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The effect of drought stress on three clary sage (*Salvia sclarea* L.) populations from different habitats

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Abstract

Clary sage (*Salvia sclarea* L.) has various endemic populations in Iran. The main compound in essential oil of this plant is linalyl acetate. To compare three main populations from Iran and to assess their response to drought stress, this experiment was conducted at Alborz Station, Research Institute of Forests and Rangelands, Karaj, Iran. The experiment was conducted in split plot in the form of a randomized complete block design with three replications. The main factor was three clary sage populations (Karaj, Semnan and Esfehan) and the sub factor was three drought stress levels (irrigation at 90, 60 and 30% FC). Results showed that among the studied populations, biologic yield and essential oil yield were the highest in Esfehan population (3407.7 and 14.3 kg/ha, respectively). Increasing the severity of drought stress suppressed all measured traits, except for the essential oil content and potassium content which were higher in 30% treatment. Studying the interaction of two factors showed that biologic yield was the highest in Esfehan × 90% (5403.3 kg/ha); however, essential oil yield was the highest in Semnan × 90% (24.4 kg/ha). Results generally indicated that Esfehan population is the highest yielding one, and drought stress would be beneficial when enhancement of essential oil content is desired.

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Introduction

Sage is a herbaceous plant of Lamiaceae family; a family with 58 annual and biennial species in Iran which 17 of them are endemic to the area (Mozaffarian, 2004). Clary sage (*Salvia sclarea* L.) is a diploid member of the family with the number of chromosomes 2X = 22 which lives mostly two years and rarely three years (Furia and Bellanca, 1995). This plant has very low essential oil content; the main compound in the essential oil is linalil acetate (Bernath, 1995).

To obtain the highest yield, irrigation must be conducted in the way that prevents both drought stress and water logging stress (Calvino *et al*., 2003; Abbaszadeh *et al*., 2012; Ardakani *et al*., 2012). Marzi *et al*. (1993) reported that licorice (*Glycyrrhiza glabra* L.) growth and yield were directly affected by irrigation and water availability. In another experiment it was found that in all drought stressed cultivars of palmarosa (*Cymbopogon martini*), RWC decreased when the severity of drought stress increased (Fatima *et al*., 1999).In addition to the reduction of plant growth, drought stress also reduces the diffusion rate of mineral nutrients from soil solution to plant roots (Alam, 1999). In most cases, drought stress usually increases N, K, Ca, Mg, Na and Cl content but reduces P and Fe content (Abdel Rahman *et al*., 1971). In different experiments, drought stress increased P, Ca, Mg and Zn content in alfalfa (Kidambi *et al*., 1990); however, decreased P content in pepper (Turner, 1985). Muni *et al*. (1995) found reduced N absorption and increased K and Ca absorption rate in drought stressed bergamot mint.

Essential oil content and composition are another factors affected by drought stress. Studies showed that drought stress increased essential oil content in peppermint (Charles *et al*., 1990) and *Origanum majorana* (Rizopoulous and Diamantoglon, 1991). Simon *et al*. (1992) found 100% enhancement of the essential oil content in fresh leaves of basil; however, Chatterjee *et al*. (1995) reported the reduction of essential oil yield as the result of reduced water availability in *Cymbopogon flexuosus*. In another experiments on thyme it was observed that essential oil content, essential oil yield and thymol content were all the highest when irrigation was conducted at 70% FC (Letchamo *et al*., 1994; Letchamo and Gosselin, 1996). Regarding the different response of medicinal plants to drought stress; and presence of various clary sage populations in Iran, this experiment was conducted to evaluate the effect of drought stress on growth, nutrient uptake and essential oil of Iranian clary sage populations.

Material and methods

This experiment was conducted in 2012 at the research field of Alborz Research Station, Research Institute of Forests and Rangelands, Karaj, Iran. The experiment was conducted in split plot in the form of a randomized complete block design with three replications.

Used factors

The main factor was three clary sage populations (Karaj, Semnan and Esfehan) and the sub factor was three drought stress levels (irrigation at 90, 60 and 30% FC). Climatic factors for the three habitats are listed in Table 1.

