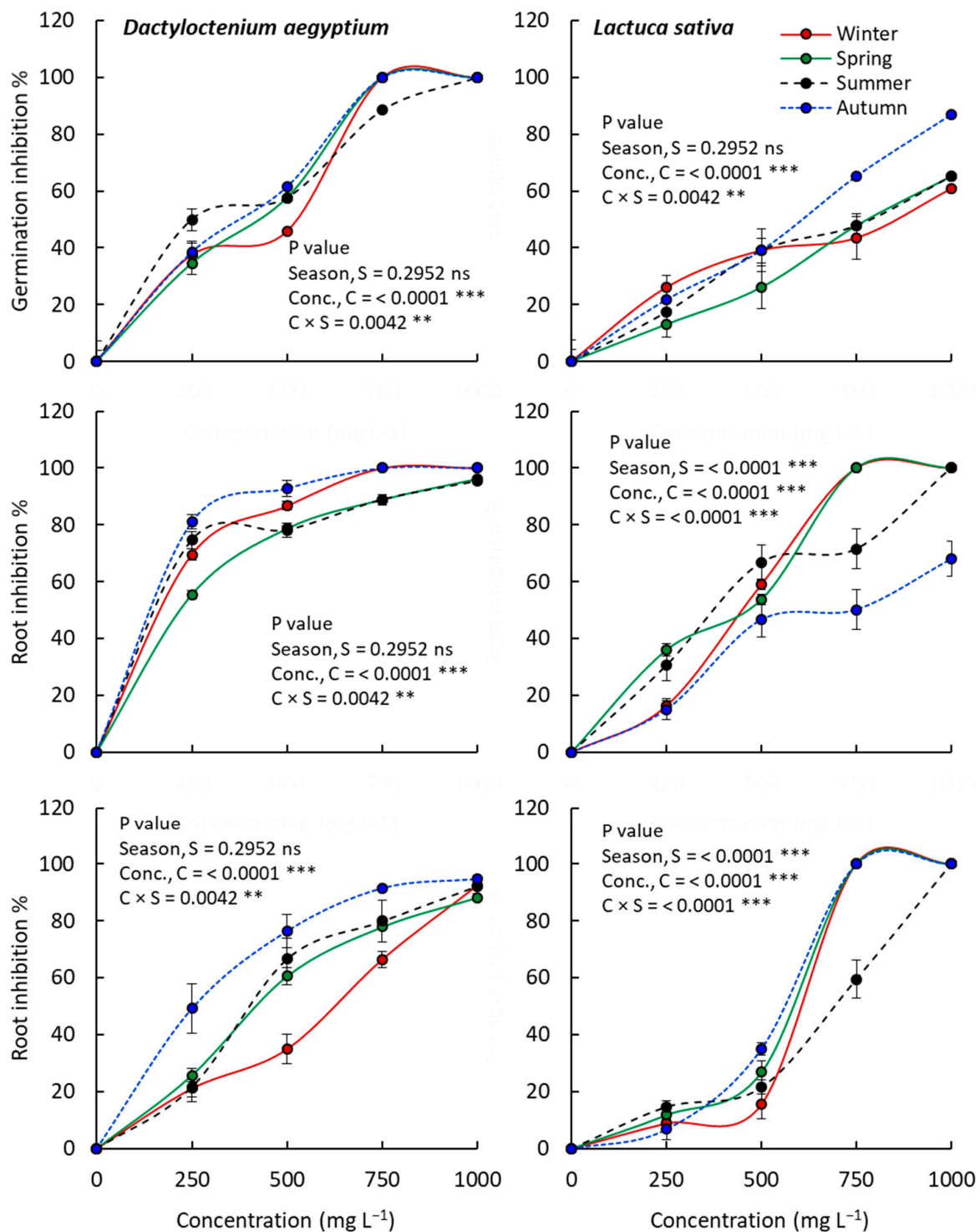


Figure 7. Allelopathic activity of the essential oils extracted from *A. monosperma* collected from Giham region during the four seasons (spring, winter, summer, and autumn) tested against the seed germination, seedling radicle growth, and seedling shoot growth of *Dactyloctenium aegyptium* (left) and *Lactuca sativa* (right). ns: nonsignificant, ** $p < 0.01$, *** $p < 0.001$.

