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Original Article

Effects of Irrigation Interval and Nitrogen Amount on Different Clary Sage (*Salvia sclarea* L.) Characters in Karaj

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Abstract

Salvia sclarea L, is an important medicinal plant that its oil has been evaluated for antioxidant, antibacterial, antifungal, anti-inflammatory, antimalarial, anticholinesterase and antiviral. Yield of plants are influenced by environmental and agricultural management factors such as drought stress and nutrition. Plant nutrition is one of the most important factors that positively influence plant production. Nitrogen, an essential plant nutrient is the most recognized in plants for its presence in the structure of the protein molecule. This experiment was conducted in 2011 in Alborz Research Station, Research Institute of Forests and Rangelands, Karaj, Iran. In order to evaluate the effect of drought stress and nitrogen on traits of Clary Sage, The experiment was conducted in split plot based on a randomized complete block design with three replications. The main factor was irrigation period (every 3, 6 and 9 days) and the sub factor was nitrogen application (0,100 and 200 and 400 kg/ha). Results indicated that irrigation interval significantly affected all the measured traits except for petiole yield, chlorophyll b and RWC. Nitrogen significantly affected all the measured traits except for leaf yield, chlorophyll b and RWC. The interaction of irrigation×nitrogen had also a significant effect on all the measured traits. Shoot yield was the highest in the interaction of 3 days×200 kg/ha (3680kg/ha). Oil percentage was the highest in the interaction of 9 days×0 kg/ha (0.05%). This experiment briefly indicated that clary sage can be cultivated in areas with low water supply, and it can produce the highest essential oil yield about 1400 kg/ ha when irrigated every 9 days without fertilizer.

Keywords: Essential oil, Drought stress, Shoot yield, *Salvia sclarea* L.

Introduction

The genus *Salvia* is the largest in the Lamiaceae with over 700 species [1] and possibly as many as 900 species [2]. The essential oil of *S. sclarea* (Clary Sage) is used in the perfumery industry, soft drink and liquor production [3,4]. The oil has shown medicinal utility in aromatherapy for its anxiolytic effects [5] as well as digestive activities [6]. Sages are used for wound treatment, bathing, washing, skin and hair care [7]. *S. sclarea* oil has been evaluated for antioxidant [8], antibacterial [9, 10], antifungal [6,11,12], anti-inflammatory [13], antimalarial [14], anticholinesterase [15] and antiviral [16] and opioid receptor activities [17].

Stress is a factor outside plant's body which damages plant growth [18]. Among the abiotic stresses, drought is the most important one which affects plants periodically in some growth stages, or permanently in all life cycle [19]. Drought stress usually occurs when available water in soil reduces and atmospheric conditions increase water loss through evapotranspiration [20]. A primary symptom of low available water to plants is the loss of turgor pressure and reduction of cell development especially in stems and leaves.

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Reduction of cell development makes the plant smaller in size, which is the characteristic of drought stressed plants. Moreover, drought stress disturbs nutrient absorption and reduces leaves growth. Lower leaf area means lower light absorption and photosynthesis. All these events finally decrease plant growth and yield [21]. Drought stress is induced when moisture at the rhizosphere falls below the permanent wilting point (PWP). So the plant is not able to take up sufficient water, resulting in cell dehydration. Dehydration is reversible until a certain point (elastic point); however, is irreversible if the water loss is too server (plastic point) [22]. However, the time, duration and frequency of drought stress incident, soil properties and so many other factors affect plant tolerance to drought, and different genotypes may also respond differently [23]. Drought stress induces some morphology biological responses in plant such as the reduction of leaf area; shoot growth, enhancement of root growth, stomata closure, and reduction of growth rate, sudden antioxidants and soluble compounds accumulation, and activation of some enzymes [24]. Stephanie *et al*. [25] reported that drought stress reduced stem length and root length of *Salvia splendens*. Lebaschi and Sharifi Ashoorabadi [26] concluded that higher drought stress levels reduced plant height and shoot weight in some medicinal plants such as *Salvia officinalis* and *Achillea millefolium*. Sangwan *et al*. [27] reported that mild drought stress decreased lemon grass height, leaf area and leaf weight. Finally, Ardakani *et al*. [28] reported that drought stress affected shoot yield, essential oil percentage and yield, leaf yield, stem yield, height, the number of tillers, leaf area, stem diameter and the length of internodes in balm (*Melissa officinalis*).