The soil profile

The soil at the test site was a loam (clay, 16%; silt, 40%; sand, 44%) with the pH of 7.36 and EC of 1.33 ds/m. Other physico-chemical properties of the soil are listed in Table 2. Experimental plots were 3 × 3 m and planting pattern was 50 (rows) × 40 (plants) cm.

Drought stress and measured traits

To apply the drought stress treatments, a TDR along with the gravimetric method was used. The following traits were measured in this study: the number of leaves, the number of defoliated leaves, total number of leaves, leaf length, leaf width, leaf area, petiole length, stem diameter, length of the longest internode, inflorescence length, the number of inflorescences, inflorescence yield, leaf yield, petiole yield, stem yield, shoot yield, biologic yield (root + shoot), plant height, essential oil content, essential oil

yield, chlorophyll content and nutrients content (NPK), chlorophyll a, chlorophyll b, total chlorophyll and the relative water content. To obtain the essential oil, samples were collected at flowering stage and essential oil was produced by hydro-distillation using a clevenger in 2.5 h. Chlorophyll content was measured by the method of Arnon (1986).

Statistical analysis

Normality of the data was tested prior to analysis of variance. Then, data were analyzed using SAS 9.1 (2002) and mean comparison was conducted by the Duncan's multiple range test at P≤0.05.

Results

Analysis of variance indicated the significant effect of population on inflorescence dry weight, leaf dry weight, petiole dry weight, main stem dry weight, biologic yield, total dry weight of leaf + petiole + inflorescence, essential oil content, essential oil yield, chlorophyll a, N and P at P≤0.01 (Table 3).

Results showed that the effect of drought stress was significant on the number of tillers, plant height, inflorescence length, the number of inflorescences, the number existing leaves, total number of leaves, leaf width, petiole length, stem diameter, length of the longest internode, inflorescence dry weight, leaf dry weight, petiole dry weight, main stem dry weight, biologic yield, total dry weight of leaf + petiole + inflorescence, essential oil content and composition, chlorophylls a, b and total, relative water content, and NPK content at P≤0.01 (Table 3).

Analysis of variance also indicated the significant effect of the interaction of population × drought stress on inflorescence dry weight, petiole dry weight, main stem dry weight, biologic yield, total dry weight of leaf + petiole + inflorescence, essential oil content and composition, chlorophyll a, total chlorophyll, relative water content, and NPK content at P≤0.01 and on leaf dry weight at P≤0.05 (Table 3).

Mean comparison of the effect of population on the measured traits (Table 4) showed that the highest inflorescence length achieved in Karaj population (37.07 cm). The highest dry weight of inflorescence achieved in Semnan population (526.67 kg/ha). Dry weight of leaf, petiole and main stem was the highest in Esfehan population (1379.2, 498.56 and 1242.0 kg/ha, respectively). Biologic yield was the highest in Esfehan population (3407.7 kg/ha). Shoot yield was the highest in Semnan and Esfehan populations (1925.6 and 2165.7 kg/ha). Essential oil content was the highest in Semnan population (0.77%). Essential oil yield was the highest in Semnan and Esfehan populations (14 and 14.3 kg/ha). The lowest content of chlorophyll b achieved in Karaj population (0.63 mg/l). The highest relative water content (80%) and P content (0.5 mg/kg) were related to Semnan population (Table 4).

Mean comparison of drought stress (Table 5) represented that the control treatment (non-stressed) had the highest number of tillers, number of inflorescences, number of existing leaves and number of total leaves (11.3, 17.17, 74.3 and 92.71, respectively). Plant height, leaf width, petiole length, stem diameter and length of the longest internode were also the highest in the control treatment (106.35, 9.55, 12.3, 1.68 and 19.73 cm, respectively). The highest value of inflorescence dry weight, leaf dry weight, petiole dry weight, stem dry weight, biologic yield and shoot yield was related to the 90% of the field capacity (705.78, 1585.0, 644.67, 1683.22, 46187.7 and 2935.4 kg/ha). Essential oil content was the highest in the severe drought stress which was irrigated at 30% FC (0.8%); however, the highest essential oil yield achieved in the control (18.3 kg/ha). Mean comparison also showed that the highest contents of chlorophyll a, total chlorophyll, relative water content, N and P were related to the control (Table 5).