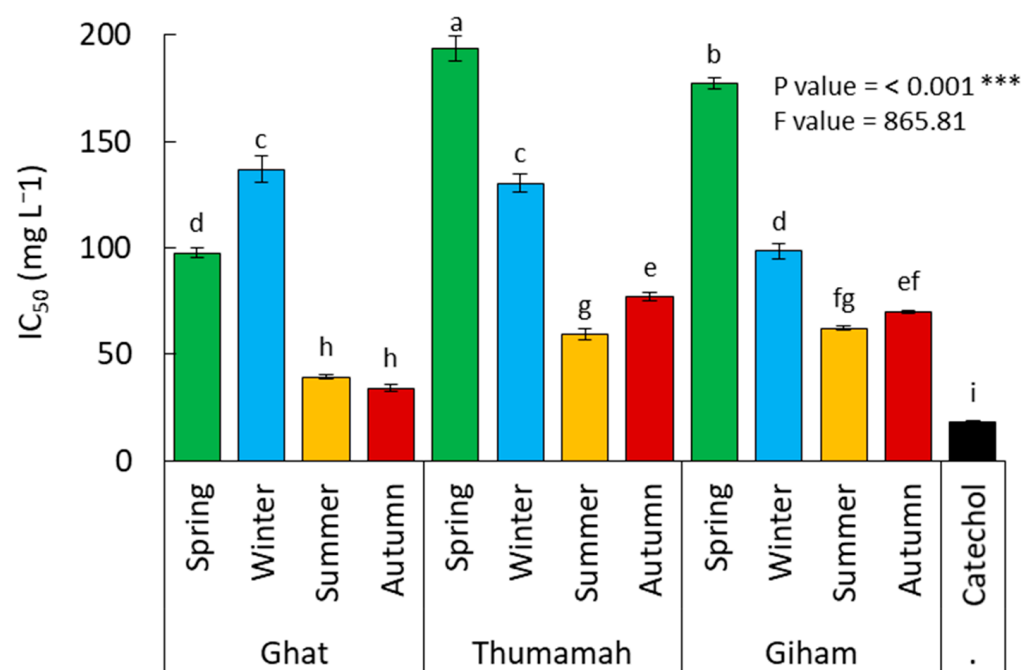


Figure 8. The IC₅₀ of the essential oils extracted from *A. monosperma* collected from three regions (Ghat, Thumamah, and Giham) during the four seasons (spring, winter, summer, and autumn) as well as catechol as standard antioxidant. *** $p < 0.001$. The different letters among columns showed significant differences at $p < 0.05$.

All of the present results revealed that all the extracted EO samples from the *A. monosperma* displayed strong to moderate DPPH radical scavenging activity with IC₅₀ ranges from 34.13 mg L⁻¹ to 177.41 mg L⁻¹. This result is in harmony with previous data concerning the antioxidant activity of the EOs from *A. monosperma* [51] and other *Artemisia* species, for example, *A. Annua* [52,53], *A. herba-alba* [54], *A. Diffusa* [55], and *A. Absinthium* [56].

In the present study, all extracted EOs from *A. monosperma* are characterized by a higher content of oxygenated compounds which have been reported to possess more antioxidant activity [48]. The stronger antioxidant activity of the oxygenated terpenes has been ascribed to the reactivity of the hydroxyl groups [57]. It has been documented that the EO components with phenolic hydroxyl (OH) are the most active constituents of EOs as free radical scavengers [12,58], where they act as free-radical scavenging agents in the EOs due to their high strong abilities to transfer the electrons during the pathway of antioxidant reaction [15]. These phenolic aromatic compounds were found with remarkable concentrations in the EO of the present studied *A. monosperma* samples. The phenolic aromatic compounds as well as the other oxygenated component of the EO could be implemented, as singular and/or synergetic, in the antioxidant process and increase the antioxidant activities [25,31].

