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Essential oil Content and Composition of *Dracocephalum Moldavica* under Different Irrigation Regimes

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Abstract
Dracocephalum moldavica L. is an annual species of Lamiacea family whose essential oil is widely used in medicinal, food, cosmetic and health industries. Drought stress is a major factor limiting plants growth and yield. The main objective of this study was to evaluate the influence of irrigation regime on essential oil content and composition of *D. moldavica* L. The Irrigation regimes were 100%, 85%, 75% and 55% of field capacity (FC). The results showed that 53 essential oil components were identified in different irrigation regimes. Thirty-six, twenty-two, twenty and thirty components were identified at 100%, 85%, 75% and 55% of FC, respectively. The amounts of identified components were 99.4%, 96.6%, 97.8% and 99.9% at 100%, 85%, 75% and 55% of FC, respectively. The highest of main components were geranyl acetate, geraniol and geranial in all the irrigation regimes. The amount of oxygen-containing monoterpenes of essential oil were 81.6%, 94.5%, 91.8% and 92.0% at 100%, 85%, 75% and 55% of FC, respectively. Our results showed that essential oil content increased significantly by 3.4%, 13.8% and 27.6% at 85%, 75% and 55% of FC, respectively. Furthermore, the amount of oxygen-containing monoterpenes of *D. moldavicum* increased by 15.8%, 12.5% and 12.7% at 85%, 75% and 55% of FC, respectively, but oxygen-containing sesquiterpenes decreased by increasing the level of drought. In conclusion, our results indicated the advantage of drought for increasing essential oil content and oxygen-containing monoterpenes (OM), especially geranyl acetate, geraniol and geranial in *D. moldavica*.

Keywords: *Dragonhead*, Oil composition, Irrigation, Field capacity.

Introduction

Dragonhead (*Dracocephalum moldavica* L.) of the Lamiaceae family is a medicinal and aromatic plant whose essential oil is used in medicinal, food, cosmetic and health industries. This plant grows in mountainous parts of Iran. The aerial parts of plant contain essential oils that have antiseptic and antibacterial properties and are applied against stomach-ache and bloat (Omidbaigi, 2005). Geranyl acetate is a natural organic compound classified as a monoterpene with strong antioxidant effect (Quintans-Junior, 2013). Geraniol is a cyclic monoterpene-alcohol and acts as a natural antioxidant that can significantly inhibit cancer growth *in vitro* and *in vivo* conditions. It has been recognized that geranial can induce apoptosis in cancer cells (Cho, 2016). Carvacrol is the other major component with antibacterial and antifungal properties. The essential oil of *Dragonhead* has also antioxidant activity (Povilaityte, 2000). About 246 compounds (e.g. terpenoids, steroids, flavonoids, phenols, alkaloids, coumarins and

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cyanogenic glycosides) have been identified from the genus of *Dracocephalum*; among them terpenoids are the dominant compounds (Shatar, 2000). The content and composition of essential oil in medicinal plants vary and depend on plant origin, culture condition and environment (Mandal, 2015).

Water scarcity is a critical environmental issue that influences plant growth and yield. A previous study on *D. moldavica* has shown that water deficit decreases the plant height, leaf area, leaf numbers, leaf chlorophyll content, fresh and dry weight of root and shoot, root length, branch number, leaf relative water content and yield (Alaei, 2013a). Hassani (2006) explained that soil water stress decreased the growth and yield of moldavian balm, but no significant change was detected in its essential oil content (Hassani, 2006). The effect of water stress on yield has been previously reported by Simon et al., (1992) in basil; Ardakani (2007) in balm; Johnson (1995) in *Spanish thyme* and Safikhani (2007) in moldavian balm. It has been shown that environmental conditions as well as phenological stages can affect the yield of medicinal plants (Anwar, 2009). To the best of our knowledge, no information is available concerning the essential oil components of *D. moldavica* under different irrigation regimes. Hence, this work aims at investigating the influence of different irrigation regimes on essential oil of *D. moldavica*.