Mean comparison of the interactions (Table 6) indicated that the highest number of tillers and plant height in all three populations achieved in the control

(non-stressed treatment). Severe drought stress reduced inflorescence length of Karaj and Esfehan populations. Results generally indicated that Esfehan × normal irrigation resulted in the highest value of most of the measured traits; some traits showed their highest values in other interactions. The highest dry weight of inflorescence, petiole dry weight, total dry weight of leaf + petiole + inflorescence and the essential oil yield achieved in Semnan population × non-stressed control (991.33, 857.33, 3554.0 and 2.44 kg/ha). Esfehan × 90% FC had also the highest leaf dry weight, stem dry weight and biologic yield (2029, 2137 and 5403.3 kg/ha). The content of the mineral nutrients was also the highest in non-stressed treatment.

Determining the correlation of the measured traits (Table 7) indicated that inflorescence dry weight had significantly positive correlation with petiole dry weight (r = 0.79**), stem dry weight (r = 0.70*), biologic yield (r = 0.78*), shoot yield (r = 0.81**) and essential oil yield (r = 0.79*). Leaf dry weight had significantly positive correlation with petiole dry weight (r = 0.93**), stem dry weight (r = 0.92**), biologic yield (r = 0.96**), shoot yield (r = 0.96**) and essential oil yield (r = 0.92**). Petiole dry weight was significantly correlated to stem dry weight (r = 0.84**), biologic yield (r = 0.95**), shoot yield (r = 0.98**) and essential oil yield (r = 0.99**). Stem dry weight had significantly positive correlation with biologic yield (r = 0.96**), shoot yield (r = 0.91**) and essential oil yield (r = 0.82**) and negative correlation with essential oil content (r = -0.81**). Biologic yield was positively correlated to shoot yield (r = 0.98**) and essential oil yield (r = 0.94**) and negatively correlated to essential oil content (r = -0.70*). A significant correlation was also observed between shoot yield and essential oil yield (r = 0.97**). Chlorophyll a had significantly positive correlation and chlorophyll b had significantly negative correlation with most of the measured traits (Table 7).

Table 1. Climatic factors (mean annual) for the three habitats seeds were collected from.

Parameter	Karaj	Semnan	Esfehan
Latitude and longitude	35°47′ N 50°56′ E	35°34′ N 53°23′ E	32°38′ N 51°39′ E
Elevation (m above the sea level)	1261	1900	1590
Average minimum air temperature (°C)	9.4	12.02	9.05
Average maximum air temperature (°C)	21.4	23.83	23.42
Average air temperature (°C)	15.4	18.4	16.4
Precipitation	262	139.5	237.6
RH (%)	49	41.8	46.2
Sunshine (h)	3029.5	3002.3	3274.2

Table 2. Physico-chemical properties of the test site soil.

Total N (%)	P_{ava} (ppm)	K_{ava} (ppm)	OC (%)	Fe (ppm)	Zn (ppm)	CU (ppm)	Mn (ppm)	B (ppm)	TNV (%)	SP (%)
0.08	8.4	278.4	0.79	7.72	0.5	1.34	17.72	0.464	10.1	24.63

Table 3a. Analysis of variance of the effect of treatments on the measured traits.

SOV	df	Mean Squares												
		The number of tillers	Plant height	Inflorescence length	Number of Inflore-scences	Number of existing leaves	Total number of leaves	Leaf length	Leaf width	Petiole length	Stem diam-eter	Length of the longest internode	Inflore-scence dry weight	Leaf dry weight
Block	2	*	*	ns	ns	ns	*	*	ns	**	**	**	**	**
Population (A)	2	ns	ns	**	ns	ns	ns	ns	ns	ns	ns	ns	**	**
Error	4	10.11	444.65	373.7	18.72	540.27	1233.44	1.39	5.8	4.74	0.23	46.3	6038.98	148248.98
Stress (B)	2	**	**	**	**	**	**	ns	**	**	**	**	**	**
A × B	4	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	**	*
Error	12	6.32	95.15	29.39	4.68	210.74	290.43	4.06	1.42	1.03	0.02	3.2	2191.48	72446.72
CV (%)	-	29.21	11.22	17.62	15.68	29.64	25.14	16.07	15.26	11.07	12.6	10.63	11.68	26.36

ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 3b. Analysis of variance of the effect of treatments on the measured traits.

