Journal of Medicinal Plants and By-Products (2025) 1: 69– 79

Comparative Analysis of the Essential Oils of *Dracocephalum moldavica* L. from Greenhouse and *In vitro* Cultured Conditions

Bahareh Allahverdi-Mamaghani[1], Seyed Mohsen Hesamzadeh Hejazi*[2], Mehdi Mirza[3] and Ali Movafeghi[1]

[1]*Faculty of Natural science, University of Tabriz, Tabriz, Iran*
[2]*Department of Biotechnology, Research Institute of Forest and Rangelands, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran*
[3]*Department of Medicinal Plants and By-products, Research Institute of Forest and Rangelands, Agricultural Research Education and Extension Organization (AREEO), Tehran, Iran*

Article Info

Article Type
Original Article

Received: 14 June 2024
Accepted: 22 June 2024

***Corresponding author**
smhessamzadeh@rifr-ac.ir

ABSTRACT

Dracocephalum moldavica L. is an aromatic annual plant belongs to the Lamiaceae family. A study was conducted to analyze the essential oils of this plant from six population cultivated in a greenhouse and four population under *in vitro* culture conditions. The essential oils were isolated using the hydro-distillation method and Clevenger apparatus. Gas Chromatography (GC) and Gas Chromatography/Mass spectrometry (GC-MS) were used for analysis. The identified components constituted approximately 91.35-96.58% of the essential oil composition under greenhouse conditions and 90.73-98.3% under *in vitro* culture conditions. The highest essential oil percentage (0.27%) was found in the Karaj (2) population under greenhouse conditions, while the maximum essential oil yield under *in vitro* culture was 0.1% in the Hamedan (2) population. The main components identified were Neral, Geraniol, Geranial, and Geranyl acetate. Hamedan (2) (greenhouse) had the highest Neral (27.05%) content, Karaj (3) (*in vitro* culture) had the highest Geraniol (34.32%) content, Karaj (2) (*in-vitro* culture) had the highest Geranial (56.12%) content, and Karaj (1) (greenhouse) had the highest geranyl acetate (24.75%) content. In conclusion, the content of Neral and Geranyl acetate increased under greenhouse conditions, while the maximum values of Geraniol and Geranial percentage were observed in the essential oils of *in vitro* culture. The results suggested that both the culture condition and the origin of the population influenced the essential oil percentage and chemical constituents of *D. moldavica* species.

Keywords: *Dracocephalum moldavica* L. Gas chromatography, Neral, Geraniol, Geranial and Geranyl acetate, Culture condition

How to cite this paper
Allahverdi Mamaghani, B., Hesamzadeh Hejazi, S. M., Mirza, M., Movafeghi, A. Comparative analysis of the essential oils of Deracocephalum moldavica L. from Greenhouse and in vitro cultured conditions. Journal of Medicinal plants and By-Products, 2025; (1): 69-79. doi: 10.22034/jmpb.2024.366414.1722

INTRODUCTION

Dracocephalum moldavica L., commonly known as Dragon's head or Moldavian balm, is an annual, herbaceous plant belonging to the Lamiaceae family [1, 2]. It is native to central Asia and has been cultivated in various regions, including parts of Europe, North America, and China [3, 4]. In Iran, this plant grows naturally in the North and North West, particularly in West Azerbaijan and the Alborz mountain regions, as well as Isfahan and Tehran provinces. Additionally, it has been widely cultivated throughout most areas of Iran [5, 6]. *D. moldavica* typically reaches a height of 55 cm and features square stems with rare hairy leaves. Its inflorescence is spike-like, and the fruits are akene and oval in shape [5]. The plant has a rich history of traditional medicinal use, particularly in treating wounds and injuries [7]. It is also employed to address various health issues, such as pain relief, kidney problems, headaches, toothaches, heart disease, blood pressure, angina, and atherosclerosis [2]. Research has highlighted the antimicrobial activity of *D. moldavica* against several bacterial strains, including *Bacillus subtilis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhimurium*, and *Listeria monocytogenes* [8, 10]. The plant's essential oil has also demonstrated pain killer and anti-depression effects [11, 12].

Phytochemical studies have identified various compounds in *Dracocephalum moldavica*, including essential oils, saturated and unsaturated fatty acids, phenolic compounds, flavonoids, and tocopherol [13]. The essential oil yield has been reported to vary between different studies, with concentrations ranging from 0.06% to 3.2% [2, 3, 14-17].

The main components of *D. moldavica* essential oil have been identified as Geranyl acetate, Geraniol, Geranial, and Neral [1-3,9,15,16,18-21]. The quantities of Geranyl acetate, Geraniol, and Geranial tend to increase during the flowering stage, while Neral content decreases.

Additionally, Geranyl acetate biosynthesis increases during the vegetative stage, while during the flowering stage, biosynthesis of chemical compounds shifts towards Geranial and Geraniol [4]. Other chemical compounds such as 1,8-cineol, 4-terpineol, cuminal alcohol, and α-terpineol have also been identified in *D. moldavica* [22].

The present research aims to investigate and compare the variation in quantity and quality of essential oils among six populations of *D. moldavica* cultivated under greenhouse conditions and four populations cultured under *in vitro* conditions. Understanding these differences may provide valuable insights into the impact of different growing conditions on the essential oil profile of this medicinal plant.

MATERIALS AND METHODS

Plant Material

Seeds of *D. moldavica* were obtained from the Natural Resources Gene Bank at the Research Institute of Forest and Rangeland (RIFR) in Tehran, Iran. Six populations were selected for cultivation under greenhouse conditions, while four out of six populations due to establishment were cultured under *in vitro* conditions (Table 1). The seeds were initially cultured in a pot with a mixture of peat moss and Perlite in November 2014. Once the seedlings grew, they were transferred to larger pots filled with a mixture of soil, sand, and mulch (1:2:1 ratio). The plants were regularly irrigated with tap water until they reached the flowering stage, which was approximately six months after cultivation.

Under *in vitro* condition, just plantlet of four population were raised in MS [23] medium containing 0.1 mgl^{-1} BAP and 0.01 mgl^{-1} NAA as plant growth regulators (PGR) and then acclimatized under greenhouse condition as described previously [24].

