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Original Article

Response of Some Agronomical and Physiological Traits of *Dracocephalum kotschyi* Boiss., under Irrigation and Dryland Farming System in Northern East of Iran

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Abstract

The *Dracocephalum kotschyi* Boiss.is a wild-growing flowering plant belonging to the Lamiaceae family and has been used as a medicinal herb for rheumatoid diseases, headaches, congestion, stomach disorders, liver treatment. In order to study the effects of dryland farming system on some agronomical and physiological traits of *D. kotschyi,* two separate experiments were conducted in three locations of, Mashad (Golmakan station), Quchan (5km in the northeast of city) and Bojnord (Sisab station) under normal irrigation and dryland farming systems in 2018. The collected data were combined analysis over three locations and mean comparisons were made using Tukey method. The results showed significant effects of locations for all of the traits except carbohydrate contents. Effect of farming system was also significant for all traits. Farming system by location interaction effects were significant for aerial fresh and dry weigh, 1000 seeds weight, proline, DPPH, flavonoid, protein, chlorophyll a, carotenoid, nitrogen and potassium. Result showed the location of Quchan had significantly higher mean values for many of traits followed by Mashad. In comparisons between two method of cultivations, the lower and higher values of plant height (20.6 vs. 25.3 cm), main branch (13.1 vs. 14.9 per plant), leaf area (38.9 vs. 48.3 mm^2), flower number (14.6 vs. 23.1 per plant), fresh weight (295 vs. 888 g/plant), dry weight (80 vs. 244 g/plant), 1000 seeds weight (0.27 vs. 0.35 g), Protein (9.74 vs. 11.06%), chlorophyll a (7.47 vs. 8.19 mg g^{-1}FW), carotenoid (0.08 vs. 0.11 mg g^{-1}FW), nitrogen (1.43 vs. 1.77%) and phosphorus (13.89 vs. 20.79 ppm) were obtained in dryland and irrigation farming, respectively. In contrast, the higher and lower values of internode length (4.11 vs. 3.71 cm), proline (1.44 vs. 0.77 mg g^{-1}FW), DPPH (0.39 vs. 0.19 mg g^{-1}FW), phenol (0.83 vs. 0.67 mg g^{-1}FW), flavonoid (0.60 vs. 0.47 mg g^{-1}FW), carbohydrate (8.2 vs. 6.2 mg g^{-1}FW), and potassium (4.2 vs. 3.7%) were obtained in dryland farming system.

Keywords: Endangered species, Essential oil, Yield.

Introduction

The *Dracocephalum kotschyi* Boiss, is a wild-growing flowering plant belonging to the Lamiaceae family. It is endemic in Iran and known as Badrandjboie- Dennaie and Zarrin-Giah. The genus of *Dracocephalum* L. is composed of about 60 species also, 9 species of *Dracocephalum* grow as wild herbs in regions of Iran with high altitude [1] of which two are endemic [2,3]. It grows wild at regions with 2000- 3200 m altitude in Isfahan, Fars, Lorestan, Mazandaran, Golestan, Hamadan, and Khorasan provinces, Iran [4]. *D. kotschyi* is a short perennial herbaceous plant that is woody at

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base part, with stems measuring between up to 20 cm length. The plant has small pubescent leaves that are calyx two-lipped, with upper lip three-toothed, stamens, and flowers in verticillaster in the upper leaves [4]. Excessive harvesting of wild plants in limited areas in Zagros highlands in Isfahan and Chaharmahal Bakhtiari are the main reasons why *D. kotschyi* is now listed as an endangered plant [5]. In recent years some attempts were made for its domestication and cultivation in Isfahan and Kohgiluyeh and Boyer-Ahmad Province, Iran. But, the area under cultivation is very low up to few hectares.

In traditional medicine, *D. kotschyi* is applied in its natural habitat as a warm herbal medicine for rheumatoid diseases and stomach disorders treatment. Its effectiveness for headaches, congestion, and liver disorders has been described as well [6]. It used as Antihyperlipidemic [7] and immunomodulatory [8].

Antioxidant activity of *D. kotschyi* was mostly due to the flavonoids such as luteolin, apigenin, cirsimaritin, xanthomicrol and rosmarinic acid [9,10]. The effect of methoxylation of flavonoids on inhibition of tumor cells has been reported from *D. kotschyi* [11]. Luteolin can induce tumor cells apoptosis such as epidermoid carcinoma, pancreatic tumor, leukemia and lung cancer [12]. The *D. kotschyi* seeds have been identified as high content of oil mostly linolenic acid [13].