Materials and Methods

Plant material and experimental plan

This experiment was carried out in the greenhouse of the Research Station of the College of Agriculture, Islamic Azad University of Kermanshah, Iran (Langitude: 47˚02′72″ E, latitude: 47˚17′87″ Altitude of the sea surface: 1494m). The seeds used in this investigation were provided from Medicinal and Aromatic Plants, Department of Corvinus University in Budapest. Similar sized pots were filled with 3750 g sand and soil mixture. The physicochemical characteristics of the soil are shown in Table 1. Following germination to 6-8 leaf stage, irrigation regimes were applied. Irrigation regimes were used till rehabilitation of plants to full flowering stage (about three months). Irrigation regimes were selected based on different percentages of field capacity (FC) which was determined by pressure plate set. The amount of FC was 21%. The pots were irrigated based on their FC every day and the amount of water was adjusted by weighting the pots.

Table 1. Physicochemical characteristics of the soil used in the present experiments.

Characteristics	Results
Sand	58 (%)
Silt	20 (%)
Clay	22 (%)
Soil texture	S-C-L (sandy clay loam)
Available K	430 (mg/kg)
Available P	51 (mg/kg)
Total N	0.092 (%)
Organic C	0.92 (%)
Zn	2.5 (mg/kg)
Fe	6.5 (mg/kg)
Mn	4.8 (mg/kg)
Acidity (pH)	7.10
Electrical conductivity (EC)	1.8 (mmhos)
Field capacity	21%

Irrigation regimes were selected based on different percentages of FC including:

1. Control – full irrigation-100% FC (3896 g) (I1)
2. Low water stress -85% FC (3814 g) (I2)
3. Moderate water stress -75% FC (3759 g) (I3)
4. Sever water stress -55% FC (3650 g) (I4)

When plants were at full flowering stage, large foliar mounds with fresh flower stalks arising from the center of each plant were harvested with a sharp knife leaving about 5 cm above the ground surface. Each complete harvested sample was placed in a separate paper bag and then was air dried. Seven to 10 days was typically required to complete the drying process in room condition (25 °C and dry shade). Shade dried aerial parts of *D. moldavica* (40 g, three times) were subjected to hydro distillation for 4 h using a Clevenger-type apparatus according to producers outlined European Pharmacopoeia. The essential oil was analyzed using GC and GC/MS for identification and quantification of the components. GC analysis was performed using an Agilent 6890 gas chromatograph equipped with a HP-5 MS column (30 m x 0.25 mm, film thickness 0.25 micrometer). Oven temperature was held at 0 °C for 5 min and then was programmed to 240 °C at a rate of 3 °C per min; holding at the final temperature of 300 °C for 3 min. Injector and detector temperature were 290 °C, and helium was used as carrier gas with a linear velocity of 0.8 mL per min. GC-MS analysis was performed using an Agilent 5973 equipped with an ionization energy 70 eV with ionization temperature of 220 °C. The components of oil were identified by comparison of their mass spectra with those of a computer library or with authentic compounds and complied with those of authentic compounds or with data published in literature (Adams, 2001).

Statistical analysis

The experiment was carried out in a randomized complete block design with four irrigation regimes in four replications. The data were analyzed by MSTATC software and the means were compared by Duncan's test at the 0.05 level of confidence.

Results

The effect of irrigation regimes on essential oil content

In this experiment, essential oil content of dragonhead changed depending on irrigation regimes. Essential oil content of *D. moldavica* increased significantly with increasing the level of drought. The highest (0.37%) and lowest (0.29%) essential oil content were 55% FC and 100% FC of irrigation levels, respectively. Our results showed that essential oil content 3.4%, 13.8% and 27.6% increased significantly ($p<0.05$) at 85%, 75% and 55% of field capacity, respectively (Fig. 1).