Abbaszadeh *et al*. [29] reported the decrease in RWC of *Melissa officenalis* L. with the increase in the severity of drought stress so that no-stress treatment had the highest RWC (93.369 % on average) and most-severe-stress treatment had the lowest one (54.75%) which was 25% of field capacity.

Yield of plants are influenced by environmental and agricultural management factors [30]. Plant nutrition is one of the most important factors that positively influence plant production. Nitrogen, an essential plant nutrient is most recognized in plants for its presence in the structure of the protein molecule [31]. Nitrogen plays an important role in the synthesis of plant constituents, which are very critical for plant growth and dry matter yield. Nitrogen limiting conditions increase volatile oil production in annual herbs [31]. Nitrogen fertilization has been reported to reduce volatile oil content in *Juniperus horizontalis* Moench. [32].

So, the objective of this experiment was to evaluate the effect of irrigation period and nitrogen application on yield and essential oil and some physiological traits of Clary Sage.

Material and Methods

This experiment was conducted in 2011 in Alborz Research Station, Research Institute of Forests and Rangelands, Karaj, Iran. In this area, average annual precipitation is 235 mm, minimum air temperature is -20 ^{o}C, maximum air temperature is 38 ^{o}C and the dominant winds blow from east and south east. The soil at the test site was loam (clay, 16%; silt, 40%; sand, 44%) with the pH of 7.36. Other physio-chemical properties of the test site soil are presented in Table 1.

The experiment was conducted in split plot in the form of a randomized complete block design with three replications and two factors: irrigation period (every 3, 6 and 9 days) and nitrogen fertilizer (0, 100, 200 and 400 kg/ha) in the sub plots. The size of the plots was 2*3 meters. The distance between the rows was 50 cm. The distance between plants on the rows was 50 cm. Seed planting was carried out directly on the soil.

Chemical fertilizers were added to the land when planting and mixed with soil. Applying drought stress treatments was started from the 6th week after seed sowing. Determine the treatment distance were based on the results of numerous research conducted on the farm), That this distance (every 3, 6 and 9 days) Irrigation equivalent was based on 90%, 60% and 30% field capacity [28, 29]. Harvest was conducted on Oct. 6th when plants entered full flowering stage. In this experiment, the following traits were measured: Leaf yield, Petiole yield and Shoot yield (via scales), essential oil percentage, the essential oil was extracted from the dried leaves using a Clevenger by the method of hydro-distillation at 2 hours [10-12], essential oil yield (shoot yield* essential oil percentage), Leaf area of one plant (leaf area meter), Chlorophyll a, Chlorophyll b, Chlorophyll a+b and Carotenoids [33], RWC [34].

Table 1 Physical and chemical characters of soil, 2011.

EC (ds/m)	TNV (%)	OC (%)	Total N (%)	P_{ava} (mg/kg)	K_{ava}(mg/kg)	Fe(mg/kg)	Zn (mg/kg)	Cu (ppm)	Mn (ppm)	B (ppm)	SP (%)
1.33	10/1	(0.79)	(0.08)	(14/4)	(178.4)	7.72	1	1.34	17.72	0.464	24.63

Table 2 Variance analysis of drought stress and Nitrogen fertilizer effect on some traits of Sage

					Mean	squares						
S.O.V	df	Leaf yield	Petiole yield	Shoot yield	Essential oil percentage	Essential oil yield	Leaf area of one plant	Chlorophyll a	Chlorophyll b	Chlorophyll a+b	Carotenoids	RWC
Block	2	338345**	16850^{ns}	473721**	0.0007**	478329**	940261^{ns}	0.2**	0.009^{ns}	0.3**	0.09^{ns}	279.7**
Drought stress (A)	2	2258501**	434721^{ns}	4649367**	0.001**	274772*	28984149**	3**	0.003^{ns}	2.9**	0.3**	1.8^{ns}
Error A	4	240660	105277	648148	0.0002	585433	4273869	0.4	0.02	0.5	0.1	74.4
N (B)	3	115777^{ns}	65132**	307967*	0.0004**	436685**	2420680**	1.5**	0.009^{ns}	1.7**	0.2**	37.7
A*B	6	456571**	51012**	789088**	0.0001*	16845*	19992237**	0.4**	0.02**	0.5**	0.2**	46.5**
Error	18	209300	46628	400981	0.0001	196302	1557131	0.05	0.005	0.06	0.05	22.2
CV (%)		11.5	13.6	19.4	18.9	21.1	16.2	11.09	11.06	29.2	12.2	6.04

^{ns}, non significant; *, significant at P≤0.05; **, significant at P≤0.01.

