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THE INFLUENCES OF DIFFERENT WATER AND NITROGEN APPLICATION ON SOME YIELD PARAMETERS AND ANTIOXIDANT ACTIVITY IN SAGE (*Salvia officinalis* L.)

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ABSTRACT

In this research *Salvia officinalis* L. var. Extrakta was used as material in the Bornova ecological conditions in 2012 and 2013. It was investigated on effect of different nitrogen and water applications (dripping irrigation) in some yield parameters and antioxidant activity in sage plants. Field trials were arranged two-factor split-plot experimental design, with four replications. Experimental factors were water regimes of main plot: S1 (irrigated when it's needed), S2 (20 days dry before harvesting) and S3 (40 days dry before harvesting) and the sub-plot nitrogen applications (nitrogen free and 6 kg da^{-1}). It was determined that plant height (cm), fresh herbage yield (kg da^{-1}), drug herbage yield (kg da^{-1}), drug leaf yield (kg da^{-1}), essential oil ratio (%), essential oil yield, essential oil content (%), antioxidant capacity (µmol FRAPgDM). The highest essential oil ratio was obtained as a mean 2.07% from S3 water doses The main component in the essential oils recovered by both experiments were α+β thujone showing a large variation. Considering all the water and nitrogen applications of field trials showed that nitrogen fertilization were distinguished, the data on plants where water applications S2. It can be said that the antioxidant activity tends to increase as the soil moisture decreases.

Keywords: Sage; *Salvia officinalis* L.; nitrogen; water application; essential oil

INTRODUCTION

Environmental conditions play an active role in plant production. Abiotic stress such as drought, high temperature, soil salinity, chilling etc. may decrease agricultural prolificacy. Plants can regulate to survive under adverse circumstances with secondary metabolites. Water is also important in the growth and productivity of medical and aromatic plants as well as in many cultivated plants. Especially water deficiency is important dilemma on medicinal and aromatic plants in growing season. Although lack of water reduced yield of aerial parts, it can enhanced accumulation of more metabolites to prevent from oxidization damage in plant tissues (Farahini et al., 2009; Bettaieb et al., 2011; Manukyan, 2011; Abdelmajeed et al., 2013). *Salvia officinalis* L. (sage) is one of the economically important species in Lamiaceae family that sage is a perennial woody sub-shrub and blue to purplish flowers. It is used for many purposes in ancient times. Leaves of *Salvia officinalis* L. are consumed in foods, drugs and perfumery industries and they have antioxidative properties. Additionally, other significant effects are antibacterial, fungistatic, virustatic, astringent, hypoglycemic and cytotoxic activities (Bernotiene et al., 2007; Bettaieb et al., 2009; Zervoudakis et al., 2012; Corell et al., 2012; Porte et al., 2013; Tosun et al., 2014; Said-Al Ahl et al., 2015)**.** *Salvia officinalis* L. is native to the Mediterranean region and sage is cultivated several countries but it is not spread out in Turkish natural flora. It can easily growth in west coast of Anatolia.

The aim of this study is to investigate the effect of irrigation arrangement and nitrogen application on herbal yields, quantity of essential oils and antioxidant capacity of *Salvia officinalis* L. in field conditions in Mediterranean climate zone.

MATERIAL AND METHODS

The field experiments were conducted in 2012 and 2013 in Department of Field Crops, Faculty of Agriculture, Ege University, İzmir. The temperature, rainfall and humidity of the growing years are shown in Table 1. . The mean annual temperature was 18.6 and 18.5°C, and the total annual precipitation was 799.3 and 854.7 mm in 2012 and 2013, respectively.