Excessive harvesting of wild plants *D. kotschyi* is now listed as an endangered plant [5]. Therefor its domestication and cultivation is now a high priority [14]. Although the effects of drought stress on crops have been extensively studied [14], the researches on the behavior of medicinal and aromatic herbs under dryland farming system here in Iran have not been so extensive. There are some studies reporting the enhancement of the concentration of secondary plant products and allelopathic activity of medicinal plants under drought stress. However, it has to be considered that drought stress also reduced the growth of most plants [15].

De Abreu and Mazzafera (2005) in *Hypercium brasiliense* showed that concentration and total content of the phenolic compounds is drastically higher in plants grown under drought stress than in the control plants. In mild stress, the amount of phenolic compounds was 10% higher than normal condition [16].

The aim of this research was to domestication and cultivation of *D. kotschyi*under dryland farming system and evaluation of agronomical and physiological traits in three location in northeast of Iran.

Material and Methods

The present study was conducted in three locations of Mashad and Quchan (In Razavi Khorasan province) and Bojnord (in North Khorasan province), Iran during 2017-2019. The annual mean rainfall and annual mean temperature of Mashad, Quchan and Bojnord were 235, 295 and 275 mm, and 17.2, 13.4 and 14.8 °C, respectively. The soil texture all of locations were silt-loamy with relatively high pH (ranged 7.2 to 8.3) and the climate of experimental sites is classified as cold semi-arid (Table 1).

Table 1 Climatic and soil of stations in three locations of Mashad and Quchan (In Razavi Khorasan province) and Bojnord (in north Khorasan province), Iran

	Mashad	Quchan	Bojnord
Latitude	36° 17′ 52.80″ N	37° 06′ 22.40″N	37° 47′ 02.64″ N
Longitude	59° 36′ 20.52″ E	58° 30′ 34.12″ E	57° 30′ 31.42″ E
Altitude (m)	1215	2032	1570
Mean annual rainfall (mm)	235	295	275
Mean annual temperature (°C)	17.2	13.4	14.8
Max. temperature (°C)	23.6	19.3	21.6
Min. temperature (°C)	8.4	6.1	6.7
Soil texture	Silty-loam	Silt-loam	Silt-loam
pH	7.5-8.2	7.5-8.3	7.2-7.8
Mean annual humidity (%)	53	59	68

Source: www.irimo.ir

Experiment Layout and Data Collection

The experiments were conducted in three locations of Mashad (Golmakan station), Quchan (5km in the northeast of city) and Bojnord (Sisab station) under two normal irrigation and dryland farming conditions. Seeds of *D. kotschyi* were provided from agriculture and natural resources research center of Isfahan province, Iran.

For both farming systems (dryland and irrigation), seeds were sown in Jiffy pots in March of 2017 in out of glasshouse. Then, the seedlings were transplanted in field in mid April 2017. The experiment layout was Randomized Completely Blocks Design (RCBD) with three replications. Each experimental unit was 2.5m × 2.5m consists of four rows with 50 cm distance between rows and 30 cm between plants within rows. Before seedbed preparation, cattle manures (22 tons/ha) were added and mixed with the soil in both trials. Under irrigation system, the plants were irrigated weekly until full flowering stage, and under dryland farming system, only four irrigations were made to aid in the establishment of the seedlings. Then irrigation stopped and water requirement provided only from precipitation (April till May 2018). Weeds were controlled mechanically by 2 times during growth period. Since *D. kotschyi* is a perennial crop and its vegetative growing in establishment year was low, therefore no data were collected in establishment year, in the second year an area of 1 m^2 in center of each plots was selected for data collection in full flowering stage as follows:

Agronomical Traits

The following agronomical traits were measured in the next year of planting in 2018. Plant height, main branch per plant, leaf area (leaf area index meter, Mod. li3100c), flower number per plant, aerial fresh weight (g/p), aerial dry weight (g/p), 1000 seeds weight (g) and internode length (cm).

Physiological Traits

Proline content: Approximately 0.5g of frozen plant material from control and treated plants was homogenized in 10mL of 3% aqueous sulfosalicylic acid and the homogenate filtered through Whatman filter paper. Free proline amount was measured by spectrophotometer at 520 nm [17].

Antioxidant Activity by DPPH assay: The scavenging effect of the hydrolysates on α-adiphenyl-β-picrylhydrazyl (DPPH) free radical was measured by using the method of Chizzola, et al. (2014) with some modifications. To do so, 200 ml ethanol 95% added to 100 mg fresh leaf and extracted by soxhelet extractor in 3 hours, 1.5 ml of this sample was added to 1.5 ml of 0.1 mM DPPH in 95% ethanol. The mixture was shaken and left for 30 min at dark room, and the absorbance of the resulting solution was measured at 517 nm. A lower absorbance represented a higher DPPH scavenging activity [18] that obtains as follows:

The scavenging effect is expressed as [(Blank absorbance - Sample absorbance)/ Blank absorbance] × 100%.