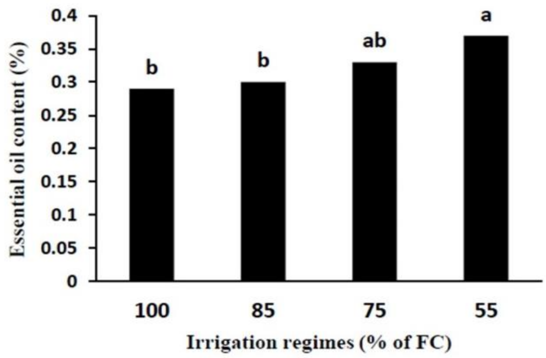

Fig. 1. Effect of different irrigation regimes (% of field capacity; FC) on essential oil content of *D. moldavica*. Means in each column with the same letter are not significantly different at 5% level; DNMRT.

Effect of irrigation regimes on essential oil components

Table 2 shows the results of GC/MS analysis of essential oil in *D. moldavica* under different irrigation regimes. It showed that not only the content of essential oil but also its constituents were affected by irrigation regimes. The numbers of identified components of essential oil in plants at each level of drought were verified. The results indicated that 53 components were identified from essential oil of *D. moldavica* in different irrigation regimes. 36, 22, 20 and 30 components were identified at 100%, 85%, 75% and 55% of FC, respectively. There were eleven compounds at all irrigation regimes. Geranyl acetate and geraniol were the major compounds (>10%) of essential oil in this plant. The intermediate constituents (5-10%) of the essential oil were geranial and neral. In this study, carvone and citral were not in drought treated and control plants, but linalool was identified (0.5% -0.6%) in all of the irrigation regimes (Table 2).

The total amount of oxygen-containing monoterpenes of essential oil were 81.6%, 94.5%, 91.8% and 92.% at 100%, 85%, 75% and 55% of FC regimes, respectively (Table 2).

Table 2. Constituents of the essential oil of *D. moldavica* grown under irrigation regimes.

