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Original article

Growth responses and essential oil profile of *Salvia officinalis* L. Influenced by water deficit and various nutrient sources in the greenhouse

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ABSTRACT

Salvia officinalis L. is a medicinal plant extensively used in foods, traditional medicine, and the pharmacological industry. In the current study, the effects of different irrigation regimes [irrigation after 70 ± 5 (regular), 105 ± 5 (moderate drought stress), and 140 ± 5 (severe drought stress) mm evaporation] and nutrient sources (control, NPK, farmyard manure, foliar fertilizer, and hydrogel) were investigated on the growth parameters and essential oil (EO) components of *S. officinalis* in the greenhouse. The plants were harvested two times. The regular irrigation treatment had the most significant effect on plant height (51 cm), fresh and dry herb weight (51.5 and 18.1 g plant^{-1}), and fresh and dry leaf weight (40.1 and 13.1 g plant^{-1}). The highest amount of EO was observed after moderate drought stress (1.48%). The NPK treatment had the greatest effect on plant height (40 cm), branch number (19 per plant), fresh and dry herb weight (53.4 and 18.9 g plant^{-1}), fresh and dry leaf weight (41.2 and 13.6 g plant^{-1}), and EO content (1.67%). The 1st cutting was superior in EO amount, while the 2nd cutting had a high agronomic yield. α-Thujone (from 21.6 to 34.2%) was identified as the predominant compound. Additionally, the content of α-thujone in the 2nd cutting was higher after moderate drought stress, NPK, and hydrogel treatments. Moreover, 1,8-cineole, β-thujone, camphene, α-pinene, α-humulene, viridiflorol, borneol, and bornyl acetate were the other main compounds. As a general result, regular irrigation and NPK treatments improved the agronomic yield of *S. officinalis*. The plants under drought stress produced high amounts of EO. The farmyard manure also improved plant yield by providing a part of the plant's nutritional needs. Therefore, it could be concluded that it is crucial to determine the effects of limited water availability and various nutrient sources on yield and chemical compositions for medicinal and aromatic plant growth.

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1. Introduction

As a perennial herb belonging to the Lamiaceae family, *Salvia officinalis* L. does not grow naturally in Turkey. But in recent years, due to the economic values of *Salvia* species, it has attracted the interest of producers. The leaves of this species are used as a raw material in the medicine, pharmaceutical, food, and perfume industry (Bruneton, 2001; Bahtiyarca Bagdat et al., 2017). Among the various species of *Salvia* that are relatively drought-tolerant, *S. officinalis* is sensitive to water deficiency (Munne Bosch et al., 2001).

The quality and production of secondary metabolites in medicinal and aromatic plants are affected by various factors such as genetic characteristics, climatic conditions, environment

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organisms, applied agro-techniques, and post-production processing (Soltanbeigi, 2020). The use of appropriate and recommended agricultural practices such as fertilization and irrigation in medicinal plants' production for achieving optimal agronomic production can provide standard bioactive ingredients to the pharmaceutical industry (Abaas, 2014). Among the limiting factors for producing crops, water deficiency or drought stress is one of the most critical factors damages the final yield. Climate change has exacerbated the water scarcity effects in many parts of the world, especially in arid and semi-arid regions (Soltanbeigi, 2019; Hatamian et al., 2020). Water deficiency is one of the main challenges of Mediterranean countries, making the selection of suitable plants and optimizing irrigation methods one of the main issues in agriculture (Ferrández et al., 2003). The average yield reduction of agricultural products due to water shortage is more than 25% globally (Delfine et al., 2005). Water deficiency causes variations in physiological and metabolic processes such as stomatal closure, diminution of photosynthesis rate, and consequently plant development (Tardieu et al., 2018). Although water deficiency reduces the agronomic yield of the medicinal plants, the biosynthesis of EO oil increases as a mechanism against environmental stresses (Sonmez and Bayram, 2017).

On the other hand, the unrestricted use of chemicals affects non-target organisms by altering biological ecosystems and challenges sustainable production and product quality (Nadjafi et al., 2014). Fertilizers are soil amendments that are used to improve a plant's growth. The primary nutrients in fertilizers are nitrogen, phosphorus, and potassium, which are known as macronutrients. In addition to the primary elements, there are micronutrients (trace elements) used in smaller amounts. The origins of these inorganic fertilizers are usually chemical sources (Naguib, 2011). On the other hand, organic fertilizers with natural origin are exceptionally acceptable in terms of interaction with natural ecosystems and human health (Birkhofera et al., 2008). Furthermore, the use of fertilizers from various nutritional sources to cultivate medicinal and aromatic plants improve their agronomic and chemical yields (Boroomand and Hosseini Grouh, 2012). In addition to using different fertilizers and irrigation methods, hydrogels as the supporting materials increase the tolerance of plants to various stresses, especially water deficiency. Hydrogels are the polymer absorbents that reduce the destructive effects of drought stress by confining considerable amounts of water in their own structure and gradually releasing moisture to the rhizosphere. The additive effect of hydrogel application on increasing the yield is probably related to the continued availability of water. Furthermore, hydrogel makes nutrients available to the plant by absorbing the elements (Kumar et al., 2018).

The present study was conducted to investigate the agronomic yield and chemical response of Salvia officinalis L. to three different irrigation levels, various nutrient sources, and culture bed as follows: NPK, farmyard manure, foliar fertilizer, and hydrogel in greenhouse conditions located at Afyonkarahisar Province, Turkey.

2. Materials and methods

2.1. Plant material, location and treatments

The plant material was *Salvia officinalis* L. (common sage) obtained from seeds. The seeds were imbibed in distilled water for 4 h and were sown into the holes of plug trays at a depth 3–7 mm on April 7th, 2017. An equal mix of peat and soil was used as the seedbed. The current study was conducted at the greenhouse located at Afyonkarahisar Medicinal and Aromatic Plants Center, Turkey (38°46′ N, 30°30′ E) in 2017. Air conditioning was performed by automatic ventilation equipped with sensitive thermometers. The average temperature in the greenhouse was kept at 25 ± 7 °C.

