



Broad Variation in Herbage Yield and Essential Oil Content Among Iranian Landraces of *Dracocephalum moldavica*

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ABSTRACT: *Dracocephalum moldavica* L. (Moldavian Balm) is a medicinal plant with pharmacological and biological properties of interest and it is a commonly cultivated plant in North Western Iran. The varied bioactive constituents of Moldavian Balm have been adduced as a contributive factor for the sedative and carminative activity of the plant. The present study was carried out to assess the differences among Iranian landraces (cultivated populations) of Moldavian balm based on some horticultural traits. Seeds of seven populations were provided from different regions of North Western Iran, included in this category are: West Azerbaijan provinces (Naghadeh, Keshtiban, Topragh Ghalee, and Baghchajogh) and East Azerbaijan provinces (Hokm Abad, Jahangir and Shiraz valley). The seeds were cultivated in the field conditions in Hamedan province. Sixteen characters associated with horticultural traits were evaluated. Horticultural characteristics included plant height, stem diameter, leaf length, leaf width, length to width Ratio, number of branches, branch length, number of leaves per node, number of nodes per stem, internode distance, dry leaf weight, dry stem weight, fresh mass, dry mass, drug fraction and essential oil content of Moldavian Balm were determined. The present study demonstrated a broad diversity among horticultural traits of Moldavian Balm landraces from North Western Iran. This research knowledge could be a useful tool in conservation blueprints, germplasm management and breeding programs of *D. moldavica*.

Key words: *Dracocephalum moldavica* L., Variation, herbage yield, essential oil

INTRODUCTION

The Moldavian balm (*Dracocephalum moldavica* L.), syn. Moldavian dragonhead is an annual, herbaceous essential oil producing, spicy and aromatic medicinal plant belonging to the Lamiaceae family, which reaches up to 80 cm in height (Dastmalchi *et al.*, 2007; Maham *et al.*, 2013). It is native to central Asia and it has been naturalized in Eastern and Central Europe. However, it has also been grown in China, Egypt, Mongolia, and the Himalayas, at altitudes up to 2700-3100 meters above sea level and originates from the Irano-Turanian phytogeographical area (Rechinger, 1982; Dastmalchi *et al.*, 2007; El-Baky & El-Baroty, 2008). In the Iranian traditional medicine, *Dracocephalum moldavica* is known as “Badershboo and Turkish Melissa” (Najafi *et al.*, 2009; Horn *et al.*, 2014). Due to its strong effects on the gastrointestinal system and other parts of the body, *D. moldavica* has traditionally been used for its culinary properties (as food ingredient) and herbal teas and

herbal drugs for treatment of diseases e. g. stomach and liver disorders, headache and congestion and as a cardiostimulant agent in folk medicine. In West Azerbaijan (Iran) this plant is reported to have been used as a general tonic, stomachic, digestive, antiemetic and diaphoretic. Dry herb of this species have been used as tincture for ages in Uyghur folk medicine to treat heart diseases, blood pressure and toothache (El-Baky & El-Baroty, 2008; Najafi *et al.*, 2009; Maham *et al.*, 2013). It has been reported that the plant possesses fungicidal, anti-microbial (Najafi *et al.*, 2009), antioxidant, antibacterial and cardioprotective (Arya & Gupta, 2011) effects. Recently published reports have buttressed its painkiller potency, sedative capacity, tranquilizer remedy (Martinez-Vazquez *et al.*, 2012) and anti-nociceptive effects (Maham *et al.*, 2013). Also, In vitro evaluation of *D. moldavica* total extract showed anti-*Helicobacter pylori* activity (Najafi *et al.*, 2009).

Other reported pharmacological properties of this plant are carminative, skin anti-inflammatory effects, spasmolytic effects and the treatment of angina, atherosclerosis, neuralgia, migraine (Dastmalchi *et al.*, 2007; Najafi *et al.*, 2009), hypertension and for immunomodulatory activities (Zeng *et al.*, 2010). Additionally it has been reported; that the essential oil and extracts of leaves of *Dracocephalum moldavica* possesses contact toxicity against two grain storage insects and had the highest SPFs due to phenolic and flavonoid compounds (Chu *et al.*, 2011; Khazaeli & Mehrabani, 2008).