The major identified oxygenated compounds in the present samples of *A. monosperma* such as 6-epi-shyobunol, dehydro-cyclolongifolene oxide, isoshyobunone, and diepicedrene-1-oxide could act as an antioxidative agent either alone or in combination. The 6-epi-shyobunol was identified as the main compound in the EO of *P. somalensis* [32] and *Teucrium polium* [59] where it showed considerable antioxidant activity. In addition, the major compound, isoshyobunone, in the present study was documented in the EOs of other plants with powerful antioxidant activity such as *Cleome amblyocarpa* [60] and *Acorus calamus* [43].

On the other hand, the main sesquiterpene hydrocarbons such as junipene, aromandendrene, dehydro-aromadendrene, α -bulnesene, and ar-curcumene have been found in the EOs with considerable antioxidant activity. For example, the aromadendrene-rich EOs of *Annona salzmannii* [61], rich with aromadendrene, have been reported to possess strong

antioxidant activity. The EO of *Pogostemon cablin* with high content of α -bulnesene (15.6%) showed strong antioxidant activity [62].

4. Conclusions

The chemical characterization of the EOs extracted from the *A. monosperma* ecospecies growing in three different regions showed slight variation among regions due to the nonsignificant variation in the soil and environmental conditions. Nevertheless, high variation was determined in the EO yield and composition among seasons due to the variation in the climatic condition and soil moisture contents. Seventy-two chemical compounds were identified, mainly as sesquiterpenes. The 7-epi-trans-sesquisabinene hydrate, 6-epi-shyobunol, dehydro-cyclolongifolene oxide, isoshyobunone, diepicedrene-1-oxide, dehydro-aromadendrene, dehydro-cyclolongifolene oxide, and junipene were determined as major compounds. The EOs of *A. monosperma* showed considerable allelopathic and antioxidant activity, which varied from season to season due to the variation in the chemical composition among samples of different seasons. These bioactivities could be ascribed to the higher content of oxygenated compounds within the EO. Although the EOs of *A. monosperma* collected during the summer and autumns seasons revealed more antioxidant and allelopathic activities, we cannot exactly attribute this effect to certain bioactive compounds, since we cannot follow a specific pattern for the concentration of the major bioactive compounds with respect to seasons. Therefore, further study is recommended to test the allelopathic activity of either the singular or combined forms of the identified major compounds against a wide range of weeds and crops. The present data showed the substantial effect of season and timing for sampling aromatic plants, which, in consequence, affects the biological activities of the EOs.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/separations10040263/s1>, Figure S1: The climatic conditions of the three studied regions during the year of sampling; Figure S2: Soil moisture content of the soil under the *A. monosperma* during the four seasons from the three regions; Figure S3: The chromatogram of the GC-MS analysis of *A. monosperma* EOs collected from the Ghat region during the four seasons; Figure S4: The chromatogram of the GC-MS analysis of *A. monosperma* EOs collected from the Thumamh region during the four seasons; Figure S5: The chromatogram of the GC-MS analysis of *A. monosperma* EOs collected from the Giham region during the four seasons.