Field trials were arranged based on a split-plot design in the randomized complete block with three replications. Five pots were considered for each treatment. Three irrigation levels [I_1: irrigation after 70 ± 5 mm (regular irrigation), I_2: 105 ± 5 mm (moderate drought stress), and I_3: 140 ± 5 mm (severe drought stress) evaporation from the soil surface] were arranged as the main plots. The evaporation was determined by the class A evaporation pan. Based on evaporation levels, the treatments were irrigated by 2 L pot^{-1} water. None fertilizer (control), chemical fertilizers (NPK; N: 120 kg ha^{-1}, P: 60 and K: 60 kg ha^{-1}), farmyard manure (60 ton ha^{-1}), foliar fertilizer (three times at each cutting stage), and superabsorbent polymer (hydrogel: 8.2 ton ha^{-1}) were considered as the sub-plots. When the seedlings reached 6–8 leaves, they were transplanted to the pots (diameter 27 cm, depth 28.5 cm) filled with 15 kg of soil on May 30th, 2017. The experimental soil was sifted and aerated for ten days. The physico-chemical properties of the soil are given in Table 1. For determining the different fertilizers and hydrogel amounts, the bulk density of soil was obtained based on the one hectare of the farm soil weight at a depth of 30 cm:

Bulk Density (g/cm^3) = Dry soil weight (g) / Soil volume (cm^3)

N: 0.44 g pot^{-1}, P and K: 0.22 g pot^{-1}, farmyard manure: 220 g pot^{-1}, and hydrogel: 30 g pot^{-1} were calculated for related treatments based on calculations. Half of N and total of PK were applied at the transplanting stage provided from 15:15:15 fertilizer type. The remaining N was added to related pots after the 1st cutting. All of farmyard manure and hydrogel were mixed into the soil of pots before transplanting. Foliar fertilizer was applied at intervals of 14 days (Table 2). Some properties of the farmyard manure and foliar fertilizer are given in Tables 3 and 4.

2.2. Harvesting and measurements

The plant height was calculated from the average of 5 plants per treatment. Two cuttings were done during the study above 5–8 cm of the soil surface when the first buds appeared throughout the experiment (Table 5). All plants of each treatment were weighed to record fresh herb weight. Then, leaves and branches were separated and weighed to calculated dry weights. The plant parts were dried at 37 °C for 72 h by a dryer.

2.3. Essential oil isolation

For isolation of the essential oil (EO) content, 50 g of dried leaves in 500 mL of distilled water were subjected to hydrodistillation using a neo-Clevenger apparatus for 3 h with three replications. The obtained EOs were dried over anhydrous sodium sulphate and stored at 4 °C in ambered vials until the chromatographic analysis.

Table 1
Physico-chemical properties of the soil in the experimental pots.

Properties	(%)	Elements	(ppm)
Organic matter content	1.06	Ca	1772
Total N	0.10	Mg	624
Sand	54.64	K	313
Clay	24.95	Na	97
Dust	20.41	Fe	0.51
Lime	0.72	P	131.08
Field capacity	19.13	Cu	2.4
Wilting point	14.55	Zn	0.59
Available moisture	4.58	Mn	1.95

Soil class: Sandy clay; EC (mS cm^{-1}): 0.13; pH: 7.97.

Table 2
Application dates of foliar fertilizer during two cuttings.

Frequentation	Date		Amount
	1st cutting	2nd cutting	
1	14 June 2016	25 August 2017	300 ml 100 L^{-1}
2	28 June 2016	08 September 2017	300 ml 100 L^{-1}
3	11 July 2016	22 September 2017	300 ml 100 L^{-1}

Table 3
Physico-chemical properties of the farmyard manure.

Properties	(%)	Elements	(mg kg^{-1})
Dry weight at 55 °C	39.1	Zn	0.33
Dry weight	31.9	Fe	198
Na	2.98	Mn	131
O.M.	47.19	Cu	22
N	0.62		
P	0.025		
K	0.17		
Mg	0.32		
Ca	0.25		
pH: 7.65			

Table 4
Chemical properties of the foliar fertilizer.

Element	(% v w^{-1})
Total N	9.2
Nitrate nitrogen (N)	4.4
Ammonium nitrate (N)	1.4
Urea nitrogen (N)	3.4
Water-soluble phosphorus pentoxide (P_2O_5)	6.8
Water-soluble potassium oxide (K_2O)	18.2
B	0.10
Cu* (EDTA chelated)	0.021
Fe** (EDTA chelated)	0.05
Mn* (EDTA chelated)	0.02
Mo	0.005
Zn* (EDTA chelated)	0.051
* pH range that chelate is stable:	pH 2–11
** pH range that chelate is stable:	pH 2–6.5

Table 5
Date of *S. officinalis* cuttings during the experiment.

Cuttings	Date	Growth Period
1st	2 August 2017	107 days
2nd	13 October 2017	72 days

2.4. Chemical analyses

A gas chromatography (GC) system (Agilent Technologies, 7890B), which was equipped with a flame ionization detector (FID) and coupled to a mass spectrometry detector (MSD) (Agilent Technologies, 5977A), was used for identifying the compositions of the oils. The column for the separation of the compositions was HP-Innowax (Agilent 19091 N-116: 60 m × 0.320 mm internal diameter and 0.25 μm film thickness). Helium (purity: 99.999%) was the carrier gas with 1.3 mL min^{-1} flow rate. The injection volume was set at 1 μL (20 μL EO was dissolved in 1 mL of n-Hexane). The solvent delay time was set to 8.20 min, and the injection was performed in split mode (40:1) by the automatic liquid sampler (ALS). The samples were analyzed with the column held initially at 70 °C after injecting with 5 min hold time. Then, the temperature was raised to 160 °C with 3 °C min^{-1} heating ramp and 5 min hold time. Eventually, the temperature reached 250 °C with 6 °C min^{-1} heating ramp and 5 min hold time. Thus, the detector, injector, and ion source temperatures were 270 °C, 250 °C, and 230 °C, respectively. MS scan range was (m z^{-1}) 50–550 atomic mass units (AMU) under electron impact (EI) ionization of 70 eV.

The retention indices (RI) were determined by injecting C7-C30 n-alkanes (Sigma-Aldrich) to the GC/FID system (Agilent Technologies, 7890B) under the same conditions of the analyses of the EOs. The EO components' identifications were determined by comparing retention indices, mass spectra by the computer library database of the US National Institute of Standards and Technology (NIST), Wiley libraries, other published mass spectra data (Adams, 2007), and our database. Relative abundance (% area) was calculated based on the ratio between the peak area of each compound and the sum of areas of all compounds.

2.5. Statistical analyses

Data were analyzed using the MSTAT-C computer software program. The means of treatments were compared using the Least Significant Difference (LSD) test at a 0.05 probability level. The analysis of variance was conducted on the samples to determine variations of parameters between the treatments.