Essential Oil Extraction

At full flowering stage, the flowering shoots were collected and dried in the shade. The dried plant material was then cut into small pieces using a mill. Essential oil extraction was performed through the hydro-distillation method using a Clevenger apparatus for duration of three hours, following the procedure specified in the British Pharmacopeia [25]. After the extraction, the essential oil was separated from any remaining water in the extract using sodium sulfate. The percentage of essential oil was calculated based on the dry weight of the plant material. The essential oil samples were stored in small containers at 4 degrees Celsius in a refrigerator until further analysis by Gas Chromatography (GC) and Gas Chromatography/Mass spectrometry (GC-MS).

Properties of GC (Gas Chromatography)

The gas chromatography was performed using a Termo-UFM (Ultra-Fast Model) from Italy, equipped with a capillary column of brand Ph-5 (semi-polar) with dimensions of 10 m in length and 1.0 mm in internal diameter. The column thickness was 4.0 mm, and it was coated with Dimetylsiloxane phenyl, 5% stationary phase. The column temperature program started at 60 °C and gradually reached the final temperature of 285 °C. During each minute of the analysis, the temperature increased to 80 °C for 3 minutes before reaching 285 °C, where it was held steady. The detector used was the Flame Ionization Detector (FID) with helium as the carrier gas at an inlet pressure of 5.0 kg/cm². The detector chamber temperature was set at 290 °C, and the injection chamber temperature was set at 280 °C.

Properties of GC-MS (Gas Chromatography-Mass Spectrometer)

The gas chromatography-mass spectrometer used was the Varian 3400 connected to a mass spectrometer Saturn II. The ionization energy of the mass spectrometer was set at 70 eV, and a DB-5 semi-polar column was employed with dimensions of 30 m in length, 25.0 mm in internal diameter, and 25.0 microns thickness of the stationary phase. The head pressure of the column was maintained at 35 pounds per square inch. The temperature increased from 40 to 250 °C at a rate of 3 °C per minute. The injection chamber temperature was set at 260 °C, and the transfer line temperature was set at 270 °C. The spectra were identified using retention indices and by comparing the mass spectra with those of standard compounds in various sources, such as [26-28], as well as the information in the GC/MS library.

Statistical analysis

In order to determine the variation between individuals on different culture methods, Completely Randomized Design (CRD) design was performed on transformed data by SINH (Arcsin) method and means were compared by Duncan's test using SAS (1996), ver.6.12 software.

RESULTS

Essential oil percentage

The essential oil percentage of the different population of *D. moldavica* under greenhouse and *in vitro* culture conditions is summarized in Table 1. Under greenhouse conditions, the studied population contained essential oil ranging from 0.13% to 0.27%. In contrast, essential oil content under *in vitro* culture ranged from 0.04% to 0.1%. The highest essential oil percentage was observed in the greenhouse condition, particularly in the Karaj (2) (G3429), while the lowest essential oil percentage was obtained in the Karaj (1) (T1089) under *in vitro* culture. This indicates that essential oil percentage was generally higher under greenhouse conditions compared than *in vitro* culture. These results are consistent with previous studies by [15, 19], who reported essential oil contents of 0.06%-0.92% and 0.1%-0.8% in *D. moldavica*, respectively. Cultivation method, whether conventional or *in vitro*, can significantly affect the essential oil content of plants.

Chemical Constituents of Essential Oil

Table 2 and 3 present the Mean of identified compounds in the essential oil of plantlets cultured *in vitro* and under greenhouse conditions, respectively. Twelve compounds were identified in the essential oil of *in vitro* cultured plantlets, accounting for 90.73%-98.3% of the essential oils. Under greenhouse conditions, eighteen chemical compounds were identified in different populations, representing 91.35%-96.58% of the essential oil. Regardless of the culture condition, oxygenated monoterpenes were the main components in both cases. The primary constituents were Neral, Geraniol, Geranial, and Geranyl acetate, with smaller amounts of γ-terpinene, Terpinolene, Linalool, cis-chrysanthenol, α-terpineol, Neryl acetate, E-caryophyllene, and Germacrene D.

Neral content ranged from 0.06%-15.25% and 14.70%-27.05% under *in vitro* culture and greenhouse conditions, respectively. Geraniol content varied from 23.56%-34.32% under *in vitro* culture and 11.54%-16.71% under greenhouse conditions. Geranial percentage ranged from 31.53%-56.12% under *in vitro* culture and 29.56%-41.75% under greenhouse conditions. Geranyl acetate content varied from 3.68%-16.6% under *in-vitro* culture and 6.48%-24.75% under greenhouse conditions. A comparison of essential oil components between greenhouse and field conditions was also done by [21] in *D. moldavica*. They reported Geranyl acetate, Geraniol, Methyl Citronellate, Geranial, and Neral as the main identified components, which is consistent with our results. They concluded that geranyl acetate percentage was higher in the field than in the greenhouse. In our experiment, geranyl acetate content was higher under greenhouse conditions compared to *in vitro* culture. Therefore, the percentage of essential oil components varied depending on environmental factors and cultivation methods.

Chemotypes

Four chemotypes were recognized under *in vitro* culture, with Karaj (2) and Karaj (3) population showing a geranial/geraniol chemotype, and Karaj (1) and Hamedan (1) populations demonstrating a Geranial/Geraniol/Geranyl acetate chemotype. In contrast, all population of *D. moldavica* grown under greenhouse conditions represented a Neral/Geraniol/Geranial/Geranyl acetate chemotype.

Analysis of variance

Statistical analysis according to Completely Randomized Design (CRD) demonstrated that there are significant differences among population on the two methods cultures just for nine out of 18 combinations of the measured essential oils in the greenhouse ($P< 0.05$ and $P< 0.01$) and 10 out of 12 combinations of the measured essential oils in the *in vitro* culture ($P< 0.05$ and $P< 0.01$) (Table 4-5). According to the results of the greenhouse, effect of population on α-terpinene, Neral, Geraniol, Geranial, Methyl Geranat, Neryl acetate, Geranyl acetate and E-caryophyllene were significant at $P< 0.01$. Also the results of *in vitro* culture showed that effect of population on almost all essential oils except Neral acetate and E-caryophyllene were significant at $P<0.01$. These differences in the volatile composition can be attributed to genetic background, environmental factors, cultivation conditions, and various plant populations [29-31, 8]. Comparison mean of essential oils composition obtained from *in vitro* culture and greenhouse plants of *D. moldavica* species in different population is shown in Table 6-7. In *in vitro* propagation method the mean value of α-terpineol, Neral, Geraniol, Geranial, methyl Geranate, Geranyl compounds were high as in similar population from greenhouse culture method. Also, the mean value of production of compounds such as Linalool and Geranyl acetate were higher in greenhouse culture and were significantly different from *in vitro* culture.