Author Contributions: Conceptualization, A.M.A.-E. and A.M.A.; validation, A.M.A.-E., G.B. and A.M.A.; formal analysis, A.M.A.-E., A.M.A., M.S.A. and A.I.E.; investigation, A.M.A.-E., A.M.A., S.L.A.-R., M.S.A., G.B. and A.I.E.; writing—original draft preparation, A.M.A.-E. and A.I.E.; writing—review and editing, A.M.A.-E., A.M.A., S.L.A.-R., M.S.A., G.B. and A.I.E. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by the Researchers Supporting Project number (RSPD2023R676) King Saud University, Riyadh, Saudi Arabia.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors extend their appreciation to The Researchers Supporting Project number (RSPD2023R676) King Saud University, Riyadh, Saudi Arabia.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. de Oliveira, M.S.; Almeida, M.M.; Salazar, M.; Pires, F.C.S.; Bezerra, F.W.F.; Cunha, V.M.B.; Cordeiro, R.M.; Urbina, G.R.O.; da Silva, M.P.; e Silva, A.P.S. Potential of medicinal use of essential oils from aromatic plants. In *Potential of Essential Oils*; IntechOpen: London, UK, 2018; pp. 1–21.
2. Pavela, R.; Benelli, G. Essential oils as ecofriendly biopesticides? Challenges and constraints. *Trends Plant Sci.* **2016**, *21*, 1000–1007. [CrossRef] [PubMed]