Table 3 Mean comparison of drought stress effect on some traits of Sage

Drought stress	Leaf yield (kg/ha)	Petiole yield (kg/ha)	Shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Leaf area of one plant (cm^2)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a+b (mg/g)	Carotenoids (mg/g)	RWC (%)
3 day	2322.3 a	966.2 a	3288.6 a	0.03 b	1107.6 ab	4462.4 a	2.2 a	0.6 a	2.9 a	1.7 b	78.2 a
6 day	2129.7 b	850.3 b	2980.1 b	0.03 b	1010.9 b	3803.2 b	2.06 b	0.7 a	2.7 b	1.8 a	77.8 a
9 day	1825.5 c	746.5 c	2572.1 c	0.04 a	1185.2 a	2687.2 c	1.7 c	0.7 a	2.4 c	1.8 a	77.8 a

Means in each column with the same letter are not significantly (P≤0.05) different.

Table 4 Mean comparison of nitrogen fertilizer effect on some traits of Sage

Nitrogen fertilizer	Leaf yield (kg/ha)	Petiole yield (kg/ha)	Shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Leaf area of one plant (cm^2)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a+b (mg/g)	Carotenoids (mg/g)	RWC (%)
0 kg/ha (Control)	2045 a	813 b	2858.1 b	0.04 a	1214.6 a	3249.9 b	1.78 c	0.6 a	2.4 c	1.8 b	78.2 ab
100 kg/ha	2028.1 a	838.2 b	2866.4 b	0.03 b	1071.9 b	3684.9 a	1.9 bc	0.6 a	2.5 c	1.7 b	79.3 a
200 kg/ha	2158.3 a	925.6 a	3084 a	0.03 b	1184.3 ab	3975.2 a	2.5 b	0.7 a	2.7 b	1.8 b	76.4 b
400 kg/ha	2138.6 a	840.6 b	2979.3 ab	0.03 c	934.2 c	3693.8 a	2.3 a	0.7 a	2.9 a	1.9 a	77.8 b

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

Table 5 Mean comparison of the interaction between drought stress and nitrogen fertilizer effect on some traits of Sage

Treatments	Leaf yield (kg/ha)	Petiole yield (kg/ha)	Shoot yield (kg/ha)	Essential oil percentage (%)	Essential oil yield (kg/ha)	Leaf area of one plant (cm^2)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Chlorophyll a+b (mg/g)	Carotenoids (mg/g)	RWC (%)
3 day × 0 kg/ha	2025.8 bcd	854.2 bcd	2880 bcd	0.04 bc	1154 b	3692.2 cde	1.7 ef	0.64 bc	2.4 de	1.6 cd	77.5 ab
3 day × 100 kg/ha	2198.2 bc	913.7 bc	3112 bc	0.03 cd	1073.5 b	4234.8 bc	2.1 c	0.62 c	2.7 c	1.5 d	79.2 ab
3 day × 200 kg/ha	2551.1 a	1129.7 a	3680 a	0.02 e	1393.5 a	4791.6 a	2.5 b	0.74 a	3.2 ab	1.7 cd	75.7 ab
3 day × 400 kg/ha	2514.1 a	967.1 b	3481 a	0.02 cd	809.8 c	5131 a	2.7 a	0.72 ab	3.4 a	1.8 abc	80.3 a
6 day × 0 kg/ha	2152.4 bcd	836.4 cde	2988.9 bcd	0.03 cd	1090 b	3225.8 efg	2 cd	0.75 a	2.7 c	2.06 ab	77 ab
6 day × 100 kg/ha	2174.2 bcd	882.2 bc	3056.4 bc	0.03 cd	1050.9 b	4262.3 bc	1.9 de	0.68 abc	2.5 cd	1.7 cd	78.2 ab
6 day × 200 kg/ha	1932 de	816.9 cde	2748.9 d	0.03 cd	988.9 bc	4153.8 cd	1.8 de	0.7 abc	2.5 cd	1.8 c	77.2 ab
6 day × 400 kg/ha	2260.4 b	865.7 bcd	3126.2 b	0.02 de	913.7 bc	3571 ef	2.4 b	0.67 abc	3.1 b	1.8 bc	78.9 ab
9 day × 0 kg/ha	1956.9 cd	748.4 def	2705.3 d	0.05 a	1399.9 a	2831.8 gh	1.6 ef	0.64 bc	2.2 e	1.6 cd	80.1 a
9 day × 100 kg/ha	1712 e	718.6 ef	2430.7 e	0.04 abc	1091.6 b	2557.7 h	1.6 ef	0.73 ab	2.4 de	1.9 abc	80.4 a
9 day × 200 kg/ha	1992 cd	830.2 cde	2822.2 cd	0.04 bc	1170.6 ab	2980.3 fgh	1.6 ef	0.7 abc	2.3 de	1.9 abc	76.4 ab
9 day × 400 kg/ha	1641.3 f	688.9 f	2330.2 e	0.04 ab	1079.1 b	2379.4 h	1.8 de	0.72 ab	2.5 cd	2.09 a	74.1 b

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

And finally, data were analyzed via SAS 9.1 and means were compared according to the Duncan's multiple range test.