In the greenhouse: the mean value of α-terpinene was varied from 0.04% in Hamedan (2) region to 0.57% in Karaj (2) region. The mean value of Neral was varied from 4.51% in Hamedan (2) region to 21.15% in Karaj (2) region. The mean value of Graniol was varied from 2.07% in Hamedan (2) region to 16.70% in Karaj (3) region. The mean value of Granial was varied from 6.96% in Hamedan (2) region to 34.74% in Hamedan (1) region. The mean value of Methyl Geranate was varied from 0.00% in Hamedan (2) region to 1.07% in Hamedan (1) region. The mean value of Geranyl acetate was varied from 1.08% in Hamedan (2) region to 24.75% in Karaj (1) region (Table6).

In vitro culture: the mean value of α-terpineol was varied from 0.35% in Karaj (1) region to 0.95% in Karaj (3) region. The mean value of Neral was varied from 0.06% in Hamedan (1) region to 19.25% in Karaj (1) region. The mean value of Graniol was varied from 23.56% in Karaj (1) region to 34.32% in Karaj (3) region. The mean value of Granial was varied from 31.53% in Karaj (1) region to 56.02% in Karaj (2) region. The mean value of Methyl Geranate was varied from 0.00% in Karaj (3) region to 0.26% in Karaj (2) region. The mean value of Geranyl acetate varied from 3.68% in Karaj (2) region to 16.6% in Karaj (1) region (Table7).

Cluster Analysis

Using principal components analysis (PCA) the first five independent components accounted for about 92.92% of total variation. The first component emphasized α-terpinene, Limonen, Linalool, cis-chrysanthenol and Neral which had the highest coefficients of Eigen vectors and were important essential oils for classification of populations with about 52.62% of total variation. The second component emphasized Geranial, Methyl Geranate, Neryl acetate and Geranyl acetate values which had the highest coefficients of Eigen vectors and were important essential oils for classification of population

with about 13.83% of total variation. The third component emphasized α-phellandrene and E-caryophyllene values which had the highest coefficients of Eigen vectors and were important essential oils for classification of populations with about 12.34% of total variation. The fourth component emphasized p-cymene, Geraniol and Germacrene D values which had the highest coefficients of Eigen vectors and were important essential oils for classification of populations with about 8.05% of total variation. The fifth component emphasized γ-terpinene, Terpineolene and α-terpineol values which had the highest coefficients of Eigen vectors and were important essential oils for classification of populations with about 6.07% of total variation (Table 8). Grouping of studied population was based on their essential oils compound (Fig. 1, Table 9).

Table 1 Comparison of essential oil percentage among populations of *D. moldavica* under greenhouse condition and *in vitro* culture

Population code	Latitude	Longitude	Altitude	*In vitro* culture	Greenhouse
Karaj (1) (1089)	35 51 12	50 53 34	1261	0.04	0.15
Karaj (2) (3429)	35 48 16	50 59 10	1325	0.08	0.27
Karaj (3) (909)	35 47 60	51 0 48	1435	0.06	0.16
Hamedan (1) (14336)	34 46 10	48 30 0	1870	0.05	0.15
Hamedan (2) (1613)	34 46 10	48 30 0	1770	0.1	0.15
Isfahan (18173)	32 28 26	51 34 48	1628	-	0.13

Table 2 Mean chemical component of essential oil in populations of *D. moldavica* under *in vitro* condition.

Chemical compound		RI (AI) Adams, 2017	Karaj (1) (1089)	Karaj (2) (3429)	Karaj (3) (909)	Hamedan (1) (14336)
1	p-cymene	1028	0.31	0.12	0.16	t
2	Linalool	1100	0.22	t	t	0.17
3	Cis-chrysanthenol	1160	0.23	0.65	0.69	0.59
4	α-terpineol	1190	0.35	0.93	0.95	0.82
5	Neral	1242	15.25	2.59	1.28	0.06
6	Geraniol	1256	23.56	31.43	34.32	32.71
7	Geranial	1270	31.53	56.12	50.27	47.12
8	Methyl geranate	1324	0.22	0.26	t	0.21
9	Neryl acetate	1359	0.31	0.5	0.57	1.53
10	Geranyl acetate	1379	16.60	3.68	9.22	13.67
11	E-caryophyllene	1417	t	0.6	0.6	1.16
12	germacrene D	1480	2.15	0.26	0.24	0.15
Total			90.73	97.14	98.3	98.18
Monoterpene			0.31	0.12	0.16	-
Oxygenated monoterpene			71.14	91.72	87.51	81.47
Ester			17.13	4.44	9.79	15.41
Sesquiterpene			2.15	0.86	0.84	1.31

t: trace < 0.05; RI: retention indices in elution order from DB-5 column

The results showed that different population of *D. moldavica* contain of different culture methods have been grouped in separate cluster. By cutting the dendrogram resulting from cluster analysis by Average method with cophenetic correlation coefficient (r= 0.95) with a metric distance of 4.68, the population was classified into five groups. Hamedan (1) (14336T), Karaj (2) (3429T) and Karaj (3) (909T) population obtained from in-vitro culture together are classified in a separate group due to high level of Geranial, Neryl acetate compounds. Karaj (1) (1089T) population obtained from in-vitro culture is classified in a separate group due to high level of Linalool,Neral, methyl Geranate and Geranyl acetate compounds.Hamedan1(1613G) population obtained from green house culture is classified in a separate group due to high level of cis- chrysanthenol, Neryl acetate and E-caryophyllene compounds. Karaj (1)(1089G) population obtained from green house culture is classified in a separate group due to high level of Neral, Geraniol, Geranial, methyl Geranate, Neryl acetate, Geranyl acetate, Germacrene D and a Unknown compounds. Finally, the remaining four population cultivated in the green house were placed in an independent group (Fig.1, Table 9).