3. Ninkovic, V.; Markovic, D.; Rensing, M. Plant volatiles as cues and signals in plant communication. *Plant Cell Environ.* **2021**, *44*, 1030–1043. [CrossRef]
4. Raveau, R.; Fontaine, J.; Lounès-Hadj Sahraoui, A. Essential oils as potential alternative biocontrol products against plant pathogens and weeds: A review. *Foods* **2020**, *9*, 365. [CrossRef] [PubMed]
5. Catani, L.; Grassi, E.; di Montanara, A.C.; Guidi, L.; Sandulli, R.; Manachini, B.; Semprucci, F. Essential oils and their applications in agriculture and agricultural products: A literature analysis through VOSviewer. *Biocatal. Agric. Biotechnol.* **2022**, *45*, 102502. [CrossRef]
6. Valarezo, E.; Aguilera-Sarmiento, R.; Meneses, M.A.; Morocho, V. Study of essential oils from leaves of asteraceae family species *Ageratina dendroides* and *Gynoxys verrucosa*. *J. Essent. Oil Bear. Plants* **2021**, *24*, 400–407. [CrossRef]
7. Miguel, M.G. Antioxidant and anti-inflammatory activities of essential oils: A short review. *Molecules* **2010**, *15*, 9252–9287. [CrossRef] [PubMed]
8. Abd-ElGawad, A.M.; El-Amier, Y.A.; Bonanomi, G.; Gendy, A.E.-N.G.E.; Elgorban, A.M.; Alamery, S.F.; Elshamy, A.I. Chemical composition of *Kickxia aegyptiaca* essential oil and its potential antioxidant and antimicrobial activities. *Plants* **2022**, *11*, 594. [CrossRef]
9. Andrade, M.A.; Braga, M.A.; Cesar, P.H.; Trento, M.V.C.; Espósito, M.A.; Silva, L.F.; Marcussi, S. Anticancer properties of essential oils: An overview. *Curr. Cancer Drug Targets* **2018**, *18*, 957–966. [CrossRef]
10. Hazrati, H.; Saharkhiz, M.J.; Moein, M.; Khoshghalb, H. Phytotoxic effects of several essential oils on two weed species and tomato. *Biocatal. Agric. Biotechnol.* **2018**, *13*, 204–212. [CrossRef]
11. Hijazi, A.M.; Salhab, A.S. Effects of *Artemisia monosperma* ethanolic leaves extract on implantation, mid-term abortion and parturition of pregnant rats. *J. Ethnopharmacol.* **2010**, *128*, 446–451. [CrossRef]
12. Pandey, A.K.; Singh, P. The genus *Artemisia*: A 2012–2017 literature review on chemical composition, antimicrobial, insecticidal and antioxidant activities of essential oils. *Medicines* **2017**, *4*, 68. [CrossRef] [PubMed]
13. El Zalabani, S.M.; Tadros, S.H.; El Sayed, A.M.; Daboub, A.A.; Sleem, A.A. Chemical profile and biological activities of essential oil of aerial parts of *Artemisia monosperma* Del. growing in Libya. *Pharmacogn. J.* **2017**, *9*, 578–586. [CrossRef]
14. Khan, M.; Mousa, A.A.; Syamasundar, K.V.; Alkhathlan, H.Z. Determination of Chemical Constituents of Leaf and Stem Essential Oils of *Artemisia monosperma* from Central Saudi Arabia. *Nat. Prod. Commun.* **2012**, *7*, 1079–1082. [CrossRef] [PubMed]
15. Abd-ElGawad, A.M.; Elshamy, A.I.; Al-Rowaily, S.L.; El-Amier, Y.A. Habitat affects the chemical profile, allelopathy, and antioxidant properties of essential oils and phenolic enriched extracts of the invasive plant *Heliotropium curassavicum*. *Plants* **2019**, *8*, 482. [CrossRef]
16. Zhang, T.; Yang, H.; Wen, S.; Qiu, F.; Liu, X. Effects of Harvest season and storage time on the essential oil of the linalool chemotype of *Cinnamomum camphora*. *J. Essent. Oil Bear. Plants* **2019**, *22*, 1379–1385. [CrossRef]
17. Time and Dates. Climate & Weather Averages in Riyadh, Saudi Arabia. Available online: <https://www.timeanddate.com/weather/saudi-arabia/riyadh/climate> (accessed on 14 January 2023).
18. Belnap, J.; Phillips, S.L.; Miller, M.E. Response of desert biological soil crusts to alterations in precipitation frequency. *Oecologia* **2004**, *141*, 306–316. [CrossRef]
19. Bouyoucos, G.J. Hydrometer method improved for making particle size analyses of soils. *Agron. J.* **1962**, *54*, 464–465. [CrossRef]
20. Jackson, M.L. *Soil Chemical Analysis*; Constable and Co., Ltd.: London, UK, 1962.
21. Rowell, D. *Soil Science: Methods and Applications*; Longman Group: Harlow, UK, 1994.
22. Allen, S.E.; Grimshaw, H.; Parkinson, J.A.; Quarmby, C. *Chemical Analysis of Ecological Materials*; Blackwell Scientific Publications: Hoboken, NJ, USA, 1974.
23. Chaudhary, S.A. *Flora of the Kingdom of Saudi Arabia*; Ministry of Agriculture and Water: Riyadh, Saudi Arabia, 2000; Volume 2.
24. Collenette, S. *Wildflowers of Saudi Arabia*; National Commission for Wildlife Conservation and Development (NCWCD): Riyadh, Saudi Arabia, 1999.
25. Abd El-Gawad, A.M. Chemical constituents, antioxidant and potential allelopathic effect of the essential oil from the aerial parts of *Cullen plicata*. *Ind. Crops Prod.* **2016**, *80*, 36–41. [CrossRef]
26. Riaz, S.; Basharat, S.; Ahmad, F.; Hameed, M.; Fatima, S.; Ahmad, M.S.A.; Shah, S.M.R.; Asghar, A.; El-Sheikh, M.A.; Kaushik, P. *Dactyloctenium aegyptium* (L.) Willd. (Poaceae) differentially responds to pre-and post-emergence herbicides through micro-structural alterations. *Agriculture* **2022**, *12*, 1831. [CrossRef]
27. Macías, F.A.; Castellano, D.; Molinillo, J.M. Search for a standard phytotoxic bioassay for allelochemicals. Selection of standard target species. *J. Agric. Food Chem.* **2000**, *48*, 2512–2521. [CrossRef]
28. Miguel, M.G. Antioxidant activity of medicinal and aromatic plants. *Flavour Fragr. J.* **2010**, *25*, 219–312. [CrossRef]
29. El-Sheri, M.; Khaleel, A.; Motaal, A.A.; Abd-Elbaki, P. Effect of seasonal variation on the composition of the essential oil of *Solidago canadensis* cultivated in Egypt. *J. Essent. Oil Bear. Plants* **2014**, *17*, 891–898. [CrossRef]
30. Grulova, D.; De Martino, L.; Mancini, E.; Salamon, I.; De Feo, V. Seasonal variability of the main components in essential oil of *Mentha × piperita* L. *J. Sci. Food Agric.* **2015**, *95*, 621–627. [CrossRef]
31. Amin, S.M.; Hassan, H.M.; El Gendy, A.E.N.G.; El-Beih, A.A.; Mohamed, T.A.; Elshamy, A.I.; Bader, A.; Shams, K.A.; Mohammed, R.; Hegazy, M.E.F. Comparative chemical study and antimicrobial activity of essential oils of three *Artemisia* species from Egypt and Saudi Arabia. *Flavour Fragr. J.* **2019**, *34*, 450–459. [CrossRef]