SOV	df	Mean Squares												
		Petiole dry weight	Main stem dry weight	Biologic yield	Shoot yield	Essential oil content	Essential oil yield	Chlorophyll a	Chlorophyll b	Total chlorophyll	Relative water content	N	P	K
Block	2	**	**	**	**	**	ns	**	**	**	**	**	**	**
Population (A)	2	**	**	**	**	**	**	**	ns	ns	ns	**	**	ns
Error	4	4405.772	129.42	119037.1	5137512.2	20.00002	0.1	0.04	0.021	0.083	6.26	0.010	0.0005	0.188
Stress (B)	2	**	**	**	**	**	**	**	**	**	**	**	**	*
A × B	4	**	**	**	**	ns	**	**	ns	**	*	**	**	ns
Error	12	1854.8	6694.37	68263.8	80321.91	0.00001	0.07	0.04	0.009	0.052	2.98	0.02	0.007	0.045
CV (%)	-	10.5	7.9	9.11	15.47	5.48	21.99	9.7	13.97	8.54	2.2	4.8	5.96	6.22

ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 4a. The variation of the measured traits among the tested populations.

Population	The number of tillers	Plant height (cm)	Inflore-scence length (cm)	Number of Inflores-cences	Number of existing leaves	Total number of leaves	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Stem diameter (cm)	Length of the longest internode (cm)	Inflor-escence dry weight (kg/ha)	Leaf dry weight (kg/ha)
Karaj	7.41a	88.23a	37.07a	14.31a	51.66a	63.72a	13.02a	8.54a	9.25a	1.38a	16.84a	387.0b	730.4b
Semnan	7.57a	85.67a	23.15b	14.15a	46.04a	69.35a	12.08a	7.74ab	8.81a	1.27a	17.8a	526.67a	953.1b
Esfehan	7.06a	86.79a	32.03a	12.92a	49.21a	70.22a	12.53a	7.14b	9.44a	1.23a	15.88a	287.9c	1379.2a

Means in a column followed by the same letter are not significantly different (P≤0.05).

Table 4b. The variation of the measured traits among the tested populations.

Population	Petiole dry weight (kg/ha)	Main stem dry weight (kg/ha)	Biologic yield (kg/ha)	Shoot yield (kg/ha)	Essential oil content (%)	Essential oil yield (kg/ha)	Chlorophyll a (mg/l)	Chlorophyll b (mg/l)	Total chlorophyll (mg/l)	Relative water content (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)
Karaj	286.33c	329.33b	2333.1c	1403.8b	0.6b	8.8b	1.94a	0.63b	2.57a	78.08b	2.76a	0.41c	3.2844a
Semnan	445.78b	934.56b	2860.1b	1925.6a	0.77a	14.0a	1.96a	0.68ab	2.64a	80.0a	2.60b	0.50a	3.4922a
Esfehan	498.56a	1242.0a	3407.7a	2165.7a	0.7b	14.3a	2.06a	0.73a	2.8a	77.5b	2.80a	0.43b	3.4767a

Means in a column followed by the same letter are not significantly different (P≤0.05).

Table 5a. The effect of drought stress on the measured traits.

Popu-lation	The number of tillers	Plant height (cm)	Inflore-scence length (cm)	Number of Inflore-scences	Number of existing leaves	Total number of leaves	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Stem dia-meter (cm)	Length of the longest inter-node (cm)	Inflore-scence dry weight (kg/ha)	Leaf dry weight (kg/ha)
30% FC	3.91c	66.88c	23.7b	9.73c	26.1c	41.5c	11.86a	6.11c	6.54c	1.0c	13.63c	192.44c	495.2c
60% FC	6.84b	87.45b	36.72a	14.47b	46.52b	69.08b	12.41a	7.76b	8.66b	1.21b	17.16b	303.33b	982.6b
90% FC	11.3a	106.35a	31.85a	17.17a	74.3a	92.71a	13.36a	9.55a	12.3a	1.68a	19.73a	705.78a	158.0a

Means in a column followed by the same letter are not significantly different (P≤0.05).