Table 1. Some meteorological parameters of experimental area at Bornova in 2012-2013 (Anonymus 2012 and 2013)

Month	2012			2013		
	Mean Temperature (°C)	Total Rainfall (mm)	Relative Humidity (%)	Mean Temperature (°C)	Total Rainfall (mm)	Relative Humidity (%)
January	6.8	127.7	67.6	9.4	252.5	70.9
February	7.6	128.2	66.9	11.2	187	70.2
March	11.3	34.7	57.8	14	56.8	58.5
April	17.5	105	58.8	17.3	30.2	54
May	20.5	86.6	62.9	22.7	43.7	54.7
June	27.3	19.9	48.5	25.7	27.1	50.7
July	30.1	0	45.2	28.4	0	42
August	29.2	0	39.5	28.7	20.2	45.1
September	24.3	0	55.4	24	5.1	48.7
October	21.7	22.1	59.5	17.2	94.1	60.8
November	16.4	56.9	65	15	128.9	70
December	10.7	218.2	71.4	8.5	9.1	59.3
mean-total	18.6	799.3	58.2	18.5	854.7	57.1

The soil texture was classified as clayey-loamy and pH: 8.7 in soil surface, it was classified as clayey-sandy, pH: 7.8 in 20-40 cm soil depth. A summary of soil chemical properties was presented in Table 2.

Table 2. Some physical and chemical characteristics of the soils of the experimental sites (Sonmez and Bayram, 2009)

Soil depth	Sand	Silt	Clay	N (%)	P (ppm)	K (ppm)	Ca (ppm)	Na (ppm)	Fe (ppm)	OM[a] (%)	pH
0-20 cm	24.72	32.56	42.72	0.101	0.4	400	5400	20	13.6	1.130	8.2
20-40 cm	32.72	30.56	36.72	0.123	0.4	300	5100	20	16.2	1.150	7.8

[a]: Organic Matter

The seeds of *Salvia officinalis* L. var. Extrakta obtained from in Germany were sown into a mixture of sand, manure and mulch (1:1:1) on November 22, 2010. When the sage seedlings reached about 10 cm in plant height, they were transferred to the experimental plots on April 4, 2011. The field trial was arranged in two-factor split-plot experimental design with four replications. Experimental factors were water regimes of main plot: S1(irrigated when it is needed), S2(20 days dry before harvesting) and S3(40 days dry before harvesting) and the sub-plot nitrogen applications (nitrogen free and 6 kg da^{-1}). Nitrogen as ammonium sulphate was applied in two equal splits, at one split spring and other after first cutting in both years. Each plot consisted of six rows with a 3 m length and 40 cm × 20 cm of plant spacing. Total amount of water application (dripping irrigation) was measured by flow meter for each irrigation traits in the dry seasons. Table 3. gives detailed information about the experiment.

Table 3. Irrigation traits during the dry seasons of total water content applications (mm)

Years	Irrigation traits		
	S1	S2	S3
2012	875	426	198
2013	855	415	210

Three controlled irrigation treatments were started on April 27, 2012 and April 26 2013 in dry season beginning. The dates of the harvest were June 6 and September 17 in 2012 and June 6 and September 4 in 2013. The side effect was eliminated and investigated characteristics in each plot were used for the measurements set out below.

Plant height (cm): The plant was measured from the soil surface to the top level of the plant before each harvest.

*Fresh herbage yields (*kg da^{-1}:. The plants were harvested by hand with a saw knife, 10 cm above the surface, and immediately weighed for the obtained plot yields. Then the plot yield was converted to a yield for a decare.

Drug herbage yields (kg da^{-1}: A sample of 500 g of green herb was taken from each plot. The samples were dried at 35 °C for 72 h to determine its dry weight

Drug leaf yields (kg da^{-1}): A sample of 500 g green herbs was taken from each plot. The leaves of the samples were separated from the stems by hand and weighed. The leaves were dried in an oven at 35 °C for 72 h to determine the dry weight.

Essential oil content (%): Dry leaf materials of 10 g were subjected to a 3 h water-distillation using a Neo-Clevenger apparatus, and the extracted essential oils were stored at 4 °C until the gas chromatography (GC) analysis. The essential oil ratios of the plants were determined by a volumetric method (ml/100 g) (Wichtl, 1971).

*Composition of the essential oil (%):*The gas chromatography (GC) analysis for essential oil compounds was performed using an Agilent 6890 N chromatograph, equipped with flame-ionization detection (GC–FID). The compounds were separated on a 30 m × 0.25 mm, 0.25 μm film thickness, DB-Wax capillary column, with helium as a carrier gas at a constant flow rate of 1 mL/min. The oven temperature program was 45 ˚C (2 min), 45–250 ˚C (3˚/min), 250 ˚C (34 min), and the injector and detector temperatures were 250 ˚C. The identification of the components was based on the comparison of their relative retention times (RT) with those from the authentic standards found in the laboratory.