Phenol: Total phenols were determined by the method of Singleton and Rossi (1965) using the Folin–Ciocalteu reagent. An aliquot (0.25ml) of each extract was added to 3.5ml of distilled water in a screw-capped test tube, followed by 0.5ml of Folin–Ciocalteu solution. After 3min, 1ml of 1% sodium carbonate was added and the contents of the tube were thoroughly mixed before being incubated in a boiling water bath for 1min. The tube was allowed to cool in the dark then the absorbance of the blue color that developed was read at 685nm using gallic acid as standard. Results were expressed in gallic acid mg g^{-1} fresh weight [18].

Carbohydrate: To measuring the content of soluble sugars, 0.5 g of dry leaves was homogenized with 5 ml of 95% ethanol. One-tenth ml of alcoholic extract preserved in refrigerator mixed with 3 ml anthrone (150 mg anthrone, 100 ml of 72% sulphuric acid, W/W). The samples placed in boiling water bath for 10 minutes. The light absorption of the samples was measured at 625 nm using a PD-303 model spectrophotometer. Contents of soluble sugar were determined using glucose standard and expressed as mg g^{-1} DW of leaves [19].

Chlorophyll a, b and carotenoid content of the leaves were evaluated by the method of Arnon (1967) at flowering stage and absorbance was measured at 480, 510, 645 and 663 nm using spectrophotometer (Mod. Genesise 10UV) and carotenoid content was calculated by using the formula [20].

$$Carotenoides = 100(A470) - 3.27(mg\ chl.a) - 104(mg\ chl.b)/227$$

Light absorbance at 470 nm= A

Protein, was estimated as N% x 6.25

Nitrogen (%): Fourth leaves from top of the randomly selected plant in each plot were collected

for analysis of Kjeldahl Method Chapman and Pratt (1961), to estimate the plant nitrogen content [21].
Phosphorus was determined using the molybdovanadate method Chapman and Pratt using spectrophotometer (Mod. Genesise 10UV) [21].
Potassium of the extract was measured using method of Perkin (1982) with flame photometer digital 310 [22].

Statistical Analysis

This experiment was conducted using randomized complete block design in three replications. Combined analysis of variance was made between locations (Mashad, Quchan and Bojnord) as main factors and two method of farming systems (Normal irrigation and dryland farming) as second factor. Means were compared using Duncan's new multiple range test (DNMRT) at 1 and 5% probability levels. All statistical analyses were conducted through MINITAB (Version 16) software.

Results and Discussions

Agronomical Traits

Result showed significant effects of locations for all of agronomical traits. Effect of farming system was also significant for all traits (Table 2). Farming system by location interactions effects were significant for aerial fresh and dry weigh and 1000 seeds weight (Table 2).
Result showed the location of Quchan had significantly higher mean values for plant height, main branch number per plants, leaf area, flower number per plant, aerial fresh and weight, followed by Mashad. This may related to higher altitude (2032 m), coupled with higher annual precipitation and lower annual temperature in Quchan than that to other two sites (Table 1). For 1000 seeds weight and internode length, the higher values were obtained in Bojnord and Mashad, respectively (Table 3).
In comparisons between two farming system result showed that the higher values all of agronomical traits except internode length were obtained in irrigation system (Table 4). This result is excepted because drought stress has negative effects on plant growth.
The reduction in plant height due to water stress is expected and slowing growth rate [23], reducing leaf number and area [24,25], reduction of shoot dry weight, leaf area, stem length and root length [26].
Result of means comparisons for farming method by location interactions effects showed that the trend of aerial fresh and dry weight in two farming systems were not similar and the ratio of irrigation to dryland farming for aerial fresh weight in Quchan were higher than two other locations. For 1000 seeds weight the differences between yield of two farming system in Bojnord location were lower than two other locations (Figure 1). This may related to higher altitude and mean annual humidity in these areas (Table 1).

Physiological Traits

Result of analysis of variance for physiological traits showed significant effects of locations for all of physiological traits except carbohydrate contents and significant effect of farming system for all traits (Table 5). Farming by location interactions effects were significant for proline, antioxidant activity (DPPH), flavonoid, protein, chlorophyll a, carotenoid, nitrogen and potassium (Table 5).
Result of mean comparisons between locations showed that Quchan had significantly higher mean values for all of traits except flavonoid, carotenoid, phosphorus and potassium.