No	Component	Class	RT	KI	I1	I2	I3	I4
1	6-Methyl-5-Hepten-2-one	Others	14.0	985	0.2	0.2	-	0.2
2	(*p*) Cymene	MH	16.8	1026	-	0.1	-	-
3	Linalool oxide	OM	18.4	1074	0.3	0.4	0.3	0.4
4	(*trans*) Linalool oxide	OM	19.2	1088	-	0.2	-	0.2
5	Linalool	OM	19.8	1098	0.5	0.6	0.6	0.6
6	(2,2-dimethyl-3,4-) Octadienal	OM	20	1100	0.2	0.2	0.2	-
7	(*cis*) Limonene oxide	OM	22.2	1134	-	0.1	-	-
8	(4-Keto) Isophorone	OM	22.4	1142	0.2	-	-	-
9	Nerol oxide	OM	22.5	1153	-	0.3	-	0.3
10	Karahanaenone	OM	23	1155	0.2	0.2	-	0.2
11	Sabine ketone	OM	23.5	1156	-	0.1	-	-
12	(*cis*) Chrysanthenol	OM	23.8	1162	0.3	0.3	0.2	0.3
13	2- Decenal (E-4)	OM	25.7	1193	-	-	-	0.3
14	Nerol	OM	26	1228	0.3	0.3	0.3	0.3
15	(*cis*) Carveol	OM	26.7	1229	-	-	5.7	-
16	Neral	OM	26.7	1240	5.8	7.7	-	7
17	Geraniol	OM	27.4	1255	15.9	15.4	15.9	16.7
18	Geranial	OM	28.1	1270	8.4	10.5	8.3	9.8
19	(dihydro) Linalol acetate	OM	28.9	1275	-	-	-	0.2
20	Methyl geranate	OM	30.4	1323	0.2	0.3	0.3	0.6
21	Neryl acetate	OM	32.2	1365	2.6	2.8	3.4	2.9
22	Geranyl acetate	OM	33.3	1383	46.7	55.1	56.6	52.2
23	Beta- elemene	SH	33.5	1391	-		0.2	-
24	trans-Caryophyllene	SH	34.7	1404	0.2	0.3	0.3	0.3
25	(*neo*) Methyl lactate	Others	36.5	1465	-	-	0.2	-
26	Germacrene-D	SH	37.2	1480	0.6	-	1.4	0.6
27	(*β*) Selinene	SH	37.8	1485	-	-	0.1	-
28	Spathulenol	OS	41.0	1576	0.5	-	-	0.2
29	Caryophyllene oxide	OS	41.0	1581	0.5	-	0.2	0.3
30	Veridiflorol	OS	41.6	1590	1.1	-	-	0.3
31	Longiborneol	OS	42.1	1592	0.2	-	-	-
32	(*cis*) Arteannuic alcohol	OS	42.2	1593	0.1	-	-	-
33	Carotol	OS	42.4	1594	0.3	-	-	-
34	Cedrol	OS	43.0	1596	0.3	-	-	-
35	(*Z*) Sesquilavandulol	OS	43.1	1606	0.3	-	-	-
36	(1,10-Di-epi) Cubenol	OS	43.2	1614	1.1	-	-	-
37	(10-epi-Ɣ) Eudesmol	OS	43.3	1619	0.4	-	-	-
38	(*α*) Acorenol	OS	43.4	1630	1	-	-	-
39	(*β*) Acorenol	OS	43.8	1634	0.7	-	-	0.3
40	Hinesol	OS	43.9	1638	2.4	-	-	-
41	(*α*) cadinol	OS	44.0	1640	-	-	-	0.3
42	(*α*) Eudesmol	OS	44.2	1652	-	-	-	0.3
43	Seline-11-en-4-α-ol	OS	44.3	1652	-	-	-	0.9
44	(14-Hydroxy-9-epi-(E) Caryophyllen	OS	45.1	1664	1.5	-	-	-
45	Khusinol	OS	45.2	1674	-	-	-	0.4
46	Cedroxyde	OS	47.4	1704	3.4	-	-	-
47	(6R,7R) Bisabolone	OS	47.2	1737	-	-	-	1.2
48	Cryptomeridiol	OS	54.4	1808	0.2	0.2	0.2	-
49	Methyl Hexadecanoate	OS	54.5	1927	0.4	-	-	-
50	Phytol	OD	59.1	1949	0.5	1.1	2.9	2.0
51	(Tetrahydro) Rimuene	DH	59.9	1956	0.4	-	-	0.3
52	Eicosene (*n*)	OD	73.8	2000	-	0.2	0.5	0.3
53	Heneicosane (*n*)	Others	73.9	2100	1.5	-		
54	Monoterpene hydrocarbons (MH)				-	0.1	-	-
55	Oxygen-containing monoterpenes (OM)				81.6	94.5	91.8	92.0
56	Sesquiterpene hydrocarbons (SH)				0.8	0.3	2.0	0.9
57	Oxygen-containing sesquiterpenes (OS)				14.4	0.2	0.4	4.2
58	Diterpene hydrocarbons (DH)				0.4	-	-	0.3
59	Oxygen-containing diterpenes (OD)				0.5	1.3	3.4	2.3
60	Others				1.7	0.2	0.2	0.2
61	Total				99.4	96.6	97.8	99.9

The amount of oxygen-containing monoterpenes of *D. moldavicum* increased by 15.8%, 12.5% and 12.7% at 85%, 75% and 55% of FC, respectively. With increasing drought level, the amount of geranyl acetate, geraniol and geranial components of essential oil increased. In the 75% of FC, geranyl acetate percentage (56.6%) was higher than that of the others, but the highest amount of geraniol was 16.7% at 55% of FC. The highest amount of neral (7.7%) and geranial (10.5%) was observed in plants treated with 85% FC level. Some oxygen-containing sesquiterpenes (OS) such as Cadinol, (*α*) eudesmol, seline-11-en-4- *α*-ol, khusinol and (6R, 7R) bisabolone compositions were identified in low amounts at 55% of FC. The highest amounts of those were concerned with (6R, 7R) bisabolone (1.2%). The other oxygen-containing sesquiterpenes (OS) such as longiborneol, (*cis*) arteannuic alcohol, caratol, cedrol, (z) sesqui lavandolul, (1, 10-Di-epi) cubenol, (10-epi-Ɣ) eudesmol, (*α*) acorenol, hinesol, methyl hexadecanoate were not identified at high drought level. The total amounts of oxygen-containing sesquiterpenes of essential oil were 14.4%, 0.2%, 0.4% and 4.2% at 100%, 85%, 75% and 55% of FC, respectively (Table 2).