Assessment of the genetic diversity of specie is an important aspect in establishing conservation programs; because this issue has been in the front burner of issues associated with the ability of specie to adapt to environmental changes including biotic and abiotic changes due to the level of genetic variability it contains (Rodrigues *et al.*, 2013). Recognition of the levels and distribution of the genetic diversity is the base and it is crucial for selection, management and the development of effective conservation strategies of plant genotypes in breeding programs and therefore its evolution. Differentiation of the genetic diversity is performed by morphological and biochemical markers. These markers are complementary in determining the genetic similarity of inter and intra species (Solouki *et al.*, 2008; Rodrigues *et al.*, 2013; Shafeei-Hajiabad *et al.*, 2014). Recently, the most extensively used markers are the horticultural traits which are used for the study of the genetic diversity issues in medicinal plants (Azizi *et al.*, 2014). For medicinal and aromatic herb, herbage yield and essential oil content are economically very important (Azizi *et al.*, 2009). The methods related to horticultural traits assessments are simple, effective, and more rapid methods than other methods and are also common procedures for identifying and classifying the populations and plant cultivars. This analysis may be used for many plant samples using small quantities to study genetic variety (Azizi *et al.*, 2014). However, according to previous studies, it has to be stated that morphological techniques have been used for genotyping and also for studies on genetic diversity in medicinal plants of Lamiaceae family, e.g. in *Mentha cervina* (Rodrigues *et al.*, 2013), *Origanum vulgare* L. (Azizi *et al.*, 2012; Shafeei-Hajiabad, 2014) and *Ocimum bacilicum* L. (Nurzynska-Wierdak, 2014). A study was also carried out on the structure, micromorphology and distribution of trichomes on different parts of the shoot of *D. moldavica* (Dmitruk & Weryszko-Chmielewska, 2010).

Nowadays, much attention has been paid to *D. moldavica* and its chemical constituents because of their diverse and manifold activities, such as anticancer, antioxidant, antihypoxic, and immunomodulatory activities (Zeng *et al.*, 2010). The analysis of lemon-like scented essential oil revealed a wide variation in essential oil production, with the maximal level attained during flowering (Davazdahemami *et al.* 2008, Omidbaigi *et al.* 2010, Alaei & Mahna 2013, Maham *et al.*, 2013). Medicinal properties of the specie increase the importance of diversity studies in this plant. For example in China, the percentage of essential oil reached 0.31% (Chu *et al.*, 2011). The previous studies have shown that high morphological and phytochemical diversity exists among *D. moldavica* populations (Dmitruk & Weryszko-Chmielewska, 2010; Zeng *et al.*, 2010), which may be used in planning the breeding and conservation of this important plant. The present work is the first report about the assessment of variation in herbage yield and essential oil content (horticultural traits) of *Dracocephalum moldavica* among 7 landraces of Northwestern Iran.

MATERIAL AND METHODS

A. Plant material and cultivation conditions

The seeds of moldavian balm plants were obtained from 7 landraces in the North-Western part of Iran. The following populations were sampled and subjected to study: West Azerbaijan provinces (Naghadeh, Keshtiban, Topragh Ghalee, and Baghchajogh) and East Azerbaijan provinces (Hokm Abad, Jahangir and Shiraz valley) (Fig. 1. and Table 1). Seeds of 7 landraces in 10 replications were used, totaling 70 plots with dimensions of 1.5 × 1.5 meters and 1.5 meters from each other as the spacing between the plots. Seeds of *D. moldavica* were sown on the 21 of May and after sowing the seeds, a fine layer of compost was spread on top of the beds. The plants were ready by 26th May. The cultivation was carried out in 2013 at the farm of the Research Center of Agriculture and Natural Resources of Hamedan, Hamedan, Iran (altitude of 1814 m above sea level).

In this study, the 7 landraces of *D. moldavica* were kept in the same culture conditions. The soil was soft and well drained. Dripping wings for irrigation were placed among the lanes throughout their length. The cultural operations, until harvesting, were composed of manual removal of weeds without the addition of any synthetic agrochemical manure.