The diagram of populations' dispersion, based on the first two components, showed that the population separated into five groups, which completely fits with results obtained through the grouping analysis by Average's method (Fig. 2). This study confirms that the essential oils of plants such as *D. moldavica* species in different methods of cultures (*in vitro* or greenhouse) and various individuals are different. Depending on what kind of essential oils compound we expect to produce by the plant; different methods of reproduction should be used. For example, for the production of Geraniol and Geranial, it is better to collect this plant from a suitable area and propagate it by *in vitro* culture.

Table 3 Mean chemical component of essential oil in population of *D. moldavica* under greenhouse condition

No.	Compound	RI (AI) (Adams, 2017)	Hamedan (1) (14336)	Hamedan (2) (1613)	Karaj (1) (1089)	Karaj (2) (3429)	Karaj (3) (909)	Isfahan (18173)
1	α-phellandrene	1002	0.04	t	t	0.26	0.35	t
2	α-terpinene	1014	0.21	0.23	0.13	0.69	0.64	0.18
3	p-cymene	1028	0.37	0.42	0.24	0.23	0.63	0.26
4	Limonene	1032	0.38	0.61	0.26	0.32	0.45	0.32
5	γ-terpinene	1065	0.28	1.08	0.19	0.43	0.94	0.32
6	Terpineolene	1091	1.10	t	0.62	1.24	0.69	0.71
7	Linalool	1100	1.72	1.58	0.80	1.75	1.33	1.35
8	Cis-chrysanthenol	1160	0.09	t	0.08	t	0.09	0.11
9	α-terpineol	1190	2.59	2.45	1.43	2.82	2.00	2.98
10	Neral	1242	19.38	27.05	14.70	25.38	18.18	17.40
11	Geraniol	1256	15.24	12.45	14.50	11.54	16.71	15.44
12	Geranial	1270	34.74	41.75	29.56	38.76	31.68	32.59
13	methyl geranate	1324	1.08	t	0.83	0.42	0.38	0.45
14	neryl acetate	1359	0.77	0.81	1.48	0.46	1.47	1.93
15	Unknown	-	t	t	1.70	t	t	t
16	Geranyl acetate	1379	15.74	6.48	24.75	12.28	17.66	19.49
17	E-caryophyllene	1417	0.04	0.04	t	t	0.04	0.03
18	Germacrene D	1480	0.05	0.05	0.08	t	0.07	0.07
Total			93.82	95	91.35	96.58	93.31	93.63
Monoterpene			2.38	2.34	1.44	3.17	3.7	1.79
Oxygenated Monoterpene			73.76	85.28	61.07	80.25	69.99	69.47
Ester			17.59	7.29	28.76	13.16	19.51	21.87
Sesquiterpene			0.09	0.09	0.08	0	0.11	0.1

t: trace< 0.05; RI:retention indices in elution order from DB-5 column

Table 4 The results of analysis of variance for essential oil data of *D. moldavica* under greenhouse condition based on CRD design

Source of variation	Freedom degree	α-phellandrene	α-terpinene	p-cymene	Limonene	γ-terpinene	Terpineolene	Linalool	Cis-chrysanthenol	α-terpineol	Neral
Replication	5	0.06 ^{ns}	0.02 **	0.01 ^{ns}	0.01*	0.0 1^{ns}	0.04 ^{ns}	0.04 ^{ns}	0.01 ^{ns}	0.1 ^{ns}	1.23 **
Population	5	0.01*	0.02 **	0.01 ^{ns}	0.007 ^{ns}	0.01 ^{ns}	0.05 ^{ns}	0.007 ^{ns}	0.001 ^{ns}	0.1 3 ^{ns}	0.77 **
Error	-	0.003	0.004	0.0006	0.0004	0.01	0.007	0.01	0.001	0.01	0.09
C. V %	-	6.76	7.42	8.97	7.11	14.08	8.52	9.12	4.25	11.80	16.67

ns: non-significant, **: significant at 1% ,* : significant at 5%

Continued table 4 The results of analysis of variance for essential oil data of *D. moldavica* under greenhouse condition based on CRD design

Source of variation	Freedom degree	Geraniol	Geranial	Methyl geranate	Neryl acetate	Unknown	Geranyl acetate	E-caryophyllene	germacrene D
Replication	5	0.1 ^{ns}	0.35 *	0.02 *	0.01 ^{ns}	0.02 ^{ns}	0.02 ^{ns}	0.01 ^{ns}	0.001 **
Population	5	0.85 **	1.52 **	0.04 **	0.09 **	0.02 ^{ns}	1.24 **	1.24 **	0.0003 ^{ns}
Error	-	0.1	0.13	0.008	0.01	0.025	0.090	0.09	0.0003
C. V %	-	18.66	17.26	9.65	12.90	18.45	16.4	16.39	2.04

Table 5 The results of analysis of variance for essential oil data of *D. moldavica* under *in vitro* condition based on CRD design

ns: non-significant, **: significant at 1% ,* : significant at 5%

Source of variatio	Freedom degree	p-cymene	Linalool	Cis-chrysanthenol	α-terpineol	Neral	Geraniol	Geranial	Methyl geranate	neryl acetate
Replication	2	0.0001 ^{ns}	0.0002 ^{ns}	0.003 ^{ns}	0.003 ^{ns}	0.0005 ^{ns}	0.0004 ^{ns}	0.005 ^{ns}	0.005^{ns}	0.08 ^{ns}
Population	3	0.034 **	0.030 **	0.069 **	0.105 **	4.54 **	0.84 **	0.182 **	0.029 *	0.22 ^{ns}
C. V %	-	0.001	0.0002	0.0026	0.004	0.005	0.003	0.123	0.006	0.062
Error	-	1.65	2.65	5.68	5.95	4.08	1.29	2.46	12.09	25.40

Continued table 5 The results of analysis of variance for essential oil data of *D. moldavica* under *in vitro* condition based on CRD design

Source of variation	Freedom degree	Geranyl acetate	E-caryophyllene	Germacrene D
Replication	2	0.095 ^{ns}	0.082 ^{ns}	0.01 ^{ns}
Population	3	1.10 **	0.23 ^{ns}	0.808 **
C. V %	-	0.041	0.060	0.004
Error	-	6.83	27.56	6.55

Table 6 Mean comparison of chemical compounds of essential oil in population of *D. moldavica* under greenhouse condition.