Antioxidant activity (μmol FRAPgDM):): The analysis of antioxidant capacity by Ferric Reducing Antioxidant Power (FRAP) was conducted in accordance with Benzie and Strain (1999). Dry leaves (0.5g) were ground with 20 mL 80% (v/v) ethanol and then centrifuged for 20 min at 4 ºC. The reaction was started by adding 0.05 mL extract to 1.50 mL FRAP reagent and 0.15 mL distilled water. The absorbance of the reaction mixture was measured spectrophotometrically (Cary 50®) at 593 nm. The results were expressed as μmoles FRAP in 1 g dry matter.

Statistical analysis: All data were statistically analyzed using the TOTEMSTAT Statistical program (Acikgoz et al., 2004). The treatment means were compared by using the LSD test as described by Steel and Torrie (1980).

RESULTS AND DISCUSSION

The result of plant height was summarized in Table 4. In general, the plant height was affected significantly by irrigation applications and nitrogen doses. According to general average the highest plant height was obtained during S1 application as 48.91 cm and nitrogen application increased the plant height in 2012. In second year, not only S1 and S2 irrigation treatments but also nitrogen application incerased the plant height. For both study years, the average mean of the plant heights decreased significantly with decreasing of water amount and without nitrogen. Analysis of data collected during two years revealed that plant height increased significantly and positively with irrigation frequency and nitrogen doses.

Table 4. Influence of irrigation treatments and nitrogen doses on *Salvia officinalis* L. var. Extrakta plant height in 2012 and 2013 (cm)

N App. (kg da^{-1})	1st cutting				2nd cutting				Average			
	Irrigation Applications											
	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean
2012												
0	56.65	47.35	48.4	50.80^{b}	34.6	30.5	26.28	30.46	45.63	38.93	37.34	40.63^{b}
6	70.25	64.83	61.1	65.39^{a}	34.13	31.1	27.83	31.02	52.19	47.96	44.46	48.20^{a}
Mean	63.45	56.09	54.75		34.36^{a}	30.8^{b}	27.05^{c}		48.91^{a}	43.44^{b}	40.90^{b}	
LSD		I=ns		N=7.63**		I=2.92**		N=ns		I=5.27*		N=7.63**
2013												
0	50.9	52.63	50.25	51.25^{b}	29.35^{bc}	27.2^{c}	21.7^{d}	26.08	40125	39.91	35.97	38.67b
6	61.55	59.2	53.45	58.06^{a}	34.05^{a}	30.25^{b}	22.5^{d}	28.93	47.8	44.72	37.97	43.50^{a}
Mean	56.23	55.91	51.85		31.7	28.73	22.1		43.96^{a}	42.32^{a}	36.98^{b}	
LSD	N=ns			I=4.88**		NxI= 1.90*				N=4.76**		I=2.93**

N: nitrogen doses, I: irrigation applications, NxI: interaction, *: significant differences $p<0.05$, **: significant differences $p<0.01$, ns: no significant differences.

When both cutting and annual general averages for the 2012 and 2013 years of this study were examined, it was determined that the nitrogen application increased the plant height. The increase of water restriction before the harvest caused to decrease of plant height values. According to the comparison of the first cutting and the second cutting of the research years, the highest of plant height was observed in first cutting, it is considered to be the effect of the winter and spring rains before the irrigations treatments. Many researchers (Koc, 2000; Ozturk et al., 2004; Oktay Koc., 2006; Ipek, 2007; Bettaieb et al., 2009; Ekren et al., 2011; Fathi et al., 2012; Bahreininejad et al., 2013) reported similar result on *Salvia officinalis* L. and members of Lamiaceae family, too.