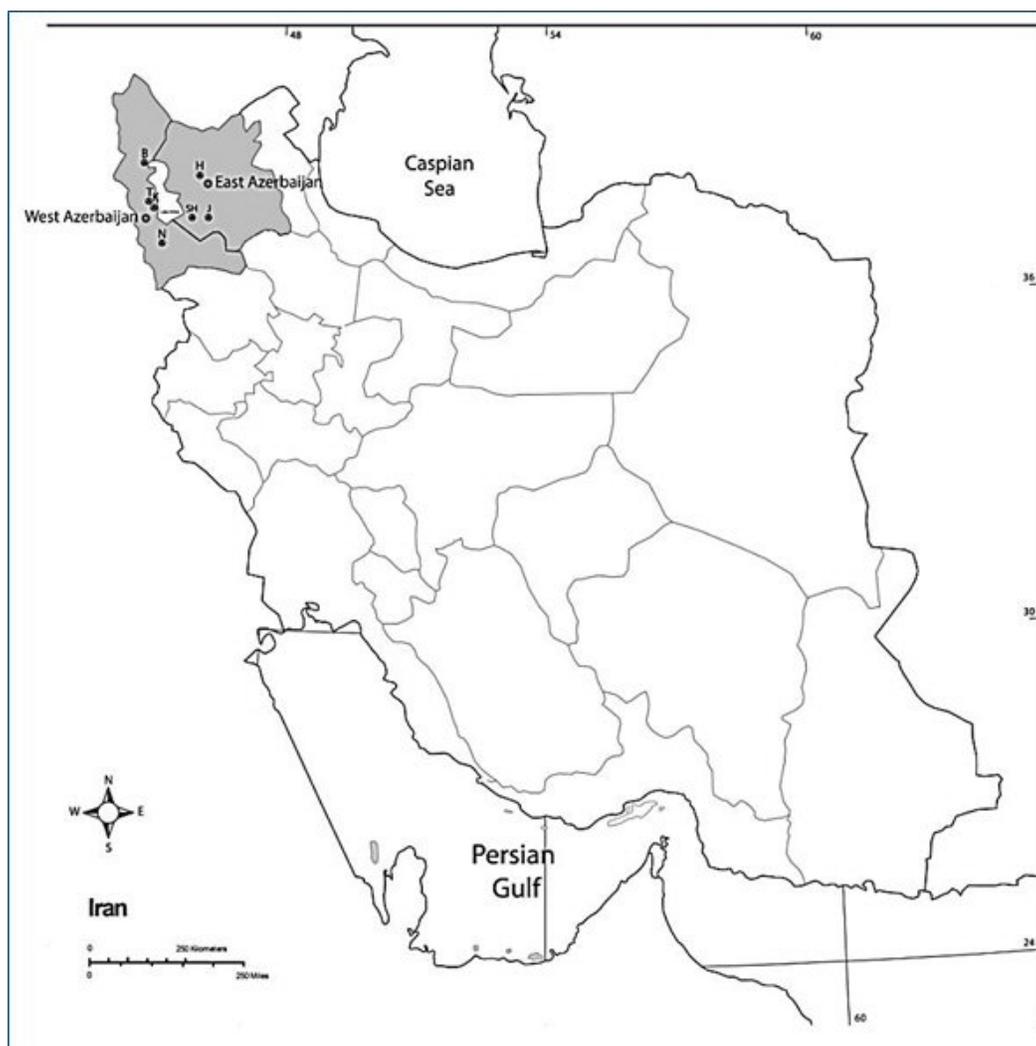


Fig. 1. Locations of *Dracocephalum* landraces from West Azerbaijan provinces (N: *Naghadeh*, K: *Keshtiban*, T: *Topragh Ghalee*, and B: *Baghchajogh*) and East Azerbaijan provinces (H: *Hokm Abad*, J: *Jahangir* and SH: *Shiraz valley*).

Table 1. Geographical coordinates of locations of *Dracocephalum moldavica* L. landraces.