Results

Analysis of variance indicated that irrigation interval significantly affected leaf yield, shoot yield, essential oil percentage and shoot yield, leaf area, chlorophyll a, chlorophyll a+b and carotenoids (P≤0.01; Table 2). Mean comparison indicated that shoot yield, leaf yield, petiole yield and leaf area were the highest in 3 days irrigation period; however, essential oil percentage and yield were the highest in 9 days irrigation (Table 3). Reducing the interval of irrigation from 9 days to 3 days increased shoot yield from 2572.1 kg/ha to 3288.6 kg/ha.

Analysis of variance of nitrogen application indicated the significant effect on petiole yield, shoot yield, essential oil percent, yield, leaf area, chlorophyll a, chlorophyll a+b and carotenoids (Table 2); Mean comparison indicated that the highest value of shoot and petiole yield were achieved when 200 kg/ha nitrogen was applied. Oil percentage and yield was maximum in control (irrigation every 3 days) with 0.04% and 1214.6 kg/ha. Chlorophyll a, a+b and carotenoids with 2.3, 2.9 and 1.9 mg/g, respectively were high in 400 kg/ha Nitrogen (Table 4).

Results also indicated that the interaction of irrigation period×Nitrogen had significant effect on leaf yield, shoot yield, petiole yield, oil percent and yield chloropyll a, b and a+b and cartenoids (Table 2). Mean comparison indicated that both shoot yield and essential oil yield were the highest in 3 days period×200 kg/ha nitrogen. However, essential oil percentage was the highest in 9 days period×nonuse to nitrogen (control), with 0.05% (Table 5).

Discussion

Tougher pressure reduction is one of the primary symptoms of drought stress, low available water to plants is the loss of turgor pressure and reduction of cell development especially stems elongation and leaves extend. Reduction of cell development makes the plant smaller in size, which is the characteristic of drought stressed plants. Moreover, drought stress disturbs nutrient absorption and reduces leaves growth. Lower leaf area means lower light absorption and photosynthesis. All these events finally decrease plant growth and yield [21], find Reduction in plant growth and yield to be expected. Drought stress induces some morph-physiological responses in plant such as the reduction of leaf area, shoot growth, enhancement of root growth, stomata closure, reduction of growth rate[24]. Sage in this study also had reduced in leaf area, shoot growth and the other traits. Lebaschi and Sharifi Ashoorabadi [26] concluded that higher drought stress levels reduced plant high and shoot weight in some medicinal plants such as *Salvia officinalis* and *Achillea millefolium*. Abbaszadeh *et al*. [29] reported the decrease in RWC of *Melissa officenalis* L. with the increase in the severity of drought stress so that no-stress treatment had the highest RWC (93.369 % on average) and most-severe-stress treatment had the lowest one (54.75%) which was 25% of field capacity. But this trait had no significant difference by drought stress in this research. Increased drought stress reduced chlorophyll a and a+b content (table 3). Chlorophyll has a large molecule and its production is stopped under drought stress conditions or the plant is preferred Avoid chlorophyll production [28]. Ardakani, *et al*., 2007 reported the decrease in chlorophylls under drought stress at *Melissa officinalis* L. [28].

Nitrogen plays an important role in the synthesis of plant constituents, which are very critical for plant growth and dry matter yield [31]. So petiole and shoot yield of sage increased with use of Nitrogen up to 200 kg/ha. Nitrogen limiting conditions increase volatile oil production in annual herbs [31]. One of the reasons is the negative effect of nitrogen on the amount of essential oil production can be used to stimulate plant growth [28, 29]. In *S. sclarea* oil percentage and yield reduced with nitrogen consumption. Nitrogen fertilization has been reported to reduce volatile oil content in *juniperus horizontalis* Moench. [32]. This experiment briefly indicated that clary sage can be cultivated in areas with low water supply, and it can produce the highest essential oil yield about 1400 kg/ ha when irrigated every 9 days without using Nitrogen.

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