No	Compound	RI (retention index)	Karaj (1) (1089)	Karaj (2) (3429)	Karaj (3) (909)	Hamedan (1 (14336)	Hamedan (2 (1613)	Isfahan (18173)
1	α-phellandrene	1002	0.00 b	0.21 ab	0.34 a	0.37b	0.00 b	0.00 b
2	α-terpinene	1014	0.13 b	0.57 a	0.64 a	0.21 b	0.04 b	0.18 b
3	p-cymene	1020	0.24 ab	0.23 ab	0.63 a	0.37 ab	0.07 b	0.26 ab
4	Limonene	1024	0.26 ab	0.26 ab	0.45 a	0.38a	0.10 b	0.32 ab
5	γ- terpinene	1054	0.19 a	0.36 a	0.94 a	0.28 a	0.18 a	0.32 a
6	Terpineolene	1086	0.62 a	1.03 a	0.69 a	1.1 a	0.00 b	0.71 a
7	Linalool	1088	0.79 b	1.46 ab	1.33 ab	1.72 a	0.26 c	1.34 ab
8	Cis-chrysanthenol	1160	0.08 a	0 a	0.08 a	0.09a	0.00 a	0.11 a
9	α-terpineol	1186	1.43 a	2.35 a	1.99 a	2.59 a	0.41b	2.97 a
10	Neral	1235	14.70 a	21.15 a	18.17a	19.38 a	4.51 b	17.39 a
11	Geraniol	1249	14.49 a	9.61 a	16.70 a	15.24 a	2.07 b	15.43 a
12	Geranial	1264	29.56 a	32.29 a	31.68 a	34.74 a	6.96 b	32.59 a
13	methyl geranate	1322	0.82 ab	0.35 bc	0.38 bc	1.07 a	0.00 c	0.45 b
14	neryl acetate	1359	1.48 a	0.38 bc	1.47 a	0.77 ab	0.12 c	1.92 a
15	Unknown	-	1.69 a	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
16	Geranyl acetate	1379	24.75 a	10.23 b	17.65 ab	15.73 ab	1.08 ab	19.49 ab
17	E-caryophyllene	1417	0.12 a	0.02 b	0.038 ab	0.04 ab	0.00 c	0.030 ab
18	Germacrene D	1480	0.08 a	0.00 a	0.07 a	0.05 a	0.00 a	0.07 a

Table 7 Mean comparison of chemical compound of essential oil in population of *D. moldavica* under *in vitro* condition. In each column the same letters are not significantly different at P≤0.05

No	Chemical compound	RI (retention index)	Karaj (1) (1089)	Hamedan (1) (14336)	Karaj (2) (3429)	Karaj (3) (909)
1	p-cymene	1028	0.31 a	0.00 d	0.12 c	0.16 b
2	Linalool	1100	0.22 a	0.17 b	0.00 c	0.00 c
3	Cis-chrysanthenol	1160	0.23 b	0.59 a	0.65 a	0.69 a
4	α-terpineol	1190	0.35 b	0.82 a	0.93 a	0.95 a
5	Neral	1242	19.25 a	0.06 d	2.59 b	1.28 c
6	Geraniol	1256	23.56 b	32.71 a	31.43 a	34.32 a
7	Geranial	1270	31.53 b	47.12 a	56.02 a	50.27 a
8	Methyl geranate	1324	0.22 a	0.21 a	0.26 a	0.00 b
9	Neryl acetate	1359	0.31 b	1.53 a	0.50 ab	0.57 ab
10	Geranyl acetate	1379	16.6 a	13.67 ab	3.68 c	9.22 b
11	E-caryophyllene	1417	0.00 b	1.16 a	0.6 ab	0.6 ab
12	Germacrene D	1480	2.15 a	0.15 b	0.26 b	0.26 b

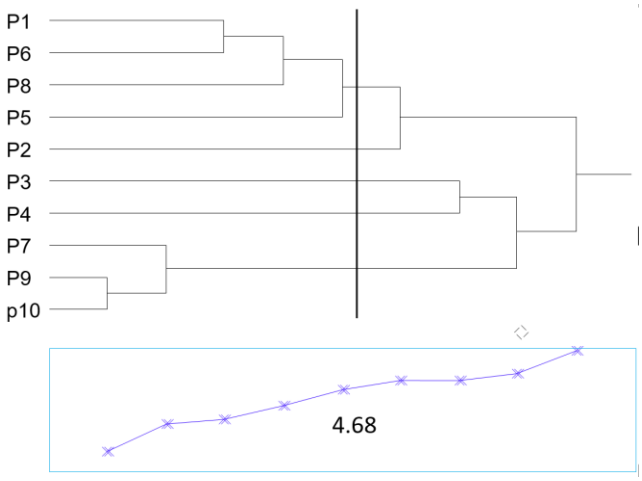

Fig. 1 Dendrogram of 10 populations of *D. moldavica* L. by analyzing 12 essential oils compound from *in vitro* plantlet and greenhouse plants using Average cluster analysis method.
Cophenetic correlation *r* = 0.95. (G: greenhouse; T: *in vitro*).
P1: Isfahan- greenhouse (G18173); P2: Karaj (1)-greenhouse (G1089); P3: Karaj (1)-tissue culture (T1089),
P4: Hamedan (1)- greenhouse (G1613), P5: Karaj (3)-greenhouse (G909); P6: Hamedan (1)- greenhouse (G14336);
P7: Hamedan (1)-Tissue culture (T14336); P8: Karaj (2) -greenhouse (G3429); P9: Karaj (2)-tissue culture (T3429),
P10: Karaj (3)- tissue culture (T909)