Table 2 ANOVA of agronomical traits in *Dracocephalum kotschyi* Boiss. evaluated in three locations under two farming systems

Source	DF	Plant height	Main branch per plant	Leaf area	Flower number per plant	Aerial fresh weight	Aerial dry weight	1000 Seeds weight	Internode length
Location (L)	2	340.2 **	48.17 **	1367.3 **	251.1 **	396048 **	30806 **	4.421**	1.777 **
Error 1	6	4.78	1.44	84.0	8.78	6469	586	0.085	0.030
Farming (F)	1	98.0 **	14.22 *	399.4 **	320.8 **	1585237 **	121327 **	2.977 **	0.761 **
L x F system	2	6.17	0.06	5.80	17.39	105919 **	6464 **	0.083 *	0.017
Error 2	6	3.11	1.44	1.68	3.56	3453	364	0.010	0.030
CV		7.67	8.58	2.97	9.98	9.93	11.77	31.98	4.43

*significant at the 0.05 probability level; ** significant at the 0.01 probability level.

Table 3 Means of agronomical traits in *Dracocephalum kotschyi* Boiss. evaluated in three locations.

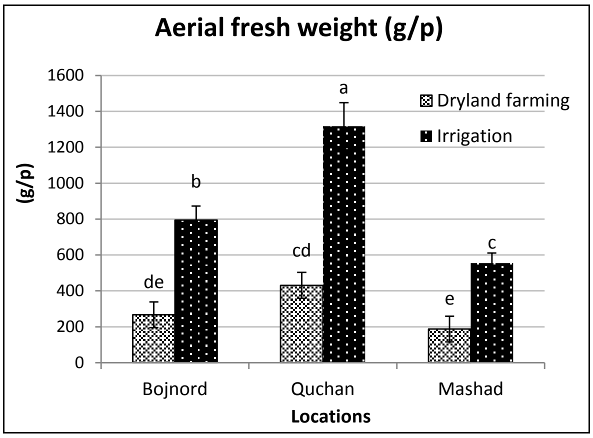
locations	Plant height (cm)	Main branch per plant	Leaf area (mm^2)	Flower number per plant	Aerial fresh weight (g/p)	Aerial dry weight (g/p)	1000 seeds weight (g)	Internode length (cm)
Bojnord	21.51 b	14.00 b	51.30 a	19.83 b	530.58 b	143.6 b	0.39 a	3.97 b
Quchan	31.17 a	16.83 a	53.35 a	24.83 a	874.09 a	241.21 a	0.33 b	3.33 c
Mashad	16.33 c	11.17 c	26.24 b	12.00 c	371.39 c	101.53 c	0.22 c	4.42 a

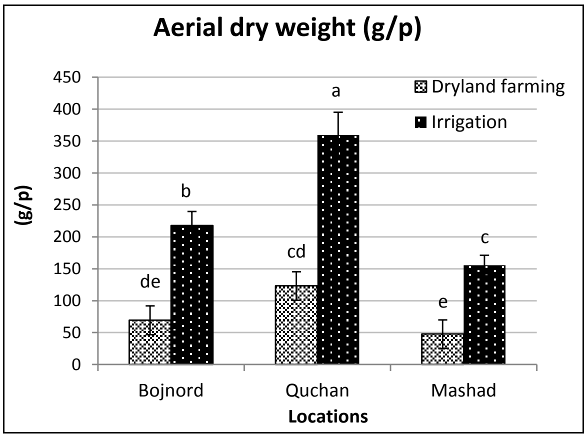
The means of the column with same letters were not significantly different based on Tukey method ($p<0.05$).

Table 4 Means of agronomical traits in *Dracocephalum kotschyi* Boiss. under two farming systems

Farming System	Plant height (cm)	Main branch per plant	Leaf area (mm^2)	Flower number per plant	Aerial fresh weight (g/p)	Aerial dry weight (g/p)	1000 seeds weight (g)	Inter node length (cm)
Irrigation	25.33 a	14.89 a	48.34 a	23.11 a	888.78 a	244.21 a	0.35 a	3.71 b
Dryland	20.67 b	13.11 b	38.92 b	14.67 b	295.26 b	80.01 b	0.27 b	4.11 a

The means of the column with same letters were not significantly different based on Tukey method ($p<0.05$).





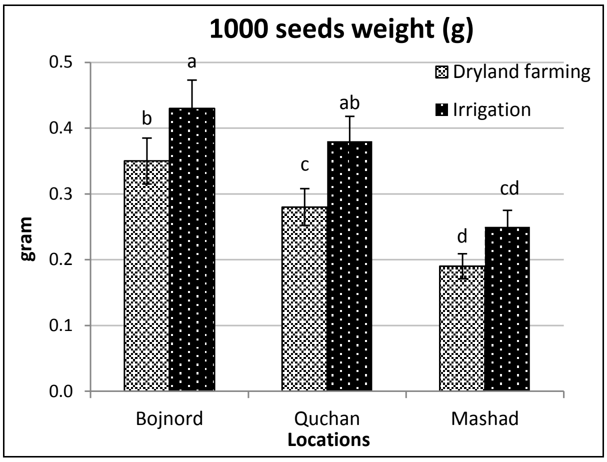


Fig. 1 Means of agronomical traits in *Dracocephalum kotschyi* Boiss. evaluated in three locations over two farming systems

For flavonoid, carotenoid and potassium, the higher values were obtained in Bojnord and for chlorophyll a and b the higher mean values were related to Quchan followed by Mashad (Table 6).