3. Results

3.1. Plant height and branch number

A significant difference was found in irrigation, fertilizer, and cuttings regarding plant height. The shortest plants were found in water deficit treatments (Table 6). Accordingly, the tallest plants were determined after regular irrigation and the 1st cutting. The tallest plant height was observed in the application of NPK, foliar fertilizer, farmyard manure, and hydrogel, respectively. Branch number was affected by various fertilizer applications and cuttings. The irrigation frequency variation did not alter branch numbers. In contrast, the NPK application increased this parameter. Other treatments ranked in a similar statistical group. The number of branches in the 2nd cutting was higher than the 1st. The interaction effect of fertilizer and cutting was also significant on branch number. The highest and lowest branch number was observed by the NPK × 2nd cutting and control × 1st cutting interaction, respectively (Table 6 and 7).

3.2. Herb yield and leaf weight

The fresh and dry weights of the aerial parts of the plants were evaluated as the herb yield. The means of herb and leaf weights as the main yield components revealed significant differences in the various irrigation levels, fertilizers, and cuttings. Prolongation of irrigation intervals deteriorated the yield components studied. The highest fresh and dry weights were recorded after the application of NPK fertilizers. Farmyard manure and hydrogel treatments at a similar statistical group were ranked lower than NPK. The lowest fresh and dry weights were also obtained in both foliar and control treatments. The fresh and dry weights were higher in the 2nd cutting (Table 6). According to Table 7, the interaction effect of irrigation × cutting on fresh herb weight, fresh and dry leaf weights was significant. The 2nd cutting that had been regularly irrigated had a higher fresh weight. The lowest fresh weight was also obtained from severe drought stress treatment in the 1st cutting. The highest and lowest fresh and dry weights of leaves were observed by regular irrigation × 2nd cutting and severe drought stress × 1st cutting interactions, respectively. Also, the interaction of NPK × 2nd cutting had the highest amount of fresh leaves. The lowest fresh leaf was also obtained by the no fertilizing × 1st cutting interaction. The dominance of 2nd cuttings in each treatment was apparent because of water availability and the increasing

Table 6
The effects of water deficit, various nutrient sources and cutting times on some yield components and essential oil content of *S. officinalis* in the greenhouse.

Treatment	Plant Height (cm)	Branch Number (no p^{-1})[a]	Fresh Herb (g p^{-1})[b]	Dry Herb (g p^{-1})	Fresh Leaf (g p^{-1})	Dry Leaf (g p^{-1})	Essential Oil (%)
Irrigation (I)							
I_1: 70 ± 5[a]	51.34a	15.10	51.50a	18.15a	40.14a	13.18a	1.32c
I_2: 105 ± 5[b]	45.97b	14.50	42.80ab	15.26ab	33.07a	11.05a	1.48a
I_3: 140 ± 5[c]	40.86c	13.83	30.07b	11.12b	23.10b	7.95b	1.36b
Probability level (%)	≤0.01	ns	≤0.05	≤0.05	≤0.05	≤0.05	≤0.01
$LSD_{(p \leq 0.05)}$	1.231	–	13.66	5.839	9.480	2.902	0.02267
Fertilizer (F)							
Control	40.00e	12.22b	33.12c	11.77d	25.70c	8.48d	1.18e
NPK	51.85a	19.22a	53.47a	18.96a	41.22a	13.62a	1.67a
Farmyard	45.99c	14.00b	45.86b	16.53b	35.70b	11.96b	1.38c
Hydrogel	42.66d	12.94b	41.12b	14.56c	31.65b	10.48c	1.28d
Foliar	49.77b	14.00b	33.70c	12.35d	26.25c	9.09cd	1.42b
Probability level (%)	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01
$LSD_{(p \leq 0.05)}$	1.165	1.934	4.912	1.930	4.221	1.460	0.02176
Cutting (C)							
1st cutting	61.74a	12.91b	34.74b	13.34b	24.14b	8.62b	1.51a
2nd cutting	30.37b	19.04a	48.17a	16.35a	40.07a	12.83a	1.27b
Probability level (%)	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01
C.V. (%)	5.65	14.49	19.98	21.18	21.70	21.03	4.01

[a]: regular irrigation, [b]: moderate drought stress, [c]: severe drought stress.
The means which have no letters are statistically non-significant at ≤ 0.05% and ≤ 0.01% probability level; ns: non-significant; LSD: least significant difference; C.V.: coefficient of variation.

Table 7
The interactions effects of irrigation level × cutting time and fertilizer × cutting time on some yield components and essential oil content of *S. officinalis* in the greenhouse.

Treatment	Plant Height (cm)	Branch Number (no p^{-1}) [a]	Fresh Herb (g p^{-1}) [b]	Dry Herb (g p^{-1})	Fresh Leaf (g p^{-1})	Dry Leaf (g p^{-1})	Essential Oil (%)
Irrigation (I) × Cutting (C)							
$I_1 \times C_1$	67.70	13.20	42.50c	16.08	29.81c	10.48c	1.45c
$I_1 \times C_2$	34.97	17.00	60.50a	20.21	50.46a	15.89a	1.19e
$I_2 \times C_1$	61.66	12.80	35.13d	13.44	24.47d	8.71d	1.57a
$I_2 \times C_2$	30.29	16.20	50.47b	17.08	41.68b	13.39b	1.38d
$I_3 \times C_1$	55.86	12.73	26.60e	10.49	18.14e	6.68e	1.49b
$I_3 \times C_2$	25.85	14.93	33.54d	11.75	28.07cd	9.21cd	1.23e
Probability level (%)	ns	ns	≤0.05	ns	≤0.05	≤0.05	≤0.05
$LSD_{(p \leq 0.05)}$	–	–	6.178	–	5.196	1.682	0.04085
Fertilizer (F) × Cutting (C)							
$F_1 \times C_1$	55.58	10.89e	28.95	10.70	20.31e	6.96	1.28
$F_1 \times C_2$	24.42	13.56bd	37.29	12.84	31.09c	10.00	1.07
$F_2 \times C_1$	68.22	15.44b	42.37	16.54	29.08cd	10.52	1.80
$F_2 \times C_2$	35.47	23.00a	64.57	21.38	53.35a	16.72	1.54
$F_3 \times C_1$	61.59	13.00cd	37.82	15.13	26.16ce	9.80	1.49
$F_3 \times C_2$	30.40	15.00bc	53.89	17.94	45.25b	14.13	1.27
$F_4 \times C_1$	58.15	11.89de	33.96	13.06	23.42de	8.37	1.42
$F_4 \times C_2$	27.17	14.00bc	48.28	16.14	39.88b	12.60	1.14
$F_5 \times C_1$	65.15	13.33cd	30.60	11.25	21.73e	7.47	1.52
$F_5 \times C_2$	34.40	14.67bc	36.79	13.44	30.77c	10.70	1.31
Probability level (%)	ns	≤0.01	ns	ns	≤0.05	ns	ns
$LSD_{(p \leq 0.05)}$	–	2.019**	–	–	6.708*	–	–