Table 5b. The effect of drought stress on the measured traits.

Popula-tion	Petiole dry weight (kg/ha)	Main stem dry weight (kg/ha)	Biologic yield (kg/ha)	Shoot yield (kg/ha)	Essen-tial oil content (%)	Essential oil yield (kg/ha)	Chlorophyll a (mg/l)	Chlorophyll b (mg/l)	Total chlorophyll (mg/l)	Relative water content (%)	N (mg/kg)	P (mg/kg)	K (mg/kg)
30% FC	299.67c	458.67c	1376.0c	917.3c	0.8a	7.1c	1.47c	0.77a	2.25c	70.16c	2.05c	0.31c	3.88a
60% FC	356.33b	964.0b	2606.2b	1642.2b	0.7b	11.6b	2.028b	0.71a	2.74b	79.61b	2.73b	0.4b	3.43b
90% FC	644.67a	1683.22a	4618.7a	2935.4a	0.6c	18.3a	2.46a	0.55b	3.02a	85.87a	3.4a	0.63a	2.95c

Means in a column followed by the same letter are not significantly different (P≤0.05).

Table 6a. The effect of interaction of population × drought stress on the measured traits.

Treatments	The number of tillers	Plant height (cm)	Inflore-scence length (cm)	Number of Inflore-scences	Number of existing leaves	Total number of leaves	Leaf length (cm)	Leaf width (cm)	Petiole length (cm)	Stem diameter (cm)	Length of the longest internode (cm)	Inflore-scence dry weight (kg/ha)	Leaf dry weight (kg/ha)
Karaj × 30% FC	4.86cd	71.66cde	28.66bc	9.76c	28.2bc	41.63d	12.26a	7.26cd	6.73de	1.03de	14.7de	237.33d	429.7d
Karaj × 60% FC	6.96bcd	84.16bcd	43.16a	14.63ab	50.53abc	65.67abcd	13.5a	8.46abcd	8.9c	1.26cd	16.1cd	333.33c	741.0cd
Karaj × 90% FC	10.4ab	108.86a	39.4a	18.53a	76.27a	83.87ab	13.3a	9.9a	12.13ab	1.86a	19.73ab	590.33b	1020.7c
Semnan × 30% FC	4.43cd	68.33de	19.33c	11.43bc	23.33c	48.0cd	11.73a	6.66d	6.43e	1.06de	14.2de	235.0d	469.0d
Semnan × 60% FC	7.8bc	90.6b	26.96bc	14.36ab	36.37bc	61.5bcd	11.13a	7.43bcd	8.56cd	1.52bc	19.03abc	353.67c	685.0cd
Semnan × 90% FC	10.5ab	98.1ab	23.16bc	16.66a	78.43a	98.57a	13.4a	9.13abc	11.43b	1.53bc	20.16a	991.33a	1705.3ab
Esfehan × 30% FC	2.43d	60.66e	23.08bc	8.0c	26.77bc	34.87d	11.6a	4.4e	6.46e	0.9e	12.0e	105.0e	587.0cd
Esfehan × 60% FC	5.76bcd	87.61bc	40.03a	14.43ab	52.67ab	80.1abc	12.6a	7.4bcd	8.53cd	1.13de	16.36bcd	223.0d	1521.7b
Esfehan × 90% FC	13.0a	112.11a	33.0ab	16.33a	68.2a	95.7a	13.4a	9.63ab	13.34a	1.66ab	19.3abc	535.67b	2029.0a

Means in a column followed by the same letter are not significantly different (P≤0.05).

Table 6b. The effect of interaction of population × drought stress on the measured traits.