32. Assaeed, A.; Elshamy, A.; El Gendy, A.E.-N.; Dar, B.; Al-Rowaily, S.; Abd-ElGawad, A. Sesquiterpenes-rich essential oil from above ground parts of *Pulicaria somalensis* exhibited antioxidant activity and allelopathic effect on weeds. *Agronomy* **2020**, *10*, 399. [\[CrossRef\]](#)
33. Keshavarz-Mirzamohammadi, H.; Tohidi-Moghadam, H.R.; Hosseini, S.J. Is there any relationship between agronomic traits, soil properties and essential oil profile of peppermint (*Mentha piperita* L.) treated by fertiliser treatments and irrigation regimes? *Ann. Appl. Biol.* **2021**, *179*, 331–344. [\[CrossRef\]](#)
34. Azizi, A.; Yan, F.; Honermeier, B. Herbage yield, essential oil content and composition of three oregano (*Origanum vulgare* L.) populations as affected by soil moisture regimes and nitrogen supply. *Ind. Crops Prod.* **2009**, *29*, 554–561. [\[CrossRef\]](#)
35. Momeni, M.; Pirbalouti, A.G.; Mousavi, A.; Badi, H.N. Effect of foliar applications of salicylic acid and chitosan on the essential oil of *Thymbra spicata* L. Under different soil moisture conditions. *J. Essent. Oil Bear. Plants* **2020**, *23*, 1142–1153. [\[CrossRef\]](#)
36. Ammar, N.M.; Hassan, H.A.; Ahmed, R.F.; El-Gendy, A.E.-N.G.; Abd-ElGawad, A.M.; Farrag, A.R.H.; Farag, M.A.; Elshamy, A.I.; Afifi, S.M. Gastro-protective effect of *Artemisia sieberi* essential oil against ethanol-induced ulcer in rats as revealed via biochemical, histopathological and metabolomics analysis. *Biomarkers* **2022**, *27*, 247–257. [\[CrossRef\]](#)
37. Guetat, A.; Al-Ghamdi, F.A.; Osman, A.K. The genus *Artemisia* L. in the northern region of Saudi Arabia: Essential oil variability and antibacterial activities. *Nat. Prod. Res.* **2017**, *31*, 598–603. [\[CrossRef\]](#)
38. Aćimović, M.; Lončar, B.; Stanković Jeremić, J.; Cvetković, M.; Pezo, L.; Pezo, M.; Todosijević, M.; Tešević, V. Weather conditions Influence on lavandin essential oil and hydrolate quality. *Horticulturae* **2022**, *8*, 281. [\[CrossRef\]](#)
39. Kaul, P.; Rajeswara Rao, B.; Bhattacharya, A.; Singh, K. Effect of weather parameters on yield and quality of the essential oil of rose-scented geranium (*Pelargonium* species). *Agric. Sci. Dig.* **1999**, *19*, 84–86.
40. Chang, X.; Alderson, P.; Wright, C. Effect of temperature integration on the growth and volatile oil content of basil (*Ocimum basilicum* L.). *J. Hortic. Sci. Biotechnol.* **2005**, *80*, 593–598. [\[CrossRef\]](#)
41. Murakami, S.; Li, W.; Matsuura, M.; Satou, T.; Hayashi, S.; Koike, K. Composition and seasonal variation of essential oil in *Alpinia zerumbet* from Okinawa Island. *J. Nat. Med.* **2009**, *63*, 204–208. [\[CrossRef\]](#) [\[PubMed\]](#)
42. Shoshtari, Z.V.; Rahimmalek, M.; Sabzalian, M.R.; Hosseini, H. Essential oil and bioactive compounds variation in myrtle (*Myrtus communis* L.) as affected by seasonal variation and salt stress. *Chem. Biodivers.* **2017**, *14*, e1600365. [\[CrossRef\]](#)
43. Parki, A.; Chaubey, P.; Prakash, O.; Kumar, R.; Pant, A.K. Seasonal variation in essential oil compositions and antioxidant properties of *Acorus calamus* L. accessions. *Medicines* **2017**, *4*, 81. [\[CrossRef\]](#)
44. Heap, I. Global perspective of herbicide-Resistant weeds. *Pest Manag. Sci.* **2014**, *70*, 1306–1315. [\[CrossRef\]](#) [\[PubMed\]](#)