The means which have no letters are statistically non-significant at ≤ 0.05% and ≤ 0.01% probability level; ns: non-significant; LSD: least significant difference; C.V.: coefficient of variation.

effects of NPK and farmyard manure on leaf fresh weight rising (Table 7). When the effects of irrigation and cutting were compared, the cutting was more effective on fresh weight than irrigation.

3.3. Essential oil content and chemical components

The results indicated that the EO content of *S. officinalis* was significantly affected by irrigation regimes, different nutrient sources, and cuttings (Table 6). Plants exposed to moderate drought stress had high efficiency in producing EOs. Accordingly, moderate drought stress treatment produced the highest content of the EO (1.48%). The levels of severe drought stress and regular irrigation treatments were subdivided into the following groups. The maximum EO content was obtained in NPK treatment (1.67%). The foliar fertilizer and farmyard manure treatments were placed in the next rank. Hydrogel and control treatments had the lowest content of EO, respectively. Also, the EO content at the 1st cutting was more than the 2nd cutting (Table 6). The interaction effect of irrigation × fertilizer on the EO content of *S. officinalis* was also significant. The highest EO content was observed in NPK × moderate drought stress and NPK × severe drought stress. The minor content was related to no fertilizing treatment (control) and regular irrigation regime (Fig. 1). Although the regular irrigation did not enhance *S. officinalis* EO content, the consumption of different nutrient sources showed an additive effect on improving the EO

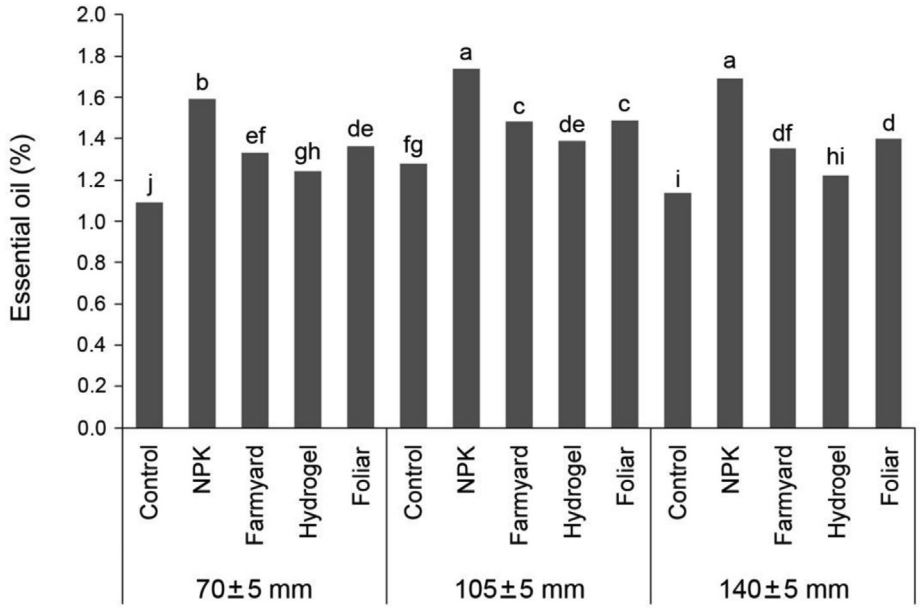

Fig. 1. The interaction effects of irrigation level × fertilizer on the essential oil content of S. officinalis in the greenhouse (LSD = 0.06527; $P \leq 0.05$).

production. Also, considering the EO content of the hydrogel treatment, the efficiency of fertilizer application becomes more apparent.

In total, 32 and 31 compounds were identified in the 1st and 2nd cuttings, respectively, by chromatographic analysis (Table 8 and 9). Based on the chromatographic analysis of *S. officinalis* EO samples, α-thujone (from 21.6 to 34.2%) was identified as the most major compound in both cuttings (Table 8 and 9). The amounts of α-thujone in the 1st and 2nd cuttings were from 21.6 to 27.8% and 24.4 to 34.2%. The biosynthesis of α-thujone in the 2nd cuttings was higher than in the 1st (Fig. 2). NPK and hydrogel treatments in regular irrigation and severe drought stress levels had the highest accumulation of α-thujone in both cuttings. Additionally, the amounts of α-thujone in the moderate drought stress treatment were higher than the other irrigation regimes in both cuttings. Camphor (from 19.4 to 27.0%) was the second most important compound of *S. officinalis* in terms of concentration (Table 8 and 9). This compound also showed a relative increase in the 2nd cutting. Also, regular irrigation treatment had higher camphor contents in both cuttings than the other irrigation regimes (Table 8 and 9). In terms of maximum accumulation of the EO oil compounds at both cuttings, 1,8-cineole (from 7.9 to 13.3%) was the third constituent. The highest values of this compound were observed in moderate drought stress treatment with NPK consumption in both cuttings. A comparison of data showed that NPK fertilizers had a significant effect on increasing 1,8-cineole. β-Thujone (from 4.0 to 8.4%), camphene (from 3.9 to 6.8%), α-pinene (from 3.1 to 7.2%), α-humulene (from 3.0 to 5.3%), viridiflorol (from 2.7 to 5.2%), borneol (from 2.7 to 4.6%) and bornyl acetate (from 2.4 to 4.2%) were the other main compounds of the 1st cutting (Table 8). Also, β-thujone (from 3.9 to 9.9%), camphene (from 3.5 to 6.3%), α-pinene (from 2.7 to 4.0%), borneol (from 2.7 to 4.5%), viridiflorol (from 2.2 to 4.4%) and α-humulene (from 2.2 to 3.4%) were identified as the other major components of *S. officinalis* in the 2nd cutting. The sum of α-thujone and β-thujone amounts as ketone was also higher in the 2nd cutting treatment than the 1st (Fig. 2).