Treatments	Petiole dry weight (kg/ha)	Main stem dry weight (kg/ha)	Biologic yield (kg/ha)	Shoot yield (kg/ha)	Essen-tial oil content (%)	Essential oil yield (kg/ha)	Chloro-phyll a (mg/l)	Chlorop-hyll b (mg/l)	Total chlorophyll (mg/l)	Relative water content (%)	N (mg/ kg)	P (mg/ kg)	K (mg/ kg)
Karaj × 30% FC	204.0e	387.0f	1258.0d	871.0c	0.7b	6.5c	1.46e	0.723abc	2.18e	71.6d	2.25de	0.28e	3.89a
Karaj × 60% FC	280.0e	1067.0d	2422.3c	1354.3c	0.6c	8.7c	2.04bc	0.687abc	2.73bc	77.71c	2.85c	0.35d	3.27bc
Karaj × 90% FC	375.0d	1333.0c	3319.0b	1986.0b	0.5e	11.2c	2.31b	0.474d	2.78b	84.95a	3.2b	0.57b	2.69d
Semnan × 30% FC	211.0e	521.0f	1436.0d	915.0c	0.8a	7.6c	1.57de	0.762ab	2.33cde	71.66d	2.02ef	0.32de	3.85a
Semnan × 60% FC	269.0e	703.0e	2010.7c	1307.7c	0.7b	9.8c	2.21b	0.720abc	2.93b	81.63b	2.47d	0.49c	3.5ab
Semnan × 90% FC	857.33a	1579.67b	5133.7a	3554.0a	0.6c	24.4a	2.11bc	0.550d	2.7bcd	86.61a	3.34b	0.66a	3.12bc
Esfehan × 30% FC	274.0e	468.0f	1434.0d	966.0c	0.7b	7.3c	1.39e	0.84a	2.22de	67.2e	1.87f	0.30de	3.9a
Esfehan × 60% FC	520.0c	1121.0d	3385.7b	2264.7b	0.7b	16.2b	1.84cd	0.73abc	2.6bcde	79.5bc	2.85c	0.35d	3.5ab
Esfehan × 90% FC	701.67b	2137.0a	5403.3a	3266.3a	0.6c	19.3b	2.97a	0.64bcd	3.6a	86.0a	3.68a	0.65a	3.03cd

Means in a column followed by the same letter are not significantly different (P≤0.05).

Table 7a. The correlation of the measured traits.

Ttraits	Number of tillers	Plant height	INF length	Number of INF	Number of existing leaves	Total number of leaves	Leaf length	Leaf width	Petiole length	Stem diameter	Longest internode	INF DW	Leaf DW	Petiole DW	Stem DW	Biologic yield	Shoot yield	EO content
Number of tillers	1																	
Plant height	0.96**	1																
INF length	0.3ns	0.46ns	1															
Number of INF	0.87**	0.93**	0.50ns	1														
Number of existing leaves	0.86**	0.89**	0.44ns	0.89**	1													
Total number of leaves	0.89**	0.91**	0.38ns	0.90**	0.93**	1												
Leaf length	0.66ns	0.65ns	0.59ns	0.67*	0.82**	0.73*	1											
Leaf width	0.91**	0.91**	0.49ns	0.91**	0.85**	0.84**	0.78*	1										
Petiole length	0.96**	0.96**	0.38ns	0.88**	0.92**	0.91**	0.73*	0.87**	1									
Stem diameter	0.92**	0.93**	0.38ns	0.91**	0.89**	0.82**	0.70*	0.90**	0.94**	1								
Longest internode	0.91**	0.93**	0.28ns	0.92**	0.83**	0.87**	0.5ns	0.87**	0.86**	0.86**	1							
INF DW	0.79*	0.71*	0.01ns	0.74*	0.83**	0.80**	0.62ns	0.74*	0.76*	0.76*	0.82**	1						
Leaf DW	0.76*	0.75*	0.15ns	0.65ns	0.79**	0.90**	0.63ns	0.61ns	0.81**	0.61ns	0.65ns	0.63ns	1					
Petiole DW	0.71*	0.65ns	0.04ns	0.59ns	0.79**	0.86**	0.61ns	0.55ns	0.74*	0.57ns	0.64ns	0.79**	0.93**	1				