In comparisons between two farming methods, the higher values of proline, antioxidant activity (DPPH), phenol, flavonoid, carbohydrate and Potassium content were obtained in dryland farming systems. In contrast, the higher values of protein, chlorophyll a and chlorophyll b, carotenoid, nitrogen and Potassium content were obtained in irrigation experiments (Table 7).

One of the known effects of water stress is the reduction of the photosynthetic rate in response to decreased CO_2 availability due to stomatal closure [27]. The decrease of chlorophyll and carotenoids in drought stressed plants is documented [28,29]. The decrease of chlorophyll content might cause a reduction in growth parameters of plants under water stress condition then, drought stressed plants significantly reduced their growth [30,31].

In comparisons between locations the higher values of chlorophyll a and b were obtained in Quchan this may related to higher altitude, higher precipitation and mean annual humidity in these areas (Table 1). Olennikov *et al*. (2017) showed that the low temperature treatment caused the reorientation of chlorophyll synthesis also with respect to total carotene level [32].

The higher and lower total flavonoid content was measured in the dry land farming and irrigation system, respectively. Similar to our result, Halimeh *et al*. (2017) in *Dracocephalum moldavica* L. found the severe drought increased the flavonoid content [33].

In the experiment, we determined significant influence drought stress on the total phenolic content (Table 7). The highest and lowest total phenolic content was measured in dryland farming and irrigation system, respectively. Polyphenols and flavonoids are among the most adaptable PSMs, helping plants to cope with different stress conditions [34]. Drought stressed may to increase in secondary metabolites content. Similar to our result, Bettaieb *et al*. (2009) in *Salvia officinalis*, found an increase in phenols contents in half irrigated plants [35]. Similarly, to the total phenolic content, the highest and lowest antioxidant activities of the samples were determined via DPPH method was measured under the drought conditions (antioxidant capacity of the dryland farming was two times higher than normal irrigation).

Also proline, an amino acid, is a compatible solute involved in cell osmotic adjustment and protection of cell components during dehydration [36].

Drought induces oxidative stress in plants, in which reactive oxygen species (ROS) are produced, Plant resistance to ROS is associated with an increase in antioxidant activity to prevent stress damage [37]. In a wide range of experiments was highlighted that plants under drought stress showed an increase in secondary metabolites content [14]. The amount of proline in dryland farming was two times higher than irrigated system. This indicated that this plant is largely resistant to drought stress [38]. Drought stress causes oxidative damage by the accumulation of reactive oxygen species (ROS) that inhibit photosynthesis, stomatal closure and alter the activities of enzymes, water stress increased the endogenous levels of sugars, proline and other osmolytes [38]. Kabiri *et al*. (2018) showed that the activity soluble sugar and protein in *D. moldavica* increased in drought-stressed plants rather than in the control groups [39].

Our results also showed that plants grown in Quchan had significantly higher mean values of agronomical and physiological traits, which may be related to higher altitude (2032 m) and climatic condition. There is some reports that environmental factors as (temperature, drought, soil properties) could influence essential oils composition [40,41]. Similarly, Lebaschy and Sharifi (2010) reported that the secondary metabolite production in plants fluctuated with changing environmental conditions [42].

In comparisons between two farming methods, the higher values of proline, antioxidant activity (DPPH) phenol, flavonoid, carbohydrate and potassium content were obtained in dryland farming systems than that of normal irrigation. It is expected since medicinal plants unlike other crops that are in the stress conditions may produce more active components in dry conditions and thereby increase their economic efficiency. Sabih *et al*. (1999) in assessing *Cymbopogon martini* found oil production and growth are influenced by environmental factors such as drought stress [41]. There is some reports that oil yields of it which is obtained moderate stress. But, severe stress by altering the metabolic pathways and reducing plant growth reduce soil yield [3,44].