No.	Locations	Provinces	Longitude	Latitude	Altitude (m)
1	<i>Naghadeh</i>	East Azerbaijan	45° 23' E	36° 57' N	1325
2	<i>Keshtiban</i>	East Azerbaijan	45° 15' E	37° 33' N	1278
3	<i>Topragh Ghalee</i>	East Azerbaijan	45° 06' E	37° 35' N	1302
4	<i>Baghchajogh</i>	West Azerbaijan	44° 49' E	38° 10' N	1339
5	<i>Hokm Abad</i>	West Azerbaijan	46° 15' E	38° 50' N	1353
6	<i>Jahangir</i>	West Azerbaijan	46° 16' E	37° 24' N	1567
7	<i>Shiraz Valley</i>	West Azerbaijan	45° 54' E	37° 26' N	1282

B. Evaluation of horticultural traits

At the beginning of the flowering stage, data were collected for 16 quantitative traits on the *Dracocephalum moldavica* L. Horticultural characteristics included plant height (PH), stem diameter (SD), leaf length (LL), leaf width (LW),

length to width Ratio (L/W Ratio), number of branches (NB), branch length (BL), number of leaves per nodes (NL), number of nodes per stem (NN), internode distance (ID), fresh mass (FM), dry mass (DM), drug fraction (DF) and essential oil content (EOC) of Moldavian Balm were determined.

Table 2: Horticultural traits studied in 7 *Dracocephalum moldavica* L. landraces.

No	Traits	Code	Unit
1	Plant height	PH	cm
2	Stem diameter	SD	mm
3	Leaf length	LL	mm
4	Leaf width	LW	mm
5	Leaf to Width Ratio	L/W	Ratio
6	Number of Branches	NB	N
7	Branch Length	BL	cm
8	Number of leaves per nodes	NL	N
9	Number of nodes per stem	NN	N
10	Internode distance	ID	cm
11	Fresh Mass	FM	g/plant
12	Dry Mass	DM	g/plant
13	Dry Leaf Weight	DLW	g/plant
14	Dry Stem Weight	DSW	g/plant
15	Drug Fraction	DF	g/plant
16	Essential Oil Content	EOC	w/w%

Dry leaf weights (DLW) and dry stem weight (DSW) were evaluated using total plants per plot. For each landraces, 10 plants (3 plants per plot) were observed (Table 2).

C. Extraction of essential oil

Harvest was performed at the beginning of flowering by cutting above ground level over the ligneous shoot parts. Fresh aerial parts (leaves, stems and flowers) of *Dracocephalum moldavica* were harvested in September 2013 from fields of the Research Center of Agriculture and Natural Resources of Hamedan. The fresh material parts were naturally air-dried in the shadow for one week. Essential oil content was extracted from flowering aerial parts of the collected samples of each population in 3 replications by hydro-distillation, using a Clevenger-type apparatus. In this study, each 100 g portion of ground powder air-dried herbs of *D. moldavica* L. was dissolved in 1000 ml of distilled water. The mixture was then boiled in a round-bottom flask, and steam distilled for 3 hr.

D. Statistical analyses

The data were analyzed by one-way Analysis Of Variance (ANOVA) using the Statistical Analysis System (SPSS) and mean values were compared by LSD test at 5% probability level. For each trait measured the least significant difference (LSD) was calculated from the corresponding ANOVA.

For each cultivar group the average and standard error (SE) were calculated. Pearson correlations at 5% probability level were performed between pairs of horticultural traits and essential oil compounds using the SPSS software, version 16.0.

RESULTS

A. Diversities in horticultural traits of moldavian balm

The mean values of the twelve measured quantitative traits related to horticultural characters for all the investigated landraces are presented in Table 3.

The results of analysis of variance (ANOVA) between the 16 horticultural traits in the seven *Dracocephalum moldavica* L. landraces, using statistical analyses, confirmed highly significant differences among all the investigated traits ($P < 0.05$) except number of leaves per node and fresh mass (Table 3). The significant differences were obtained for many of the characters, indicating that a wide variation exists in the landraces for these evaluated characters. The landraces from Shiraz valley presented remarkably high mean values for plant height, number of nodes per stem, leaf length and length to width ratio and the lowest mean value for leaf width. The Baghchajogh landraces had the highest values for branch length, stem diameter and leaf dry weight. The landraces from Hokm Abad were characterized by very high leaf width (Table 3) and essential oil content (Fig. 2).