The irrigation treatment x applying nitrogen interaction affected the fresh herbage yield significantly ($p<0.01$) in 2012 and 2013 (Table 5). The maximum fresh herbage yield (4307.39-3982.75 kg da^{-1}) were observed in plots which received S1 (irrigated when it's needed) with 6 kg da^{-1} of nitrogen fertilizer. It can be said that interactions of S2 x 6 kg da^{-1} N in both year's and S1 x 0 kg da^{-1} N in the first year at second harvest and each harvest in the second year's values had second higher fresh herbage yield**s**. Also, the treatment of S3 with no fertilizer application had the lowest fresh herbage yield. This can be related to the fact that nitrogen fertilizer and soil moisture improve soil physical and chemical properties which are effective on vegetative growth.

Table 5. Influence of irrigation treatments and nitrogen doses on *Salvia officinalis* L. var. Extrakta fresh herbage yield in 2012 and 2013 (kg da^{-1})

N App. (kg/da^{-1})	1st cutting				2nd cutting				Total Yield			
	Irrigation Applications											
	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean
2012												
0	1548.2^{c}	1392.13^{d}	1136.12^{f}	1358.82	1436.9^{b}	1090.08^{c}	840.87^{e}	1122.62	2985.10^{c}	2482.21^{d}	1976.98^{f}	2481.43
6	2235.64^{a}	1876.2^{b}	1274.04^{e}	1795.29	2071.75^{a}	1446.21^{b}	996.45^{d}	1504.81	4307.39^{a}	3322.41^{b}	2270.49^{e}	3300.1
Mean	1548.2	1392.13	1136.12	1358.82	1754.32	1268.15	918.66		3646.24	2902.31	2123.74	
LSD		NxI= 83.97**				NxI= 138.07**				NxI= 154.67**		
2013												
0	1533.65^{b}	1245.19^{c}	852.76^{d}	1210.54	1342.13^{b}	923.08^{c}	684.5^{d}	983.23	2875.78^{b}	2168.27^{c}	1537.26^{e}	2193.77
6	2111.18^{a}	1643.63^{b}	1075.12^{c}	1609.98	1871.57^{a}	1170.67^{b}	838.1^{cd}	1293.45	3982.75^{a}	2814.3^{b}	1913.22^{d}	2903.43
Mean	1822.42	1444.41	963.94		1606.85	1046.88	761.3		3429.27	2491.29	1725.24	
LSD		NxI= 182.21**				NxI= 178.85**				NxI= 229.65**		

N: nitrogen doses, I: irrigation applications, NxI: interaction, *: significant differences $p<0.05$, **: significant differences $p<0.01$

Several studies showed that nitrogen application enhanced biomass on *Salvia officinalis* L. plants of aerial parts (Ceylan et al., 1979; Putievsky et al., 1986; Ceylan et al., 1994; Koc, 2000; Oktay Koc, 2006; Ipek, 2007) until critical dosage. Bettaieb et al. (2009) investigated different irrigation water levels (I_{100}:100%, I_{50}:50% and I_{25}:25% of field capacity) on *Salvia officinalis* L. They found that fresh biomass increasingly correlated with amount of soil water so finds that the amount of soil water enhanced plant biomass. In other study, Bettaieb et al. (2011) determined the effect of water deficit (moderate and severe water deficits) on sage plant. Their result show that increasing in water scarcity led to the reduced production of fresh weight. Lier (1999) point out that water deficit can affect related to growth properties and water deficits cause to close stomata and limit the photosynthesis. So the most significant general effects of water deficiency are the reduction in plant size, leaf area, and crop yield. Fresh herbage yield was ascended by diminishing of irrigation interval and applying of nitrogen fertilizer in our research. In the presented study, it can be claimed that our result is harmonies in the light of these literatures.

The result of drug herbage yield was shown in Table 6. Irrigation x nitrogen applications interaction recorded significantly higher drug herbage yield of 1162.90 kg da^{-1} S1x6 kg da^{-1} as compared to the rest of the combination in first year of total yield. Before harvesting, 40 days dry treatment coupled with no nitrogen application resulted in the lowest drug herbage yield (650.32 kg da^{-1}). Various irrigation intervals had highly significant effect ($p<0.01$) on drug herbage yield in 2013. The maximum drug herbage yield was obtained from S1 applications as 1017.89 kg da^{-1}. It also evident from data that drug herbage yield was affected ($p<0.01$) by nitrogen. Nitrogen was increased total yield as 1017.56 kg da^{-1}.