Ttraits	Number of tillers	Plant height	INF length	Number of INF	Number of existing leaves	Total number of leaves	Leaf length	Leaf width	Petiole length	Stem diameter	Longest internode	INF DW	Leaf DW	Petiole DW	Stem DW	Biologic yield	Shoot yield	EO content
Stem DW	0.90**	0.88**	0.37ns	0.79**	0.88**	0.93**	0.77*	0.79**	0.94**	0.81ns	0.75*	0.70*	0.92**	0.84**	1			
Biologic yield	0.86**	0.83**	0.23ns	0.76*	0.89**	0.95**	0.72*	0.73*	0.90**	0.75ns	0.76*	0.78*	0.96**	0.95**	0.96**	1		
Shoot yield	0.81**	0.78*	0.15ns	0.71*	0.86**	0.93**	0.67*	0.67*	0.84**	0.69ns	0.74*	0.81**	0.96**	0.98**	0.91**	0.98**	1	
EO content	-0.78*	-0.83**	-0.69*	-0.76*	-0.86**	-0.73*	-0.83**	-0.78*	-0.87**	-0.85**	-0.64ns	-0.53ns	-0.63ns	-0.51ns	-0.81**	-0.70*	-0.62ns	1
EO yield	0.71*	0.67*	0.04ns	0.63ns	0.78*	0.88**	0.57ns	0.57ns	0.73*	0.56ns	0.68*	0.79*	0.92**	0.99**	0.82**	0.94**	0.97**	-0.47ns
CHL a	0.93**	0.92**	0.38ns	0.81**	0.73*	0.80**	0.55ns	0.80**	0.91**	0.82**	0.82**	0.57ns	0.73*	0.59ns	0.87**	0.79*	0.71*	-0.75*
CHL b	-0.81**	-0.83**	0.35ns	-0.87**	-0.89**	-0.77*	-0.71*	-0.89**	-0.83**	-0.93**	-0.84**	-0.83**	-0.49ns	-0.53ns	-0.66*	-0.65*	-0.63ns	0.76*
Total CHL	0.86**	0.84**	0.34ns	0.70*	0.61ns	0.72*	0.44ns	0.68*	0.82**	0.70*	0.72*	0.43ns	0.70*	0.53ns	0.84**	0.73*	0.64*	-0.67*
RWC	0.94**	0.96**	0.37ns	0.95**	0.89**	0.94**	0.61ns	0.89**	0.91**	0.87**	0.80**	0.81**	0.77*	0.73*	0.85**	0.85**	0.83**	-0.72*
N	0.94**	0.94**	0.49ns	0.88**	0.93**	0.95**	0.82**	0.90**	0.96**	0.86**	0.85**	0.75*	0.87**	0.80**	0.96**	0.93**	0.89**	-0.85**
P	0.93**	0.88**	0.05ns	0.81**	0.84**	0.86**	0.50ns	0.76*	0.92**	0.87**	0.90**	0.87**	0.74*	0.77*	0.84**	0.86**	0.84**	-0.65*
K	-0.87**	-0.93**	0.54ns	-0.94**	-0.94**	-0.85**	-0.71*	-0.89**	-0.93**	-0.97**	-0.84**	-0.72*	0.63ns	-0.57ns	-0.82*	0.75*	0.69*	-0.90**

INF, inflorescence; EO, essential oil; DW, dry weight.

ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 7a. The correlation of the measured traits.

Traits	Essential oil yield	Chlorophyll a	Chlorophyll b	Total chlorophyll	RWC	N	P	K
Essential oil yield	1							
Chlorophyll a	0.59ns	1						
Chlorophyll b	-0.53ns	-0.61ns	1					
Total chlorophyll	0.53ns	0.98**	-0.45ns	1				
RWC	0.76*	0.86**	-0.82**	0.77*	1			
N	0.79**	0.87**	-0.78*	0.80**	0.92**	1		
P	0.77*	0.84**	-0.77*	0.76*	0.90**	0.83**	1	
K	-0.56ns	-0.8**	-0.91**	-0.68*	-0.88**	-0.88**	-0.81**	1

CHL, chlorophyll; RWC, relative water content;

ns, nonsignificant; *, significant at P≤0.05; **, significant at P≤0.01.