Oxygenated monoterpenes were the greatest chemical classes in all EO samples. Except for the hydrogel application with regular irrigation and the NPK with moderate drought stress treatment, the oxygenated monoterpenes in the other treatments increased in the 2nd cutting. Conversely, monoterpene hydrocarbons decreased in most of the 2nd cutting treatments. The majority of the reduction in monoterpene hydrocarbons was observed at the moderate drought stress level. Except for the hydrogel application in regular irrigation treatment, the sesquiterpene hydrocarbons accumulation in the 2nd cutting had a decreasing trend. Oxygenated sesquiterpenes, ethers, and other compounds in both cuttings did not follow a distinct tendency. While esters constituted a small fraction of the EO, the reduction of these compounds was partially apparent at the 2nd cutting. Epimanool as an oxygenated diterpene compound was only observed in the control treatment of the 1st cutting. The only identified alkyl aldehyde compound, Nonanal, was also detected in all treatments of 2nd cutting.

4. Discussion

4.1. Plant height and branch number

This study showed that drought stress applied by increasing irrigation interval significantly decreased plant height of *S. officinalis*. The additive effect of different fertilizers was also evident in the number of branches. The number of branches is closely related to genetic characteristics and climatic factors (Mamnabi et al., 2020). In the present study, it seems that the genetic makeup was the more dominant factor because drought stress did not affect the number of branches. Additionally, the number of branches was increased by the applications of different nutrient sources. One of the first symptoms of water deficiency is the loss of turgor pressure and consequently decreasing cell expansion in plants. The movement of nutrients from the soil matrix to the root and their uptake by the plants is reduced due to the decreasing soil moisture under drought stress (Hu et al., 2007). The macro elements such as N, P, and K have critical functions in plant development. Especially N has a vital role in cell division and enlargement, which increases plant height and branch number (Purbajanti et al., 2019). The application of farmyard manure and foliar fertilizer prevented the reduction in plant height by providing plant access to different elements compared with the control treatment. Moreover, the hydrogel provides part of the water required by the plant

Table 8
The effects of water deficit and various nutrient sources on the essential oil compounds and compound classes of *S. officinalis* in the 1st cutting.

RI*	Compounds (%)	70 ± 5 mm evaporation					105 ± 5 mm evaporation					140 ± 5 mm evaporation				
		Ctrl.[a]	NPK	FY[b]	Hyd.[c]	Fol.[d]	Ctrl.	NPK	FY	Hyd.	Fol.	Ctrl.	NPK	FY	Hyd.	Fol.
1019	Tricyclene	0.13	0.18	0.2		0.18	0.14				0.15	0.23	0.17	0.19		0.22
1031	**α-Pinene**	**3.78**	**4.81**	**4.7**	**3.78**	**4.3**	**5.45**	**3.19**	**4.29**	**3.11**	**3.56**	**7.24**	**4.02**	**5.31**	**4.55**	**5.3**
1072	α-Thujene	0.15	0.21	0.2	0.18	0.18	0.2	0.16	0.18	0.13	0.35	0.21	0.22	0.2	0.18	0.2
1076	**Camphene**	**4.7**	**5.67**	**6.24**	**4.79**	**5.92**	**4.88**	**4.75**	**4.04**	**4**	**5.11**	**6.41**	**5.4**	**5.76**	**4.61**	**6.89**
1120	**β-Pinene**	**1.94**	**2.28**	**1.93**	**1.74**	**2.14**	**2.08**	**2**	**1.74**	**1.64**	**1.87**	**2.3**	**2.17**	**2.18**	**1.87**	**0.16**
1130	Sabinene		0.15				0.14						0.16	0.15	0.14	2.13
1167	β-Myrcene	0.99	1.07	1.07	1.04	1.07	1.14	1.02	1.01	0.97	1.06	1.02	1.15	1.07	1.11	1.1
1190	α-Terpinene	0.22	0.23	0.24	0.23	0.23	0.25	0.22	0.25	0.22	0.23	0.23	0.23	0.23	0.22	0.25
1209	**Limonene**	**2.27**	**2.43**	**2.56**	**2.15**	**2.51**	**2.41**	**2.21**	**2.11**	**2.33**	**2.28**	**2.37**	**2.42**	**2.42**	**2.37**	**2.63**
1219	**1,8-Cineole**	**10.45**	**11.34**	**8.75**	**11.03**	**10.66**	**11.3**	**12.79**	**8.64**	**8.5**	**9.22**	**8.88**	**11.5**	**10.83**	**10.37**	**10.09**
1254	γ-Terpinene	0.44	0.45	0.45	0.44	0.45	0.48	0.43	0.51	0.45	0.46	0.43	0.44	0.44	0.43	0.47
1281	o-Cymene	0.29	0.3	0.32	0.3	0.32	0.32	0.3	0.31	0.31	0.32	0.28	0.33	0.31	0.3	0.32
1292	α-Terpinolen	0.59	0.6	0.64	0.54	0.61	0.62	0.57	0.6	0.8	0.74	0.6	0.6	0.59	0.61	0.64
1435	**α-Thujone**	**21.69**	**24.57**	**22.84**	**26.52**	**23.73**	**26.12**	**25.61**	**27.86**	**26.6**	**26.5**	**23.09**	**27.75**	**22.17**	**24.11**	**23.03**
1454	**β-Thujone**	**8.5**	**4.77**	**5.76**	**7.78**	**6.32**	**5.86**	**6.96**	**6.23**	**4.34**	**6.64**	**4.03**	**4.85**	**6.87**	**7.27**	**5.29**
1469	*cis*-Sesquisabinene hydrate	0.2	0.37	0.19	0.18	0.18	0.21	0.19	0.21	0.24		0.4	0.24	0.22	0.26	
1530	**Camphor**	**19.76**	**22.35**	**24.42**	**22.61**	**24.72**	**20.78**	**20.72**	**19.43**	**23.65**	**22.46**	**21.97**	**21.69**	**20.29**	**20.52**	**23.53**
1550	Linalool	0.47	0.36	0.36	0.38	0.41	0.39	0.33	0.41	0.36	0.37	0.38	0.3	0.37	0.43	0.35
1553	*trans*-Sabinene hydrate	0.2		0.2	0.18		0.19	0.21	0.23	0.26	0.44	0.15	0.2	0.21	0.24	
1590	**Bornyl acetate**	**4.23**	**2.94**	**3.26**	**2.48**	**2.83**	**2.56**	**3.35**	**2.97**	**3.74**	**3.22**	**3.58**	**2.79**	**3.23**	**3.12**	**3.76**
1608	**Caryophyllene**	**2.32**	**2.04**	**2.19**	**2.06**	**1.86**	**2.05**	**1.85**	**2.69**	**2.61**	**1.65**	**2.94**	**1.79**	**2.09**	**2.76**	**1.93**
1618	(+)-Aromadendrene											0.26			0.19	
1659	Sabinyl acetate	0.31	0.26	0.28	0.26	0.27	0.33	0.28	0.4	0.31	0.31	0.34	0.29	0.29	0.28	0.29
1681	**α-Humulene**	**3.47**	**3.54**	**3.81**	**3.14**	**3.07**	**3.92**	**3.37**	**5.4**	**4.47**	**3.58**	**4.04**	**3.52**	**3.74**	**4.74**	**3.35**
1703	α-Terpineol	0.44	0.25	0.33	0.29	0.36	0.26	0.29	0.34	0.35	0.29	0.33	0.36	0.3	0.32	0.34
1709	**Borneol**	**4.64**	**3.55**	**3.82**	**3.46**	**3.23**	**2.91**	**3.69**	**2.75**	**3.81**	**3.83**	**3.54**	**3.75**	**4.51**	**3.21**	**4.01**
1995	Caryophyllene oxide	0.26	0.21	0.22	0.25	0.2	0.2	0.21	0.22	0.23	0.18	0.25	0.15	0.26	0.28	0.18
2053	Humulene oxide	0.55	0.48	0.53	0.46	0.49	0.48	0.61	0.67	0.65	0.56	0.43	0.41	0.58	0.62	0.45
2076	Acetophloroglucine	0.49	0.21	0.34	0.21	0.28	0.29	0.46	0.58	0.61	0.32	0.37	0.23	0.3	0.57	0.31
2093	**Viridiflorol**	**3.68**	**4.16**	**3.38**	**3.38**	**3.23**	**3.87**	**4.05**	**5.24**	**4.93**	**3.71**	**3.04**	**2.77**	**4.73**	**3.93**	**2.77**
2388	Unknown	0.21	0.19	0.75	0.12	0.21	0.15	0.16	0.67	0.35	0.56	0.63	0.1	0.15	0.37	
2691	**Epimanool**	**2.6**														
Number of identified compounds		30	29	29	28	28	30	28	28	28	28	30	30	30	30	27
Grouped compounds (%)																
Oxygenated monoterpenes		61.07	63.39	62.33	68.5	65.84	64.64	66.62	62.8	63.71	65.63	58.5	66.29	60.74	62.94	62.29
Monoterpene hydrocarbons		15.5	18.38	18.55	15.19	17.91	18.11	14.85	15.04	13.96	16.13	21.32	17.31	18.85	16.39	20.31
Oxygenated sesquiterpenes		9.33	8.77	8.14	7.73	7.33	7.67	8.75	9.09	9.86	8.28	7.66	7.32	10.3	8.3	7.41
Sesquiterpene hydrocarbons		6.23	5.83	6.33	5.49	5.29	6.23	5.51	8.43	7.43	5.52	7.57	5.67	6.13	8.01	5.62
Oxygenated diterpene		2.6														
Esters		4.54	3.2	3.54	2.74	3.1	2.89	3.63	3.37	4.05	3.53	3.92	3.08	3.52	3.4	4.05
Ketones		0.49	0.21	0.34	0.21	0.28	0.29	0.46	0.58	0.61	0.32	0.37	0.23	0.3	0.57	0.31
Others		0.21	0.19	0.75	0.12	0.21	0.15	0.16	0.67	0.35	0.56	0.63	0.1	0.15	0.37	
Total (%)		99.97	99.97	99.98	99.98	99.96	99.98	99.98	99.98	99.97	99.97	99.97	100	99.99	99.98	99.99