Table 6- Influence of irrigation treatments and nitrogen doses on *Salvia officinalis* L. var. Extrakta drug herbage yield in 2012 and 2013 (kg da^{-1})

N App. (kg da^{-1})	1st cutting				2nd cutting				Total Yield			
	Irrigation Aplications											
	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean
2012												
0	373.17^{c}	377.59^{c}	323.84^{d}	358.2	394.76^{cd}	362.09^{de}	326.48^{e}	361.11	767.93^{c}	739.68^{c}	650.32^{d}	719.31
6	567.65^{a}	528.47^{b}	375.56^{c}	490.56	595.25^{a}	508.00^{b}	429.92^{c}	511.06	1162.90^{a}	1036.47^{b}	805.49^{c}	1001.62
Mean	470.41	453.03	349.7		495	435.04	378.2		965.42	888.07	727.9	
LSD		NxI= 35.25**				NxI=42.99*				NxI=66.40**		
2013												
0	454.84	426.74	325.10	402.23^{b}	382.02	357.42	302.14	347.19^{b}	836.87	784.16	627.24	749.42^{b}
6	648.79	565.90	430.04	548.24^{a}	550.12	456.53	401.31	469.32^{a}	1198.91	1022.43	831.35	1017.56^{a}
Mean	551.82^{a}	496.32^{a}	377.57^{b}		466.07^{a}	406.98^{b}	351.72^{c}		1017.89^{a}	903.29^{b}	729.30^{c}	
LSD	I=66.19**			N=68.19**	I=40.32**			N=47.95**	I=62.44**			N=100.94**

N: nitrogen doses, I: irrigation applications, NxI: interaction, **: significant differences $p<0.01$

Ceylan et al. (1994) researched different nitrogen doses on sage and they found drug herbage yield varied from 558 to 713 kg da^{-1}. Koc (2000) mentioned that increasing of nitrogen quantities enhanced the drug herbage yields. Khalil et al. (2008) reported the same results on sage. Bettaieb et al. (2009) found that the increases in water deficiency affected negatively the amount of drug herbage in sage. Many studies showed production of aerial parts of Lamieceae member plants related with water volumes. Water deficiency in soil reduced the drug herbage yield (Arabaci, 1995; Baher et al. 2002; Ozturk et al. 2004; Bettaieb et al. 2009; Farahani et al.2009; Ekren et al. 2012; Bahreininejad et al. 2013). This could be a result of reduction in the chlorophyll content and photosynthesis activity. The result of this study was generally in agreement with the ones reported by Arabaci. (1995); Baher et al. (2002); Ozturk et al. (2004); Farahani et al. (2009); Ekren et al. (2012);

Bahreininejad et al. (2013). Our dry herbage yield values were higher than the values reported by Ceylan et al. (1994); Koc, (2000) and Khalil et al. (2008) because of different climate, environmental factors and the homogeneous material used in this research.

The yields of drug leaf yields of different nitrogen and water applications for 2012 and 2013 are evaluated statistically and the results of analysis are shown in Table 7. Interaction between two factors was found significant for drug leaf yields in 2012. The annual total leaf yield data are statistically important, it was determined that the S1 water treatment with nitrogen application had the highest drug leaf yield as 812.35 kg da^{-1}. In terms of total drug leaf yield main effect of irrigations intervals and nitrogen levels have showed great variations at $p<0.01$ in 2013. The highest drug leaf yields were observed from S1 water treatments as 766.29 kg da^{-1}. Nitrogen fertilizer application was increased total drug leaf yield as763.14 kg da^{-1}.

Table 7. Influence of irrigation treatments and nitrogen doses on *Salvia officinalis* L. var. Extrakta drug leaf yields in 2012 and 2013 (kg da^{-1}).