Discussion

Variation in inflorescence yield, leaf yield, petiole yield shoot yield and essential oil percentage and yield among the populations shows that the study and comparison of populations in order to detect the superior populations is an important field of research. A significant variation was observed in the populations of artemisia and camphor (Abbaszadeh, 2011) and basil (Asadollahi, 2011; Hajimohammad, 2011). In countries with different climatic conditions such as Iran, various types of gene expression in different climatic conditions results in the formation of different population; populations must be evaluated in order to select the most yielding ones. Advantages of Semnan population in traits such as inflorescence yield and essential oil percentage, and advantages of Esfehan population in most of the measured traits compared with the Karaj population indicates the adaptability of the plant to new environment and also shows that some features of the plant are controlled by the genetic factors.

As the results of this experiment indicate, drought stress reduced most of the measured traits including the morphologic ones. However, essential oil benefited from the drought stress which shows that plants have different mechanisms to cope with the stress. Reduction of leaf area which means the reduction of the evaporating surface and reduction of photosynthesis rate is a plant response to drought stress. Reduction of plant shoot growth is also due to the reduction of photosynthesis surface and chlorophyll a content, and enhancement of the energy level plants need to absorb water and nutrients from soil.

Generally, drought stress destroys existing chloroplasts and prevents the formation of chlorophyll (Heidari Sharifabad, 2000). Researchers have reported the effect of drought stress on the reduction of leaf chlorophyll content in mustard and anise (Zahtab Salmasi, 2001) and Japanese mint (Misra and Srivastava, 2000).

Results of this experiment showed that the highest relative water content was achieved in the non-stressed treatment; this may be due to preservation of water potential in plant cells (Irrigoyen, 1992). There

is a strong relation between leaf relative water content and photosynthesis, so the reduction of dry matter accumulation under drought stress may be attributed to the reduction of relative water content and consequently photosynthesis. Leaf relative water content is a suitable indicator of plant water status and is also considered as an index of the drought stress tolerance in plants. Different studies have indicated the effect of water stress on the reduction of leaf relative water content (Ogbonnaya *et al*., 1998; Fatima *et al*., 1999; Zahtab Salmasi, 2001). In our experiment, the reduction of leaf relative water content in severe drought stress is probably because plant cells cannot hole sufficient water without the reduction of water potential; this is in contrast with the findings of Irrigoyen *et al*. (1992).

Studying the essential oil content (Table 5) indicated that the essential oil content increased strictly under drought stress. This implies that applying medium or severe drought stress is a useful technique in order to obtain plants with high essential oil content. Although in severe drought stress the content of essential oil increases; however, shoot yield decreases. In essential oil containing plants, such as clary sage, the interaction of essential oil percentage × shoot yield determines the final essential oil yield. These findings were also achieved in other experiments on *Melissa officinalis* (Munne and Algre, 1999), *Cymbopogon martinii* (Saudan *et al*., 2000), *Origanum majorana* (Rizopoulous and Diamantoglon, 1991) and *Trigonnella foenum graecum* L. (Kumari *et al*., 1999).

Mean comparison of the plant mineral nutrients content showed that uptake of N and P increases under non-stressed conditions and uptake of K increases under drought stress. Drought stress usually disturbs nutrients absorption from soil because it reduces plant transpiration and disturbs active transport and membrane permeability which consequently decreases roots absorption ability (Levitt, 1980). Diffusion of nutrients is also much slower in dry soils; reducing the contact of plant roots with the nutrients. Moreover, some nutrients such as P and K become fixed in dry soils and plants cannot absorb them (Heidari Sharifabad, 2000).

Generally, there are conflicting reports about the effect of drought stress on nutrients absorption of different plant species. Muni *et al*. (1995) reported the reduction of plant N content, but Abdel Rahman *et al*. (1971) reported the enhancement of plant N content under drought stress condition. Kidambi *et al*. (1990) also reported that plant P content increased under drought stress.

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