Meanwhile, this landraces were the lowest for six characters including plant height, branch length, stem diameter, number of nodes per stem, distance of internodes and length to width ratio compared to the others (Table 3). Moreover, the Topragh Ghalee landraces were evaluated by highest number of branches and stem dry weight and the lowest for drug fraction. Whereas, the Keshtiban landraces had the

highest amount for drug fraction and the lowest amount for number of branches, leaf length, dry mass, leaf dry weight and stem dry weight. On the other hand, the highest value of dry mass and distance of internode were observed in two landraces including the Jahangir and Naghadeh respectively. No differences were found among the landraces, in fresh mass and Number of leaves per node (Table 3).

Table 3 : Mean Values of Sixteen Quantitative Horticultural Traits for 7 Population of *Dracocephalum moldavica* L.

Population	PH	NB	BL	SD	NN	DI	NL	LL	LW	LWRatio	FM	DM	DF	LDW	SDW
<i>Naghadeh</i>	71.43	11.19	12.10	4.52	15.46	5.04	2.0	4.48	2.26	2.12	12.64	3.06	48.35	1.46	1.59
<i>Keshtiban</i>	66.86	7.86	10.28	3.58	15.53	4.42	2.0	3.81	1.94	1.92	8.87	1.48	55.87	0.93	0.80
<i>Topragh Ghalee</i>	60.26	11.66	13.14	4.57	16.06	5.02	2.0	3.95	2.05	2.17	11.19	3.16	46.23	1.53	1.78
<i>Baghchajogh</i>	70.90	9.33	15.88	4.74	15.80	4.71	2.0	4.64	2.31	1.95	14.51	3.14	47.58	1.48	1.65
<i>Hokm Abad</i>	54.76	10.06	9.60	4.24	14.13	4.13	2.0	4.24	2.37	1.86	12.86	2.62	51.95	1.35	1.27
<i>Jahangir</i>	64.39	10.26	15.08	4.58	15.86	4.93	2.0	4.77	2.16	2.36	12.81	3.25	47.98	1.44	1.65
<i>Shiraz Valley</i>	74.76	10.39	15.69	4.70	16.93	4.50	2.0	4.82	1.77	2.56	13.55	2.63	49.55	1.28	1.35
SE	2.22	0.89	1.97	0.33	0.63	0.18	0.0	0.33	0.09	0.17	2.47	0.36	2.69	0.15	0.23
LSD (0.05)	5.76	1.54	3.86	0.65	0.98	0.36	0.0	0.60	0.34	0.31	5.00	0.72	5.49	0.32	0.44

PH: Plant height (cm). NB: Number of branches. BL: Branch length (cm). SD: Stem diameter (mm). NN: Number of nodes per stem. DI: Distance of internodes (cm). NL: Number of leaves per node. LL: Leaf length (mm). LW: Leaf width (mm). LWRatio: Length to Width ratio. FM: Fresh Mass (g/plant). DM: Dry mass (g/plant). DF: Drug fraction (g/plant). LDW: Leaf Dry Weight (g/plant). SDW: Stem Dry Weight (g/plant).