Table 5 ANOVA of physiological traits for *Dracocephalum kotschyi* Boiss. evaluated in three locations under two farming systems

Source	DF	Proline	DPPH	Phenol	Flavonoid	Carbohydrate	Protein	Chlorophyll a	Chlorophyll b	Carotenoid	N	P	K
Location (L)	2	1.201 **	0.403 **	0.080 **	11.6 **	0.46	686 **	5.41 **	145.7 **	22.42 **	154.3 **	105 **	2.570 **
Rep (Loc)	6	0.012	0.034	0.005	0.09	0.41	0.20	0.04	1.23	0.011	0.011	0.36	0.001
Farming (F)	1	1.989 **	0.182 **	0.119 *	8.2 **	16.8 **	779 **	2.37 **	173.9 **	19.03 **	51.0 **	214 **	1.115 **
L* F system	2	0.218 **	0.115 **	0.007	0.40 *	0.44	12.20 **	0.02	2.23 *	23.45 **	1.43 **	1.22	0.109 **
Error	6	0.007	0.011	0.014	0.03	0.06	0.100	0.02	0.21	0.001	0.021	0.32	0.002
CV		7.48	30.45	15.61	32.94	34.69	3.04	1.68	15.76	14.62	6.56	3.27	1.19

*significant at the 0.05 probability level; ** significant at the 0.01 probability level.

Table 6 Means of physiological traits in *Dracocephalum kotschyi* Boiss. evaluated in three locations.

Locations	Proline mg g^{-1}FW	DPPH mg g^{-1}FW	Phenol mg g^{-1}FW	Flavonoid mg g^{-1}FW	*Carbohydrate* mg g^{-1}FW	*Protein* mg g^{-1}FW	Chlorophyll a mg g-1FW	Chlorophyll b mg g-1FW	Carotenoid mg g-1FW	N %	P ppm	K %
Bojnord	0.62c	0.21 b	0.65 b	0.69 a	7.1 a	10.41 b	7.16 b	2.31 b	0.14 a	1.56 b	14.15 c	4.51 a
Quchan	1.51 a	0.57 a	0.88 a	0.42 c	7.1 a	13.78 a	8.91 a	3.10 a	0.07 b	2.13 a	15.78 b	3.23 c
Mashad	1.19 b	0.08 b	0.71 b	0.49 b	7.5 a	7.02 c	7.41 b	3.21 a	0.07 b	1.11 c	22.08 a	4.12 b

The means of the column with same letters were not significantly different based on Tukey method p<0.05).

Table 7 Means of physiological traits in *Dracocephalum kotschyi* Boiss. under two farming systems

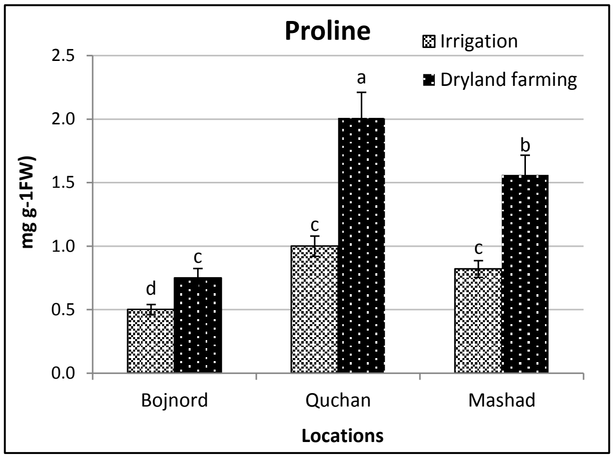
Farming system	Proline mg g^{-1}FW	DPPH mg g^{-1}FW	Phenol mg g^{-1}FW	Flavonoid mg g^{-1}FW	Carbohydrate mg g^{-1}FW	Protein mg g^{-1}FW	Chlorophyll a mg g-1FW	Chlorophyll b mg g-1FW	Carotenoid mg g-1FW	N %	P ppm	K %
Irrigation	0.77 b	0.19 b	0.67 b	0.47 b	6.2 b	11.06 a	8.19 a	3.18 a	0.11 a	1.77 a	20.79 a	3.70 b
Dryland	1.44 a	0.39 a	0.83 a	0.60 a	8.2 a	9.74 b	7.47 b	2.56 b	0.08 b	1.43 b	13.89 b	4.20 a

The means of the column with same letters were not significantly different based on Tukey method p<0.05).

Table 8 Means of physiological traits in *Dracocephalum kotschyi* Boiss. evaluated in three locations under two farming systems

Locations	Stress	Proline mg g^{-1}FW	DPPH mg g^{-1}FW	Phenol mg g^{-1}FW	Flavonoid mg g^{-1}FW	*Carbohydrate* mg g^{-1}FW	Protein mg g^{-1}FW	Chlorophyll a mg g-1FW	Chlorophyll b mg g-1FW	Carotenoid mg g-1FW	N %	P ppm	K %
Bojnord	Irrigation	0.50 d	0.17 c	0.61 b	0.61 b	5.8 d	10.97 c	7.55 cd	2.68 b	0.16 b	1.76 c	17.13 c	4.18 b
	Dryland	0.75 cd	0.25 bc	0.70 b	0.77 a	8.3 ab	9.84 d	6.76 e	1.94 c	0.12 c	1.37 d	11.17 d	4.83 a
Quchan	Irrigation	1.00 c	0.33 b	0.76 ab	0.38 d	6.4 cd	14.36 a	9.31 a	3.35 a	0.08 e	2.3 a	19.27 b	2.90 e
	Dryland	2.01 a	0.82 a	0.99 a	0.46 c	7.8 abc	13.19 b	8.52 b	2.85 b	0.07 f	1.95 b	12.30 d	3.56 d
Mashad	Irrigation	0.82 c	0.07 c	0.63 b	0.41 cd	6.6 bcd	7.84 e	7.71 c	3.52 a	0.10 d	1.25 e	25.97 a	4.02 c
	Dryland	1.56 b	0.09 c	0.80 ab	0.58 b	8.5 a	6.19 f	7.11 de	2.90 b	0.07 a	0.97 f	18.20 bc	4.21 b