	2012											
N App. (kg da^{-1})	**1st cutting**				**2nd cutting**				**Total Yield**			
	Irrigation Aplications											
	S1	S2	S3	Mean	S1	S2	S3	Mean	S1	S2	S3	Mean
0	232.86 ^{c}	236.75 ^{c}	198.26 ^{d}	222.63	297.49 ^{cd}	268.09 ^{de}	251.23^{e}	272.27	530.36 ^{c}	504.84 ^{c}	449.50 ^{d}	494.90
6	372.14^{a}	324.58 ^{b}	228.39 ^{cd}	308.37	440.21 ^{a}	371.98 ^{b}	318.94 ^{c}	377.04	812.35 ^{a}	696.56 ^{b}	547.33 ^{c}	685.41
Mean	302.50	280.67	213.33		368.85	320.03	285.09		671.35	600.70	498.42	
LSD		NxI:36.24**				NxI=34.32*				NxI=58.31**		
	2013											
0	317.95	289.26	218.61	275.27b	313.23	289.39	251.24	284.62^{b}	631.18	578.64	469.85	559.89^{b}
6	459.67	396.51	291.37	382.52^{a}	441.74	377.81	322.32	380.62^{a}	901.41	774.32	613.69	763.14^{a}
Mean	388.81^{a}	342.88^{b}	254.99^{c}		377.49^{a}	333.60^{a}	286.78^{b}		766.29^{a}	676.48^{b}	541.77^{c}	
LSD	I=43.03**			N=38.30**	I=51.28**			N=44.73**	I=77.18**			N=56.16**

N: nitrogen doses, I: irrigation applications, NxI: interaction, *: significant differences $p<0.05$, **: significant differences $p<0.01$

Baher et al. (2002) and Khazaie et al. (2008) stated in different research that reduction of irrigation frequency induced to decline vegetative yield components such as drug leaf yield. Farahani et al. (2009) and Atallah et al. (2010) who reported that the increase in water restriction resulted in a decrease in the yield of drug leaf yield. Researchers have obtained that there is a linear relationship between the increase of the soil moisture and the drug leaf yield. Fathi et al. (2012) found that *Salvia sclarea* L. has the highest values of leaf yield, flower yield and leaf area irrigated with 3 days intervals. The findings of the study were found similar trends with those reported by Baher et al. (2002); Khazaie et al. (2008); Farahani et al. (2009); Atallah et al. (2010); Fathi et al. (2012).

The essential oil ratio was given Table 8. The interaction between water treatment and nitrogen fertilizer significantly affected average of essential oil content in 2012. The highest average essential oil content of *Salvia officinalis* L. var. Extrakta was obtained from S3 x 6 kg da^{-1} application as 1.96%. Second higher value was found from same irrigation application without nitrogen as 1.74%. In 2013 growing season, the effects of different irrigation intervals with N applications were significant on essential oil ratio at 1% probability level. The treatments of water applications of general average had higher essential oil content as 2.07 and 1.97%, respectively, S3 and S2 treatment were in the same statistical group. Essential oil rate increased with decreasing in irrigation level. In other words, volatile oil accumulation was positively affected by low soil moisture.

The chemical composition of the essential oil was obtained by GC from sage leaves. The data are shown in Table 9. α-thujone was a dominant compound (25.09-34.33%) followed by camphor (15.16-34.59%), β-thujone (5.23-12.58%) and 1.8-cineole (2.85-9.48%) both harvests in 2012. Minor components including borneol (2.54-6.90%) and linalool (2.24-5.72%) were also detected. In the second year, the leaf essential oil of *Salvia officinalis* L. plants showed that main constituents were not changed but represented different quantities. The findings indicate that α-thujone was determined between 14.00 and 36.17%. The other significant compound, camphor, was varied from 18.00% to 28.87%. β-thujone (3.86-9.01%) and 1.8-cineole (0-6.40%) were also detected, however their values found to be less than the amount detected in the previous year. Bettaieb et al. (2009) reported that there is no information about how it is affected main constitutes by water deficiency on *Salvia officinalis* L. and variations of α-thujone, ß-thujone and camphor were not clear about restriction of water treatment. Also, Azizi et al. (2009) stated that it is difficult to fully explain the effects of essential oils components of the water restrictions. The different water regimes altered the content of some essential oil components. Major constituents were found in different amounts such as α-thujone (32.10-%42.72), camphor (13.93-17.09%), ß-thujone (8.62-16.17%), 1.8 cineole (4.11-7.97%) and borneol (1.62-2.60%). In the presented study, major compounds were the same as the literatures although amount of basic components were variable in water treatments on *Salvia officinalis* L. plants.