* RI: Retention indices calculated against n-alkanes (C7-C30) on HP-Innowax column; [a] Control; [b] Farmyard; [c] Hydrogel; [d] Foliar.

under drought stress and facilitates the uptake of nutrients by plants. Numerous studies have reported the positive effect of decreasing irrigation intervals, NPK, foliar fertilizers, farmyard manure, and hydrogel application on plant height (Khalid, 2012; Gerami et al., 2016; Bahtiyarca Bagdat et al., 2017; Govahi et al., 2015; Ljubojevi et al., 2017). The results of the present study suggest that the various irrigation regimes and fertilization increase plant growth by regulating plant water status and nutrient balance.

In the present experiment, plant height was higher in the 1st cutting than the 2nd. It has probably resulted from a long period of growth and vital growth factors such as nutrients and sufficient light. This result was constituent with Sonmez and Bayram (2017), who reported that adding 60 kg of N and decreasing irrigation intervals affected the height of *S. officinalis*. In the present study, the number of branches significantly increased after the 1st cutting. This increase at the 2nd cutting was probably due to eliminating apical dominance regulated by growth hormones. Similar results were reported by El-Fiky et al. (2006) and Aziz et al. (2013) in *S. officinalis*. The present study results may suggest that the effect of cutting on the number of branches is superior to fertilizer consumption.

4.2. Herb weight and leaf weight

Based on the results, water deficiency decreased the plant yield components. The critical effects of water deficiency are the decrease in plant dimension, leaf area, and yield (Sonmez and Bayram, 2017). In addition, the closure of the stomata and reducing plant cell turgor pressure under water deficit conditions reduce the photosynthesis rate and growth capability (Lier et al., 1999). Bettaieb et al. (2011) reported that the herb weight of *S. officinalis* was reduced by the increasing irrigation intervals and insufficient access to water sources. However, the dry matter of *S. officinalis* was enhanced by reducing irrigation intervals and applications of different fertilizer sources with chemical and organic origin (Govahi et al., 2015). In this experiment, along with the plant's regular access to water, the supply of various nutrient sources also improved plant yield. These results were constituent with Radnezhad et al. (2015) and Ljubojevi et al. (2017), who showed that macro fertilizers (NPK), farmyard manure, and hydrogel increased fresh herb weight in *S. officinalis*. Enhanced plant growth with different nutrient sources might be resulted from improving soil productivity by restoring structure, reviving fertility, and biodiversity of the soil. These soil structure improvements may help

Table 9
The effects of water deficit and various nutrient sources on the essential oil compounds and compound classes of *S. officinalis* in the 2st cutting.