The means of the column with same letters were not significantly different based on Tukey method p<0.05)


Proline
Irrigation
Dryland farming
mg g-1FW)
2.5
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Bojnord
Quchan
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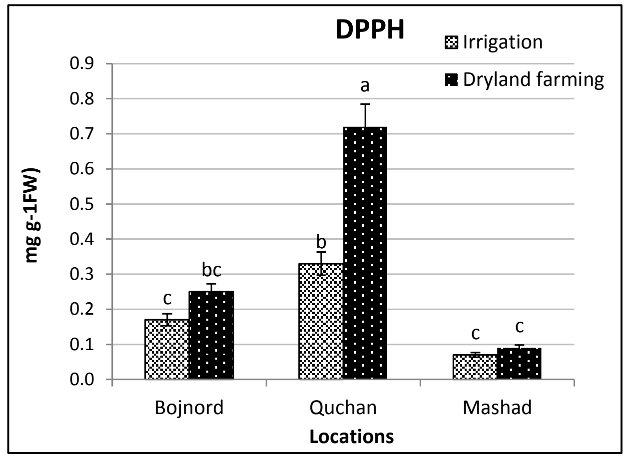
Locations


DPPH
Irrigation
Dryland farming
mg g-1FW)
0.9
0.8
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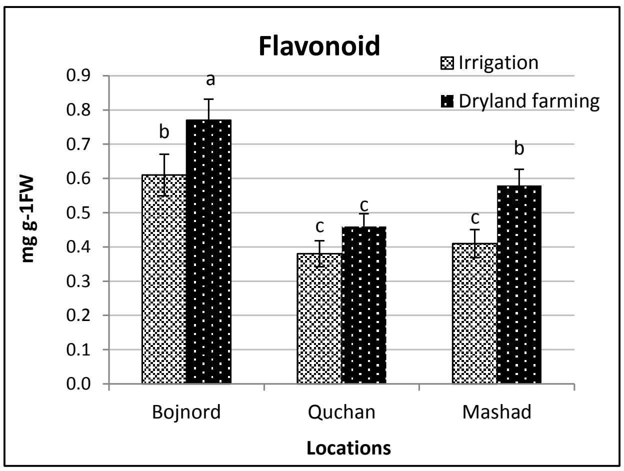
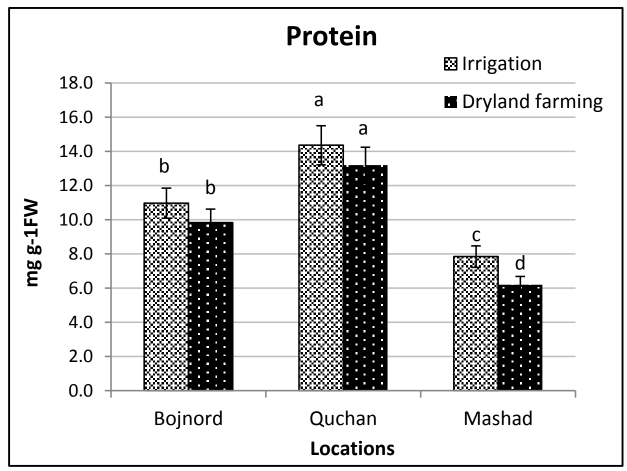
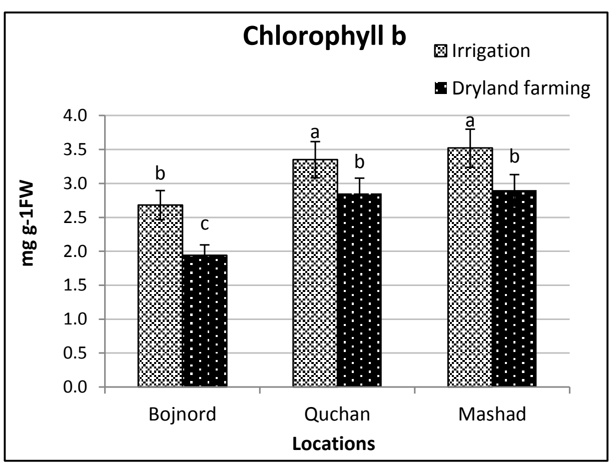
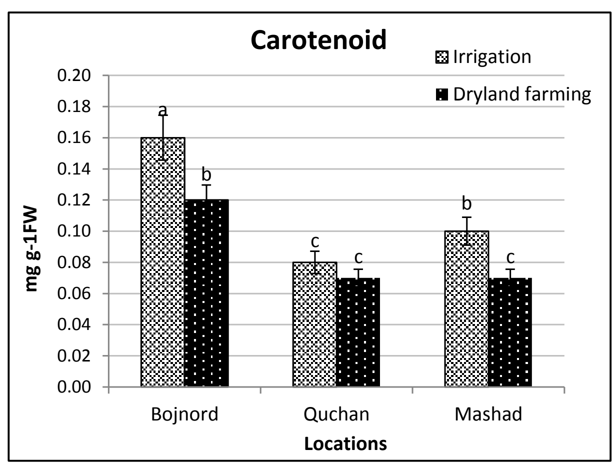