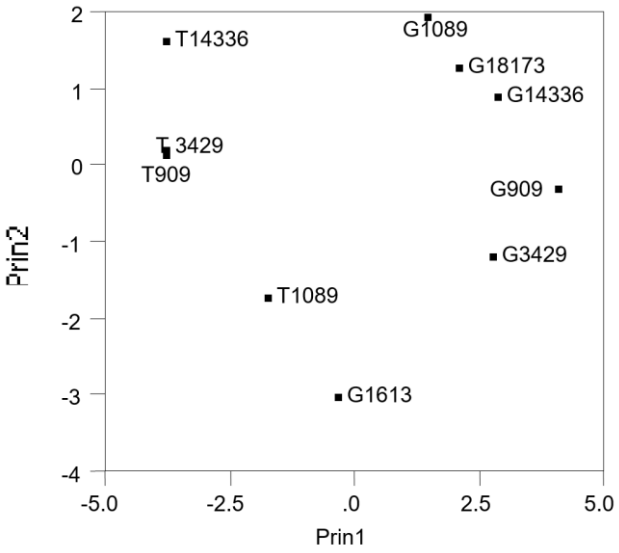

Fig. 2 Plot obtained by principle component analysis of 10 population of *D. moldavica* based on 12 essential oils compositions from *in vitro* plantlet and greenhouse plants.
P1: Isfahan- greenhouse (G18173); P2: Karaj (1)-greenhouse (G1089); P3: Karaj (1)-tissue culture (T1089),
P4: Hamedan (1)- greenhouse (G1613), P5: Karaj (3)-greenhouse (G909); P6: Hamedan (1)- greenhouse (G14336);
P7: Hamedan (1)-Tissue culture (T14336); P8: Karaj (2)-greenhouse (G3429); P9: Karaj (2)-tissue culture (T3429),
P10: Karaj (3) - tissue culture (T909)

Table 8 Eigenvectors from the first five principal components for 12 essential oils to classify 10 populations of *D. moldavica* Boiss.

Compound	First	Second	Third	Fourth	Fifth
α-phellandrene	0.21	-0.12	0.40	0.25	-0.16
α-terpinene	0.27	-0.08	0.31	0.1	-0.07
p-cymene	0.24	-0.05	0.11	0.46	-0.03
Limonene	0.31	-0.06	0.07	-0.08	0.07
γ- terpinene	0.26	-0.11	0.26	0.08	-0.33
Terpineolene	0.28	0.17	0.04	-0.09	0.31
Linalool	0.31	0.07	0.06	-0.11	0.22
Cis-chrysanthenol	-0.26	0.18	0.22	0.10	0.07
α-terpineol	0.25	0.21	0.15	-0.19	0.28
Neral	0.30	-0.07	-0.13	0.14	0.22
Geraniol	-0.25	0.26	0.19	0.30	0.11
Geranial	-0.20	0.31	0.29	0.15	0.28
Methyl geranate	0.19	0.35	-0.21	-0.01	0.31
Neryl acetate	0.10	0.48	0.05	0.04	-0.39
Geranyl acetate	0.02	0.39	-0.25	0.37	-0.13
E-caryophyllene	-0.25	0.24	0.27	-0.07	-0.08
Germacrene D	-0.10	-0.21	-0.27	0.60	0.21
EigenValue	9.47	2.49	2.22	1.45	1.09
Percent	52.62	13.83	12.34	8.05	6.07
Cum Percent	52.62	66.46	76.80	86.85	92.92

Table 9 Hierarchical Clustering, Method =Average Clustering

Number of clusters	Distance	Leader	Joiner
9	1.272932506	T 3429(P9)	T 909(P10)
8	2.6864071543	T 14336(P7)	T 3429(P8)
7	3.033553791	G 18173(P1)	G 14336(P6)
6	3.7367745267	G 18173(P1)	G 3429(P8)
5	4.6811803674	G 18173(P1)	G 909(P5)
4	5.1691920458	G 18173(P1)	G 1089(P2)
3	5.1784303512	T1089(P2)	G 1613(P4)
2	5.5630604525	T 1089(P2)	T 14336(P7)
1	6.8751498448	G 18173(P1)	T 1089(P3)

This study also introduced new chemotypes of *D. moldavica* with high levels of Geranial (Karaj (2) and Karaj (3)) compound, which were propagated by *in vitro* method. In greenhouse condition we introduced new chemotypes of *D. moldavica* with high level of Neral/Geraniol/Geranial/Geranyl acetate in Karaj (2), Karaj (3), Hamedan (1) and Karaj (1), respectively.

DISCUSSION

The study of secondary metabolites in plants is of major interest in the areas of plant biotechnology and phytochemistry [32]. Comparison of essential oils composition between field plants and *in vitro*-cultured plants provided variable results [33]. In some studies, such as *Salvia fruticosa* [34] and *Dracocephalum kotschyi* [24] variation in chemical composition of essential oils was observed. According to our result, the main compound of essential oils differed between in-vitro plantlets and greenhouse plants. In addition, the percentage of chemical compounds varied between *in vitro* plantlets and greenhouse plants. The amount of Cis-chrysanthenol, Geraniol, Geranial, E-caryophyllene and Germacrene was increased in *in vitro* plantlets. However, a high level of Neral and Geranyl acetate were found under greenhouse conditions. The variation in chemical compound of essential oils between *in vitro* plantlets and greenhouse plants is related to different environment of *in vitro* and greenhouse culture condition. Under *in vitro* conditions, the relative humidity was high. In addition, auxin and cytokinin hormones stimulate secondary metabolite accumulation [35] and rejuvenation of shoots causes accumulation of volatile compounds [36]. However, in greenhouse conditions, the relative humidity was low, light intensity was high, and non-sterile condition caused monoterpene accumulation [37]. In contrast to the results of this study, the chemical composition of essential oils between *in vitro* culture and field condition was reported to be similar in plants of *Lavandula viridis* [38], *Lavandula pedunculata* [39], *Minostachys mollis* [40], *Mentha spicata* [41], *T. mastichina* [42], *Varronia curassavica* [43]. According to Andrys and Kulpa [44] the chemical constituent of borneol was dominant in both in-vitro plantlets and *in vivo* plants of *Lavandula angustifolia.* However, the significant difference was found between *in vitro* and field conditions. The essential oils composition of intact plant and *in vitro* shoots and adventitious roots of *Caryopteris* species varied significantly which is in agreement with our results [45]. Similar results mentioned in relation to chemical composition of *Teucrium scorodonia* ssp. Scorodonia [43]. The main essential oils obtained in this research were almost the same of the main components of *D. moldavica* essential oil that have been identified by other researchers [3, 9, 20, 21]. As a result, the cultivation method, whether conventional or in-vitro culture, can significantly influence the essential oil content in plants. Also, *in vitro* plantlet production of *D. moldavica* is important for commercial production of Geraniol and Geranial chemotype. Variation in chemical composition was observed among populations of this species. This difference may be due to the genetic variation among population of this species [46]. According to cluster analysis, the essential oils compound diversity displayed by *in vitro* plantlets and greenhouse plants can be attributed to the geographical origin of populations and is affected by culture conditions. It should be mentioned that the chemical composition and percentage are affected by genotype, culture conditions, environmental factors, and their interaction.