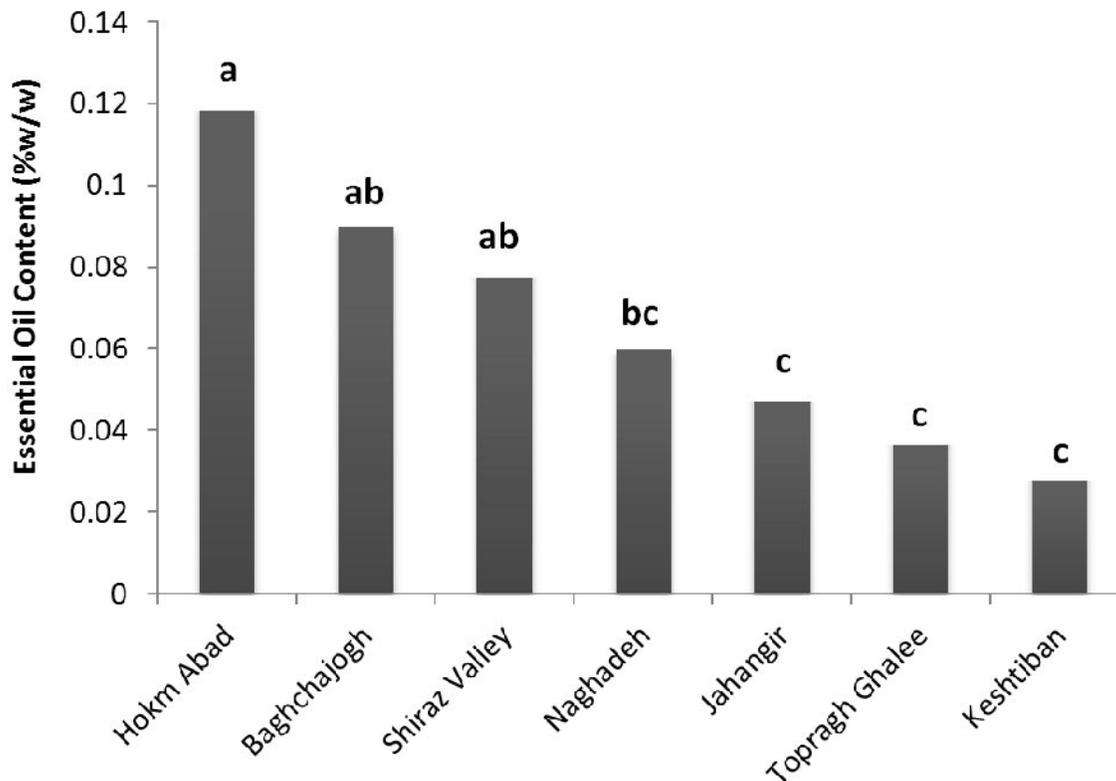


Fig. 2. Essential oil contents (percentages) of *Dracocephalum* obtained from landraces investigated.

B. Essential oil content of moldavian balm

In the 7 populations of *D. moldavica* the content of pale yellow essential oil, collected at the beginning of the flowering stage ranged from 0.03% to 0.12% (w/w) (Fig. 2). The highest amount of essential oil was recorded for landrace of Hokm Abad (0.12%) and the lowest amount was obtained from the Keshtiban landrace to the level of 0.03% (Fig. 2).

DISCUSSION

High significant differences for traits such as plant height and number of auxiliary shoots in this study were in agreement with that of Bagheri Khoulenjani and Salamati (2014). Horticultural and medicinal traits of *D. moldavica* L. could be affected by different growing conditions and geographical origin (Hassani, 2006, Davazdahemami *et al.* 2008, Mohtashami *et al.* 2013). The broad accumulated diversity in herbage yield and other horticultural traits in *D. moldavica* L. on the species level is the most important goals of breeding, which can be used in experimental efforts to produce cultivars of practical importance. The results from this essential oil content are in accordance with some oil content studies for Moldavian Balm. According to a study carried out by Kakasy *et al.*, (2006) the content of essential oil with percentage of 0.11% was obtained in *Dracocephalum moldavica*. Compared to the results of the studies on Moldavian dragonhead carried out by other authors, in the Maragheh landrace (East Azerbaijan Province) the essential oil content ranged between 0.06% and 0.92% (Maham *et al.*, 2013). The changes in composition of any plant essential oil are influenced by the presence of several factors, such as climatic, seasonal and genetic factors (Tepe *et al.*, 2005, Zhang *et al.*, 2008). Based on the results of a study on cultivated *Satureja cuneifolia* and *S. montana* in the same condition, it was indicated that the populations had main differences in the essential oil content, and it was due to genetic variation (Bezic *et al.*, 2005). In this assay, *D. moldavica* landraces were cultivated in similar environmental conditions. Accordingly, in this medicinal plant most of these variations might be related to genetic factors.

CONCLUSION

In conclusion, a significant variation was observed among 7 landraces of *Dracocephalum moldavica* L. based on horticultural traits, herbage yield and essential oil content. The present study is primarily for breeding programs and can be very useful for medicinal purposes of this plant. Consequently, Genetic diversity provides

guidelines for the conservation, development, management and sustainable use of this species.

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