The identified components can be classified in seven types: monoterpene hydrocarbons (MH), oxygen-containing monoterpenes (OM), sesquiterpene hydrocarbons (SH), oxygen-containing sesquiterpenes (OS), diterpene hydrocarbons (DH), oxygen-containing diterpenes (OD) and others.

Discussion

Our results indicated that *D. moldavica* essential oil content significantly increased by 3.4%, 13.8% and 27.6% with increasing drought level at 85%, 75% and 55% of FC, respectively. Omidbaigi, (2005), Shatar, (2000) and Safikhani et al. (2007) showed that drought stress can significantly influence essential oil in different plant species. Hassani (2006) observed that water stress had a significant effect on essential oil in Moldavian balm, and that the highest amount of essential oil content (0.35 mlg^{-1} DW) was in 70% of FC. Omidbaigi et al. (2010) also found that essential oil content and its constituents were significantly affected by sowing in dragonhead plant. In their study the highest (3.2%) and the lowest (2.3%) oil contents were obtained from the herbs sown on 20^{th} of April and 5^{th} of March, respectively (Omidbaigi, 2010).

The effect of drought stress on plants is a focus of research especially with medicinal and aromatic plants in order to increase the yield and the production of essential oils. Drought stress produces an over-reduce state that involves the synthesis of secondary metabolites, which affect the essential oil content (Kleinwächter et al., 2015).

Essential oils are the most important active ingredients in the plants. Actually, essential oils are the remains of the key processes of plant metabolism, especially in the stress conditions (Aziz et al., 2008).

It has been reported that the major compounds of the *D. moldavica* oil were found to be geranyl acetate, geranial, neryl acetate, geraniol, neral and nerol (Li, 2001). Hawthorne et al. (1993) identified geranyl acetate (65.8%), carvacrol (14.9%) and thymol (7%) as the major components of *D. moldavica* oil (Hawthorne, 1993), but Shatar and Altantseg (2000) introduced linalool (67%) and carvone (5.9%) as the main components of its oil (Shatar, 2000). Chopra (1986) found that dragonhead contained citral in its essential oil (Chopra, 1986).

Davazdahemami (2008) showed that the sum of five major components, namely, neral, geraniol, geranial, neryl acetate and geranyl acetate in the essential oil were 92% and 64% in spring and summer sowing time with maximum change being observed in geranyl acetate from 35.3% in spring to 14% in the summer (Davazdahemami, 2008).

Omidbaigi et al. (2010) showed that eighteen components were identified from the oil of plants sown on 5th of June, but nine to twelve components were analyzed from the essential oils of plants sown from 5th of March to 20th of May. The highest amount of geranyl acetate (50.1%), geranial (25.3%), neral (19.3%) and geraniol (28.8%) were obtained from the plants sown on 5th of May, 5th June, 5th June and 20th of March, respectively (Omidbaigi, 2010). Alaei (2013b) showed that 36 and 21 components identified from *D. moldavica* in field and greenhouse conditions, respectively. The major constituents of the *D. moldavica* oil were geranyl acetate (46.72%), geraniol (15.87%), geranial (8.36%), neral (5.8%), cedroxyde (3.39%), neryl acetate (2.57%) and hinesol (2.39%) (Total: 88.49%) in the field condition and geranyl acetate (39%), geraniol (27.30%), ethyl citronellate (12.92%) and neral (9.32%) (Total: 88.54%) in the greenhouse conditions. Geranyl acetate percentage in the field was higher than that in the greenhouse, but neral and geraniol decreased in the field conditions (Alaei, 2013b).