CONCLUSION

In this study, chemical compounds and their percentage were compared between *in vitro* plantlets and greenhouse plants of *D. moldavica* with different population. The main compounds identified in various population were Geraniol, Geranial, Geranyl acetate and Neral. The conducted protocol of this experiment would be applied to an investigation of volatile chemical compounds of medicinal plants especially Lamiaceae family.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the financial support given by the RIFR for this research.

REFERENCES

1. Maham M., Akbari H., Delazar A. Chemical Composition and Antinociceptive Effect of the Essential Oil of *Dracocephalum moldavica* L. Pharmaceut. Sci. 2013; 18(4), 187-192.
2. Nikitina A.S., Popva O.I., Ushakova L.S., Chumakova V.V., Ivanova L. Studies of the essential oil *Dracocephalum moldavica* L. cultivated in the Stavropol reigion. Pharmaceut. Chem. J. 2008; 42 (8): 35-39.
3. Said-Al Ahl H.A.H., Sabra A.S., El Gendy A.N.G., Aziz E.E., Tkachenko K.G. Changes in content and chemical composition of *Dracocephalum moldavica* L. essential oil at different harvest dates. J. Med. Plants Stud. 2015; 3(2): 61-64.
4. Aćimović M., Sikora V., Brdar-Jokanović M., Kiprovski B., Popović V. Koren A., Puvača N. *Dracocephalum moldovica*: Cultivation, Chemical composition and biological activity. J. Agron. Technol. Engineer. Manag. 2019; 2(1): 153-167.
5. Jamzad Z. Flora of Iran. Research Institute of Forest and Rangelands publication. 2012; 1066p.
6. Dastmalchi K., Damien-Dorman H.J., Laakso I., Hiltunen R. Chemical composition and antioxidative activity of Moldavian balm (*Dracocephalum moldavica* L.) extracts. LWT 2007; 40: 1655–1663.
7. Zargari A. Medicinal Plants (vol 4). Tehran university publication. 1997; 969p.
8. Sonboli A., Salehi P., Gharehnaghadeh S. Chemical variability in the essential oil composition of *Salvia hypoleuca*. An