Table 8. Influence of irrigation treatments and nitrogen doses on *Salvia officinalis* L. var. Extrakta essential oil content in 2012 and 2013 (%)

N App. (kg da^{-1})	1st cutting				2nd cutting				Average			
	Irrigation Aplications											
	S1	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**	**S1**	**S2**	**S3**	**Mean**
2012												
0	1.69 ^{d}	2.15 ^{b}	2.2 ^{a}	2.01	1.29	1.23	1.29	1.27	1.49 ^{d}	1.69 ^{bc}	1.74 ^{b}	1.64
6	1.9 ^{c}	1.98 ^{bc}	2.35 ^{a}	2.08	1.51	1.45	1.58	1.51	1.71 ^{b}	1.71 ^{b}	1.96 ^{a}	1.79
Mean	1.79	2.06	2.28		1.40	1.34	1.43		1.60	1.70	1.85	
LSD		NxI= 0.199**			I=0.063*			N=0.105**		NxI=0.095*		
2013												
0	1.99	2.05	2.30	2.11^{b}	1.41	1.60	1.71	1.58	1.70	1.83	2.01	1.85^{b}
6	2.03	2.61	2.61	2.42^{a}	1.60	1.59	1.63	1.60	1.81	2.10	2.12	2.01^{a}
Mean	2.01^{b}	2.33^{a}	2.46^{a}		1.51	1.59	1.67		1.76^{b}	1.97^{a}	2.07^{a}	
LSD	I=0.300**			N=0.255*	I=ns			N=ns	I=0.149**			N=0.142*

N: nitrogen doses, I: irrigation applications, NxI: interaction, *: significant differences $p<0.05$, **: significant differences $p<0.01$, ns: no significant differences.

Table 9. Influence of irrigation treatments and nitrogen doses on *Salvia officinalis* L. var. Extrakta main essential oil composition in 2012 and 2013 (%)

Years	Harvest	Irrigation Aplications	N App. (kg da^{-1})	*α*-Pinene	Camphene	1.8-Cineole	*α*- Terpinene	β-pinene	*α*-Thujone	β-Thujone	Camphor	Linalool	Borneol	Bornyl acetate
				Main Components (%)										
2012	I.	S1	0	-	1.12	8.53	0.70	1.60	32.49	8.45	22.65	3.37	6.69	1.11
			6	-	-	3.71	0.52	2.09	25.09	8.21	24.81	5.31	5.03	0.89
		S2	0	-	-	4.60	-	3.22	32.26	11.39	20.93	5.72	6.22	0.63
			6	-	-	6.12	0.62	1.40	29.83	12.58	21.72	2.60	2.72	0.89
		S3	0	1.07	1.00	6.80	0.75	1.01	32.04	8.11	22.91	2.70	3.89	-
			6	0.98	1.02	9.48	0.77	1.47	34.33	7.20	15.16	4.74	6.90	1.21
	II.	S1	0	-	-	2.85	-	2.75	32.55	6.07	29.60	2.46	3.91	1.10
			6	-	-	2.95	0.63	2.49	32.90	5.45	26.98	3.50	4.78	-
		S2	0	-	-	3.27	0.73	1.92	33.27	5.23	22.72	4.83	4.83	1.10
			6	-	-	4.16	0.64	2.15	28.31	7.44	34.59	2.68	2.54	0.93
		S3	0	-	-	3.83	-	3.13	32.46	10.88	26.94	2.24	2.76	0.90
			6	-	0.43	4.02	0.71	1.92	30.00	5.63	31.36	3.87	4.52	1.11
2013	I.	S1	0	0.34	0.53	5.03	0.52	1.04	31.95	7.83	21.12	4.6	2.78	1.02
			6	0.57	0.34	6.4	0.52	1.3	28.87	7.25	24.75	7.6	5.71	-
		S2	0	1.26	1.79	5.65	0.54	1.25	32.51	5.91	22.55	4.82	3.44	1.00
			6	0.88	1.12	6.12	0.53	1.21	31.21	6.73	23.74	5.24	2.83	0.99
		S3	0	1.34	1.84	6.36	0.61	0.70	32.37	7.63	21.31	5.02	2.72	0.79
			6	-	0.63	5.42	0.47	1.25	29.21	6.10	21.61	4.61	3.40	0.91
	II.	S1	0	-	-	2.45	0.68	2.06	36.17	7.28	24.86	4.71	2.64	1.14
			6	-	-	-	-	4.48	14.00	5.04	18.00	6.63	9.17	0.52
		S2	0	-	-	2.64	-	2.64	33.25	6.48	26.81	4.50	4.17	1.27
			6	-	-	0.69	-	1.38	28.57	9.01	21.59	3.79	3.61	0.27
		S3	0	-	-	1.54	-	2.94	29.79	3.86	28.87	5.20	4.76	-
			6	-	-	0.92	-	2.02	35.76	6.82	27.31	3.79	3.17	0.94