45. Perotti, V.E.; Larran, A.S.; Palmieri, V.E.; Martinatto, A.K.; Permingeat, H.R. Herbicide resistant weeds: A call to integrate conventional agricultural practices, molecular biology knowledge and new technologies. *Plant Sci.* **2020**, *290*, 110255. [\[CrossRef\]](#)
46. Atak, M.; Mavi, K.; Uremis, I. Bio-herbicidal effects of oregano and rosemary essential oils on germination and seedling growth of bread wheat cultivars and weeds. *Rom. Biotechnol. Lett.* **2016**, *21*, 11149–11159.
47. De Mastro, G.; El Mahdi, J.; Ruta, C. Bioherbicidal potential of the essential oils from Mediterranean Lamiaceae for weed control in organic farming. *Plants* **2021**, *10*, 818. [\[CrossRef\]](#)
48. Abd-ElGawad, A.M.; El Gendy, A.E.-N.G.; Assaeed, A.M.; Al-Rowaily, S.L.; Alharthi, A.S.; Mohamed, T.A.; Nassar, M.I.; Dewir, Y.H.; Elshamy, A.I. Phytotoxic effects of plant essential oils: A systematic review and structure-activity relationship based on chemometric analyses. *Plants* **2021**, *10*, 36. [\[CrossRef\]](#) [\[PubMed\]](#)
49. Hasan, M.; Ahmad-Hamdani, M.S.; Rosli, A.M.; Hamdan, H. Bioherbicides: An eco-friendly tool for sustainable weed management. *Plants* **2021**, *10*, 1212. [\[CrossRef\]](#) [\[PubMed\]](#)
50. Skaria, B.P. *Aromatic Plants*; New India Publishing Agency: New Delhi, India, 2007; Volume 1.
51. Romeilah, R.M.; El-Beltagi, H.S.; Shalaby, E.A.; Younes, K.M.; Hani, E.; Rajendrasozhan, S.; Mohamed, H. Antioxidant and cytotoxic activities of *Artemisia monosperma* L. and *Tamarix aphylla* L. essential oils. *Not. Bot. Horti Agrobot. Cluj-Napoca* **2021**, *49*, 12233. [\[CrossRef\]](#)
52. Juteau, F.; Masotti, V.; Bessiere, J.M.; Dherbomez, M.; Viano, J. Antibacterial and antioxidant activities of *Artemisia annua* essential oil. *Fitoterapia* **2002**, *73*, 532–535. [\[CrossRef\]](#) [\[PubMed\]](#)
53. Čavar, S.; Maksimović, M.; Vidic, D.; Parić, A. Chemical composition and antioxidant and antimicrobial activity of essential oil of *Artemisia annua* L. from Bosnia. *Ind. Crops Prod.* **2012**, *37*, 479–485. [\[CrossRef\]](#)
54. Younsi, F.; Trimech, R.; Boulila, A.; Ezzine, O.; Dhahri, S.; Boussaid, M.; Messaoud, C. Essential oil and phenolic compounds of *Artemisia herba-alba* (Asso.): Composition, antioxidant, antiacetylcholinesterase, and antibacterial activities. *Int. J. Food Prop.* **2016**, *19*, 1425–1438. [\[CrossRef\]](#)
55. Mohammadi, A.; Arianfar, A.; Noori, M. Chemical composition, antioxidant and antibacterial activity of *Artemisia diffusa* essential oil. *J. Essent. Oil Bear. Plants* **2017**, *20*, 1235–1243. [\[CrossRef\]](#)
56. Benkhaled, A.; Boudjelal, A.; Napoli, E.; Baali, F.; Ruberto, G. Phytochemical profile, antioxidant activity and wound healing properties of *Artemisia absinthium* essential oil. *Asian Pac. J. Trop. Biomed.* **2020**, *10*, 496–504.
57. Zuo, A.-R.; Dong, H.-H.; Yu, Y.-Y.; Shu, Q.-L.; Zheng, L.-X.; Yu, X.-Y.; Cao, S.-W. The antityrosinase and antioxidant activities of flavonoids dominated by the number and location of phenolic hydroxyl groups. *Chin. Med.* **2018**, *13*, 51. [\[CrossRef\]](#)
58. Lopes-Lutz, D.; Alviano, D.S.; Alviano, C.S.; Kolodziejczyk, P.P. Screening of chemical composition, antimicrobial and antioxidant activities of *Artemisia* essential oils. *Phytochemistry* **2008**, *69*, 1732–1738. [\[CrossRef\]](#)