Flavonoid
Irrigation
Dryland farming
mg g-1FW
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
b
a
c
c
c
b
Bojnord
Quchan
Mashad
Locations


Protein
Irrigation
Dryland farming
mg g-1FW
18.0
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
b
b
a
a
c
d
Bojnord
Quchan
Mashad
Locations


Chlorophyll b
Irrigation
Dryland farming
mg g-1FW
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
b
c
a
b
a
b
Bojnord
Quchan
Mashad
Locations


Carotenoid
Irrigation
Dryland farming
mg g-1FW
0.20
0.18
0.16
0.14
0.12
0.10
0.08
0.06
0.04
0.02
0.00
a
b
c
c
b
c
Bojnord
Quchan
Mashad
Locations

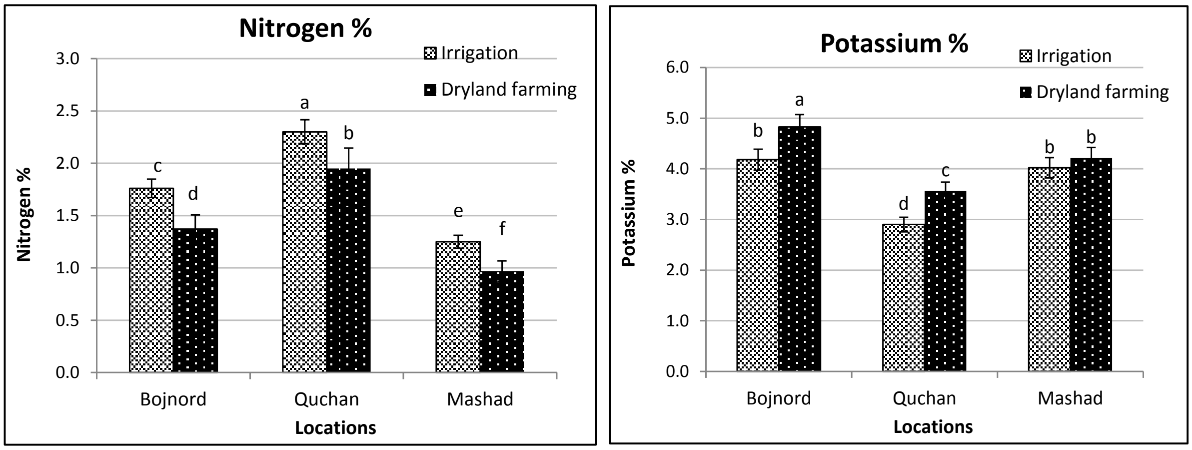


Fig. 2 Means of nitrogen, potassium and physiological traits in *Dracocephalum kotschyi* Boiss. evaluated in three locations under two farming systems.

Conclusion

It was concluded that the higher values of plant height, main branch, leaf area, flower number, fresh weight, dry weight, 1000 seeds weight, protein, chlorophyll a and b, carotenoid, nitrogen and phosphorus were obtained in normal irrigation. In contrast, the higher values of proline, antioxidant activity (DPPH), phenol, flavonoid, carbohydrate, and potassium content were obtained in dryland farming system, respectively. The higher values of antioxidant in medicinal plants are related to moderate stress conditions that may produce more active components in dry land farming conditions and thereby increase their economic efficiency. In comparison of locations, plants grown in Quchan had significantly higher means values for many agronomical and physiological traits, This was related to the area higher altitude (2032 m), coupled with higher annual precipitation and lower annual temperature than that to other two sites. Therefore domestications and cultivations of *D. kotschyi* in higher altitude (higher than 2000 m) under dryland farming system is recommended.

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