Najafpour (2003) showed that *D. kotschyi* essential oil had twenty-six components including limonene (38.2%), perillaldehyde (24.5%), geranial (7.5%), *α*-pinene (6.3%), neral (6.1%) and *cis-β*-ocimene (Najafpour, 2003).

The results of the current study showed that 36, 22, 20 and 30 components were identified at 100%, 85%, 75% and 55% of FC, respectively. Geranyl acetate and geraniol, geranial, neral and neryl acetate were the major compounds of essential oil in this plant, but carvone and citral were not in the drought-treated and control plants.

These results are in agreement with other previous studies such as Li and Ding (2001), Hawthorne et al. (1993), Yousefzadeh (2011), Mafakheri (2012), Alaei and Mahna (2013b), Omidbaigi et al. (2010) and Davazdahemami et al. (2008).

In another report, it was shown that composition and yield of essential oil of rosemary can be influenced by ecological, physiological, phenological and agronomical factors (Hassanzadeh, 2017).

The results of the effects of the elevation and phenological stage on essential oil components of *Teucrium polium* L. and *Teucrium orientale* L. confirmed the significant effects of phenological stage and elevation on the percentages of essential oil components. High elevation led to an increase in aromatic compounds such as α-pinene and limonene from monoterpene hydrocarbons, but a decrease in sesquiterpene compounds such as β-caryophyllene contents. There was a significant correlation between essential oil constituents under different elevations and phenological stages. Elevation and phenological stage led to differences in morphology, essential oil content, and essential oil composition of the populations (Reaisi, 2019).

The amount of oxygen-containing monoterpenes of *D. moldavicum* increased by 15.8%, 12.5% and 12.7%, but the amount of oxygen-containing sesquiterpenes of essential oil, decreased by 98%, 97% and 70.8% at 85%, 75% and 55% of FC, respectively.

As the level of drought increased, the amount of geranyl acetate, geraniol and geranial components of essential oil increased. The obtained results indicate that oxygen-containing monoterpenes of essential oil increased (15.8%) and oxygen-containing sesquiterpenes of essential oil decreased (98%) significantly with increasing the level of drought (85% FC).

Furthermore, with increasing drought stress, the morphological traits were negatively influenced. Environmental factors can affect the type and intensity of chemical compounds by influencing metabolism and production (Baczek et al., 2016).

Many of these factors in medicinal plants are interdependent that can be influenced by season and growth stages, geographical origin, maturity variation, genetic variation, utilized part of plant, postharvest drying and storage conditions (Anwar, 2009). Essential oil content and composition are complex traits, which are dependent on yield components and are highly influenced by many genetic as well as environmental factors. In general, indices such as ecology, edaphic, genetic and management factors, essential oil extraction method, and plant characteristics are the important factors influencing the quantity and quality of essential oil (Reaisi, 2019).

Genetic and environmental conditions like drought stress influenced the essential oil compounds in medical plants. Alternation in the composition of essential oil under drought stress was observed in the other plants (Baher Nik et al., 2008; Akhzari and Pessarakli, 2015). In addition, the stimulation of essential oil production under drought stress could have been due to higher terpene production under stress conditions (Bahreininejad et al., 2013).

Conclusion

Water deficit caused significant changes in the yield and composition of essential oils in *D. moldavicum* which can be attributed to stress severity. The main components of essential oil include oxygen-containing monoterpenes and oxygen-containing sesquiterpenes were enhanced by drought stress. The highest amount of oxygen-containing monoterpenes (94.5%) was detected in dragonhead plants treated with 85% FC level. Therefore, our results indicated the advantage of drought for increasing essential oil content by 27.6% and oxygen-containing monoterpenes especially geranyl acetate and geraniol by 15.8%. The data of the present study can clarify the effect of drought on essential oil content and composition. Further studies such as measuring the activity of enzymes that are involved in biosynthetic pathway of essential oil and the expression of their genes can lead to a better understanding of the impact of drought on regulation of essential oil metabolism.

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Conflict of interest

The author declare no conflict of interest on this work

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