RI	Compounds (%)	70 ± 5 mm evaporation					105 ± 5 mm evaporation					140 ± 5 mm evaporation				
		Ctrl.[a]	NPK	FY[b]	Hyd.[c]	Fol.[d]	Ctrl	NPK	FY	Hyd.	Fol.	Ctrl	NPK	FY	Hyd.	Fol.
1019	Tricyclene	0.16		0.16		0.16	0.17	0.17	0.15	0.18	0.15	0.21	0.13	0.20		0.17
1031	**α-Pinene**	**3.58**	**3.68**	**3.45**	**3.27**	**2.79**	**4.27**	**3.98**	**4.09**	**4.63**	**3.21**	**6.57**	**2.86**	**4.55**	**3.80**	**3.91**
1072	α-Thujene	0.19	0.16	0.16	0.17	0.17	0.19	0.23	0.18	0.21	0.21	0.16	0.23	0.21	0.19	0.17
1076	**Camphene**	**5.23**	**4.42**	**5.66**	**4.02**	**5.27**	**5.13**	**5.16**	**4.63**	**5.57**	**4.68**	**6.31**	**4.45**	**5.68**	**3.51**	**5.27**
1120	**β-Pinene**	**2.28**	**2.07**	**1.82**	**1.76**	**2.09**	**2.02**	**3.08**	**2.09**	**2.06**	**2.05**	**2.14**	**2.49**	**2.45**	**2.11**	**2.06**
1130	Sabinene	0.15						0.17	0.15	0.14	0.15		0.17	0.16		
1167	β-Myrcene	0.93	0.84	0.82	0.91	0.83	0.85	1.01	0.90	0.88	0.91	0.85	0.94	0.89	0.96	0.89
1190	α-Terpinene	0.18	0.18	0.20	0.19	0.19	0.19	0.21	0.22	0.22	0.21	0.17	0.24	0.20	0.25	0.17
1209	**Limonene**	**2.05**	**1.85**	**2.22**	**1.86**	**1.88**	**1.82**	**2.05**	**1.89**	**1.95**	**1.86**	**2.12**	**1.86**	**1.88**	**1.76**	**1.99**
1219	**1,8-Cineole**	**11.55**	**10.12**	**8.79**	**10.49**	**10.58**	**10.60**	**13.30**	**9.13**	**9.14**	**7.91**	**8.06**	**12.28**	**10.98**	**12.69**	**9.55**
1254	γ-Terpinene	0.36	0.38	0.38	0.40	0.34	0.40	0.42	0.44	0.43	0.42	0.34	0.45	0.39	0.47	0.35
1281	o-Cymene	0.33	0.33	0.34	0.39	0.38	0.36	0.38	0.42	0.38	0.40	0.34	0.37	0.35	0.42	0.37
1292	α-Terpinolen	0.42	0.38	0.46	0.36	0.34	0.36	0.40	0.39	0.40	0.37	0.40	0.39	0.35	0.36	0.38
1402	Nonanal	0.60	0.48	0.43	0.49	0.49	0.43	0.53	0.48	0.44	0.48	0.48	0.44	0.52	0.50	0.53
1435	**α-Thujone**	**24.57**	**30.69**	**24.71**	**30.54**	**29.49**	**32.14**	**27.21**	**28.38**	**29.52**	**34.29**	**26.68**	**31.11**	**27.79**	**33.22**	**24.40**
1454	**β-Thujone**	**8.13**	**4.62**	**6.67**	**6.35**	**6.36**	**4.62**	**6.11**	**7.83**	**6.66**	**4.62**	**3.95**	**4.77**	**6.27**	**7.29**	**9.99**
1469	*cis*-Sesquisabinene hydrate	0.23	0.20		0.18		0.18	0.25	0.20	0.17	0.19		0.16	0.22		0.23
1530	**Camphor**	**23.18**	**20.59**	**27.08**	**22.07**	**25.57**	**22.92**	**19.32**	**22.04**	**23.31**	**21.52**	**25.01**	**22.63**	**21.08**	**18.18**	**22.65**
1550	Linalool	0.21	0.47	0.33	0.26	0.34	0.32	0.21	0.36	0.33	0.30	0.32	0.25	0.21	0.33	0.29
1553	*trans*-Sabinene hydrate	0.20	0.19					0.21	0.18	0.15	0.19		0.15	0.19		0.21
1590	**Bornyl acetate**	**1.84**	**2.45**	**3.23**	**1.34**	**1.58**	**1.96**	**2.21**	**2.02**	**2.15**	**2.17**	**2.73**	**1.88**	**1.96**	**1.34**	**2.69**
1608	**Caryophyllene**	**1.54**	**1.84**	**1.67**	**2.07**	**1.76**	**1.59**	**1.65**	**1.75**	**1.59**	**1.43**	**1.74**	**1.77**	**1.25**	**1.96**	**1.67**
1659	Sabinyl acetate	0.21	0.24	0.23	0.23		0.23	0.22	0.24	0.24	0.28	0.23	0.26	0.20	0.21	0.29
1681	**α-Humulene**	**2.87**	**3.23**	**2.34**	**3.47**	**2.53**	**2.45**	**3.37**	**3.12**	**2.37**	**3.20**	**2.79**	**2.58**	**2.27**	**2.85**	**2.70**
1703	α-Terpineol		0.31	0.25	0.26	0.25	0.24	0.20	0.29	0.24	0.23	0.29	0.30	0.20	0.25	0.27
1709	**Borneol**	**2.85**	**4.17**	**4.58**	**3.02**	**3.23**	**3.08**	**2.78**	**2.76**	**3.24**	**3.66**	**3.90**	**2.83**	**3.52**	**2.97**	**4.24**
1995	Caryophyllene oxide	0.23	0.35	0.25	0.42	0.24	0.23	0.24	0.32	0.23	0.24	0.26	0.21	0.24	0.34	0.32
2053	Humulene oxide	0.57	0.80	0.46	0.81	0.44	0.46	0.62	0.70	0.45	0.74	0.52	0.42	0.58	0.60	0.65
2076	Acetophloroglucine	0.10	0.25	0.12			0.10		0.11	0.12	0.14	0.12		0.12		0.14
2093	**Viridiflorol**	**4.24**	**4.36**	**2.74**	**4.17**	**2.28**	**2.57**	**4.19**	**4.40**	**2.49**	**3.75**	**2.46**	**3.12**	**3.85**	**2.91**	**3.39**
2388	Unknown	0.98	0.33	0.42	0.36	0.4	0.11	0.11	0.12	0.1	0.04	0.85	0.22	1.04	0.49	0.05
Number of identified compounds		30	29	28	27	26	29	30	31	31	31	28	30	31	26	30
Grouped compounds (%)																
Oxygenated monoterpenes		67.84	66.68	67.58	69.71	72.34	70.6	66.36	67.92	69.11	68.83	64.02	71.19	66.52	71.71	67.09
Monoterpene hydrocarbons		15.86	14.29	15.67	13.33	14.44	15.76	17.26	15.55	17.05	14.62	19.61	14.58	17.31	13.83	15.73
Oxygenated sesquiterpenes		8.12	9.88	8.03	8.6	6.19	6.52	8.08	8.38	6.58	8.58	7.14	6.74	8.41	6.82	8.83
Sesquiterpene hydrocarbons		4.41	5.38	4.26	5.8	4.54	4.28	5.22	5.16	4.2	4.86	4.82	4.65	3.72	5.06	4.64
Aldehydes		0.6	0.48	0.43	0.49	0.49	0.43	0.53	0.48	0.44	0.48	0.48	0.44	0.52	0.5	0.53
Esters		2.05	2.69	3.46	1.57	1.58	2.19	2.43	2.26	2.39	2.45	2.96	2.14	2.16	1.55	2.98
Ketones		0.1	0.25	0.12			0.1		0.11	0.12	0.14	0.12		0.12		0.14
Others		0.98	0.33	0.42	0.36	0.4	0.11	0.11	0.12	0.1	0.04	0.85	0.22	1.04	0.49	0.05
Total (%)		99.96	99.98	99.97	99.86	99.98	99.99	99.99	99.98	99.99	100	100	99.96	99.8	99.96	99.99