59. Saleh, I.; Abd-ElGawad, A.; El Gendy, A.E.-N.; Abd El Aty, A.; Mohamed, T.; Kassem, H.; Aldosri, F.; Elshamy, A.; Hegazy, M.-E.F. Phytotoxic and antimicrobial activities of *Teucrium polium* and *Thymus decussatus* essential oils extracted using hydrodistillation and microwave-assisted techniques. *Plants* **2020**, *9*, 716. [[CrossRef](#)] [[PubMed](#)]
60. Abd-ElGawad, A.M.; Elgamal, A.M.; El-Amier, Y.A.; Mohamed, T.A.; El Gendy, A.E.-N.G.; Elshamy, A.I. Chemical composition, allelopathic, antioxidant, and anti-inflammatory activities of sesquiterpenes rich essential oil of *Cleome amblyocarpa* Barratte & Murb. *Plants* **2021**, *10*, 1294. [[PubMed](#)]
61. Costa, E.V.; Dutra, L.M.; de Jesus, H.C.R.; de Lima Nogueira, P.C.; de Souza Moraes, V.R.; Salvador, M.J.; de Holanda Cavalcanti, S.C.; dos Santos, R.L.C.; do Nascimento Prata, A.P. Chemical composition and antioxidant, antimicrobial, and larvicidal activities of the essential oils of *Annona salzmanii* and *A. pickelii* (Annonaceae). *Nat. Prod. Commun.* **2011**, *6*, 907–912. [[CrossRef](#)] [[PubMed](#)]
62. Luchesi, L.A.; Paulus, D.; Busso, C.; Frata, M.T.; Oliveira, J.B. Chemical composition, antifungal and antioxidant activity of essential oils from *Baccharis dracunculifolia* and *Pogostemon cablin* against *Fusarium graminearum*. *Nat. Prod. Res.* **2022**, *36*, 849–852. [[CrossRef](#)]

Disclaimer/Publisher’s Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.