endemic species from Iran. J. Essent. Oil Res. 2016; 28 (5): 421-427.
9. Ehsani A., Alizadeh O., Hashemi M., Afshari A., Aminzare M. Phytochemical, antioxidant and antibacterial properties of Melissa officinalis and *Dracocephalum moldavica* essential oils. Vet. Res. Forum 2017; 8 (3) 223-229.
10. A´cimovi´c M., Šovljanski O., Šeregelj V., Pezo, L., Zheljazkov V.D., Ljuji´C J., Tomi´C. A., Cetkovi´c G., Canadanovi´c-Brunet J., Miljkovi´c A. Chemical Composition, Antioxidant, and Antimicrobial Activity of *Dracocephalum moldavica* L. Essential Oil and Hydrolate. Plants 2022; 11: 941.
11. Olha B., Antonina P., Mariia Sh. Chemical compositions and sedative activities of the *Dracocephalum moldavica* L. and *Ocimum americanum* L. essential oil. Pharmacol. Online 2021; 2: 179-187.
12. Jiménez Zúñiga M.I., Hurtado Mariles A.J., Castrejón Flores J.L., Mondragón Herrera J.A., Ramírez Sotelo M.G., Cerón Montes G.I. Antidepressant-Like Effects of *Dracocephalum moldavica* L. in Mouse Models of Immobility Tests. Pharmacog. J. 2019; 11(5): 976-983.
13. Mafakheri S., Hallaj R., AsghariB. Study on phytochemical and antioxidant properties of dragonhead (*Dracocephalum moldavica* L.) seed oil, ethanoland aqueous extracts. Iranian J. Med. Aromatic Plant Res. 2022; 38 (1): 176-189.
14. Racz G., Tibori G., Csedo C. Composition of volatile oil from *Dracocephalum moldavica* L. Farmacia 1978;26: 93-96.
15. Holm Y., Galambosi B., Hiltunen R. Variation of the main terpenes in dragonhead *(Dracocephalum moldavica* L.) during growth. Flav. Fragr. J. 1988; 3 (3): 113-115.
16. Omidbaigi R., Borna F. Borna T., Inotai, K. Sowing dates affecting on the essential oil content of dragonhead (*Dracocephalum moldavica* L.) and its constituents. J. Essent. Oil Bear. Plants 2009; 12(5): 580-585.
17. Mafakheri S., Omidbaigi R., Sefidkon F., Rejali, F. Influence of biofertilizers on the essential oil content and constituents of *Dracocephalum moldavica* L. J. Essent. Oil Bear. Plants 2012; 15(1): 58-65.
18. Aziz E.E., El-Sherbeny S. E. Effect of some micro-nutrients on growth and chemical constituents of *Sideritis montana* as a new plant introduced into Egypt. Arab. Univ. J. Agric. Sci. Ain Shams Univ. Cairo. 2003; 12: 391-403.
19. Kakasy A.Z., Lemberkovics E., Simandi B., Lelik L., Hethelyi E., Antal I., Szoke E. Comparative study of traditional essential oil and supercritical fluid extracts of moldavian dragonhead (*Dracocephalum moldavica* L.). Flav. Fragr. J. 2006; 21(4): 598-603.
20. Yousefzadeh S., Modarres-Sanavy S.A., Sefidkon F., Asgarzadeh A., Ghalavand A., Sadat-Asilan K. Effects of azocompost and ureaon the herbage yield and contents and compositions of essential oils from two genotypes of dragonhead (*Dracocephalum moldavica* L.) in two regions of Iran. Food Chem. 2013; 138(2-3): 1407-1413.
21. Alaei S.h., Mahna N. Comparison of essential oil composition in *Dracocephalum moldavica* in green house and field. TEOP 2013;16 (3): 346-351.
22. Chu S.h., Liu S.h.L., Liu Q. Zh., Liu, Zh. L., Du S.h. Composition and toxicity of Chinese *Dracocephalum moldavica* (Labiatae) essential oil against two grain storage insects. J. Med. Plants Res. 2011; 5(18): 4621-4626
23. Murashige T., Skoog F.A. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant 1962; 15: 473-497.
24. Allahverdi-Mamaghani B., Hesamzadeh Hejazi S.M., Mirza M., Movafeghi A. Comparison of Essential Oils Composition Between in-vitro Plantlets and Greenhouse Plants from Various Populations of *Dracocephalum kotschyi* Boiss. J. Med. Plants and By-prod. 2022; 2: 219-230.
25. Brithish Pharmacopeia, vol. 2, Appendix XI F, HMSO, London. 1988,p. A138.
26. Adams R.P. Identification of essential oils by Ion trap Mass Spectroscopy. Academic Press. 2017; San Diego, CA.
27. Davies N.W. Gas chromatoghraphic retention indices of monoterpenes and sesquiterpenes on methylsilicone and carbowax 20M phases. J. Chromatogr. 1990; 503: 1-24.
28. Shibomoto T. Retention indices in essential oil analysis, In: Sandra P, and Bicchi C (eds.) Capillary Gas Chromathography in essential oil analysis. Editors New York. Alfred Heuthig Verlag. 1987; pp. 259-274.
29. Ghasemi Pirbalouti A., Nourafcan H., Solyamani-Babadi E., Variation in chemical composition and antibacterial activity of essential oils from Bakhtiari Savory (*Satureja bachtiarica* Bunge.). TEOP 2017; 20(2): 474-484.
30. Karami, A., Bohlooli, A., Essential oil chemical diversity of Ducrosia anethifolia (DC) Boiss accession from Iran. TEOP 2017; 20 (5): 1342-1348.
31. Bajalan I., Rouzbahani R., Ghasemi Pirbalouti A., Maggi F. Quali-quantitative variation of essential oil from Iranian rosemary (*Rosmarinus officinalis* L.) accessions according to environmental factors. J. Essential Oil Res. 2017; 1-9.
32. Otroshy M., Moradi K. Microporpagation of medicinal plant Dracocephalum kotschyi Boiss. Via nodal cutting technique. J. Medicinal Plants Res. 2011; 5: 5967-5972.
33. Mendes M.D., Figueriredo A.C., Oliveeria M.M., Trindade H. Essential oil production in shoot cultures versus field –grown plants of Thymus caespititius. Plant Cell Tissue Org. Cult. 2013; 113: 341-351.
34. Arikat N.A., Jawad F.M., Karam N.S., Shibil R.A. Micropropagation and accumulation of essential oil in wild sage (*Salvia fruticosa* Mill.). Sci Hortic-Amsterdam. 2004; 100: 193-202.
35. Sudria C., Pinol M.T., Palazon J., Cusido R.M., Vila R., Morales C., Bonfill M., Canigueral S. Influence of plant growth regulators on the growth and essential oil content of cultured Lavandula dentate plantlets. Plant Cell Tissue Organ Cult. 1999; 58: 177-184.
36. Al-Qudah T.S., Shibil R.A., Alali F.Q. In-vitro propagation and secondary metabolites production in wild germander. In-vitro Cell Dev. B-Plant 2011; 47: 496-505.
37. Julianijr H.R., Koroch A.R., Juliani H.R., and Trippi V.S. Micropropagation of *Lippia Junelliana* (Mold.) Tronc. Plant Cell Tissue Organ Cult. 1999; 59: 175-179.
38. Nogueira J.M.F., Romano A. Essential oils from micropropagated plants of *Lavandula viridis*. Phytochem. Anal. 2002; 13: 4-7.
39. Zuzarte M.R., Dinis A.M., Cavaleiro C., Salgueiro L.R., Canhoto J.M. Trichomes, essential oil and in-vitro propagation of *Lavandula pedunculata* (Lamiaceae). Ind. Crop Prod. 2010; 32: 580-587.
40. Chebel A.V., Koroch A.R., Juliani H.R., Trippi V.S. Micropropagation of *Minthostachys mollis* (H. B. K) Grieseb

and essential oil composition of clonally propagated plants. In-vitro Cell Dev. B- Plant 1998; 34: 249-251.

41. Hirata T., Murakami S., Ogihara K., Suga T. Volatile monoterpenoid constituents of the plantlets of *Mentha spicata* produced by shoot tip culture. Phytochemistry 1990; 29: 493-495.
42. Fraternale D., Giamperi L., Ricci D., Rocchi M.B.L., Guidi L., Epifano F., Marcotullio M.C. The effect of triacontanol on micropropagation and on secretory system of *Thymus mastichina*. Plant Cell Tissue Organ Cult. 2003; 74: 87-97.
43. Makowczyn´ska J., Sliwinska E., Kalemba D., Pia¸tczak E., Wysokin´ska H. In-vitro propagation, DNA content and essential oil composition of *Teucrium scorodonia* L. ssp. Scorodonia. Plant Cell Tissue Organ Cult. 2016; 127: 1-13.
44. Andrys D., Kulpa D. In-vitro propagation affects the composition of narrow - leaved lavender essential oil. Acta Chromatographica 2017. doi: 10.1556/1326.2017.00317.
45. Luczkiewicz M., Jesionek A., Kokotkiewicz A., Migas P., Mardarowicz M., Szreniawa-Sztajnert A., Zabiegala B., Bucinski A. Production of essential oils from in-vitro cultures of *Caryopteris* species and comparison of their concentrations with in-vivo plants. Acta Physiol. Plant 2015; 37-58.
46. Skoula M., Abbes J.E., Johnson C.B. Genetic variation of volatiles and rosmarinic acid in populations of *Salvia fruticosa* Mill growing increte. Biochem. Syst. Ecol. 2000; 28: 551-561.