The antioxidant capacity was not significantly affected by the nitrogen doses and irrigation treatments. The highest value was obtained from 20 days of drying period before harvesting with 6 kg ha^{-1} of nitrogen fertilizer in both years (Figure 1.). On the other hand, our results indicated that reducing of irrigation interval caused enhancement of stress severity.

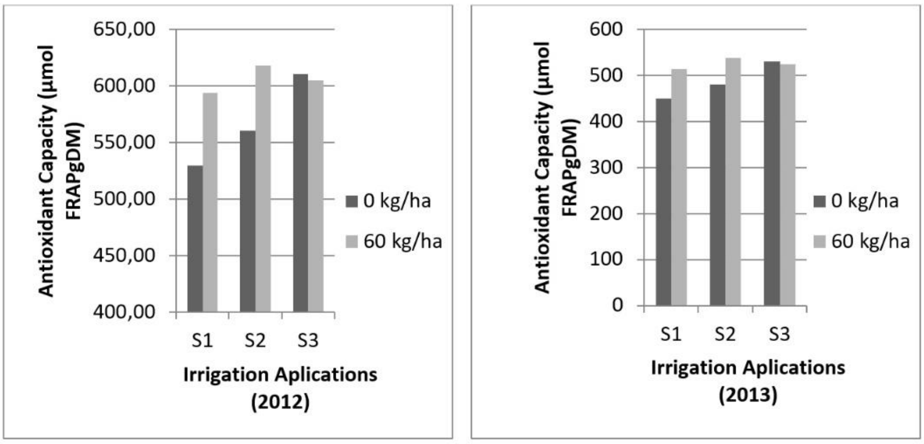

Figure 1. Influence of irrigation treatments and nitrogen doses on antioxidative capacity of *Salvia officinalis* L. var. Extrakta in 2012 and 2013.

Salvia officinalis L. plants contain important polyphenols which increase the biosynthesis under water deficit and play significant role in plant resistance (Bettaieb et al. 2011; Manukyan 2011; Pellegrini et al. 2015). Munne-Bosch et al. (2001) reported that antioxidant molecules are responsible for avoiding drought damage in plants. Antioxidant activity (Nakatani, 2003), tend to increase on sage plants in the lack of rainfall during summer season in Mediterranean field conditions. In generally our result were in accordance with literature.

CONCLUSIONS

The results of the research showed that irrigation water and nitrogen fertilizer are important effects on growth and development characteristics in sage. When water restriction was increased and nitrogen application is not done, the plant growth was decreased as the fresh herbage and drug herbage yield were reduced. One of the most important indicators of the defense mechanism of plants in stress conditions is the synthesis of volatile oils. It can be said that decreasing of soil moisture may lead to raise essential oil amount and antioxidant capacity at the same time. It can be said that decreasing of soil moisture may lead to raise essential oil amount and antioxidant capacity at the same time.

In conclusion, it is important to determine the effects of limited water use and crop water relations on yield and quality criteria for medicinal and aromatic plant growth when the global warming threat increases and the water resources are reduced. It can be concluded that the yield and quality characteristics of plants with nitrogen fertilizer and S2 water application are distinguished.

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