*RI: Retention indices calculated against n-alkanes (C7-C30) on HP-Innowax column; [a] Control; [b] Farmyard; [c] Hydrogel; [d] Foliar.

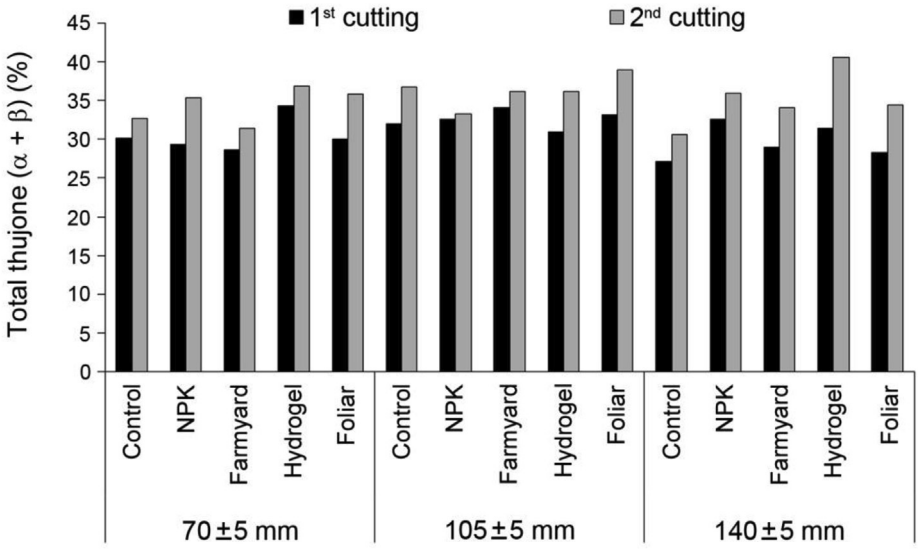

Fig. 2. The effects of water deficit, various nutrient sources and cutting times on sum of α-thujone and β-thujone amounts of S. officinalis in the greenhouse.

plants assimilate nutrients and optimal growth (Ahmadian, 2011). Furthermore, superabsorbent polymers may increase the growth of plants by gradually releasing the nutrients during the drought period (Kumar et al., 2018). Moreover, foliar application of nutrients did not affect yield components. In contrast, Salman et al. (2019) have shown that foliar consumption of nutrients increased the fresh herb of *Salvia hispanica*. In the present study, the dry yield weight in the 2nd cutting was higher than the 1st cutting in a growing season. Hegab et al. (2018) reported that the yield increase in the 2nd cutting was due to the gradual and slow release of N and other nutrients.

4.3. Essential oil content and chemical components

It has been well-known that the biosynthesis of secondary metabolites is genetically determined (Sedlakova et al., 2003). Additionally, climatic and edaphic factors and agricultural practices (fertilizing, irrigation, post-harvest processing, etc.) also affect the production of these metabolites and the chemical constituents of medicinal plants (Ozguven et al., 2008). *Salvia* is one of the plants that produce more EO in response to drought stress (Holtzer et al., 1988). In the present study, irrigation regimes and fertilization affected the biosynthesis of EO in *S. officinalis* which is consistent with the results of Rioba et al. (2015). Garcia-Caparros et al. (2019) have suggested that the enhanced levels of EO under drought stress may be due to the higher density of oil glands. A previous study showed that irrigation after depletion of 60% available water (moderate stress) led to the highest increase in EO content of *S. officinalis* as compared to irrigation after depletion of 40% (low stress) and 80% available water (high stress) under field conditions (Govahi et al., 2015). In contrast, Kleinwachtera et al. (2015) suggested that severe drought stress led to a reduction in the biosynthesis of secondary metabolites. The decrease in EO content under extreme drought stress might be attributed to high consumption of the assimilate to the production of osmotic regulator compounds (Munns, 1993). In the present study, the EO content was found higher in the 1st cutting as compared to the 2nd cutting. A similar result was reported by Sonmez and Bayram (2017). The decrease in EO content at the 2nd cutting is probably due to the light quality, average temperature, and nutrition availability during growth after the 1st cutting.

The availability of minerals for medicinal and aromatic plants improves many metabolic processes and the biosynthesis of secondary metabolites (Janmohammadi et al., 2014). Also, the EOs are terpenoid compounds, and their structural constituents such as isopentenyl pyrophosphate and dimethylallyl pyrophosphate are in dire need of NADPH and ATP. Moreover, elements such as nitrogen are essential for the formation of the mentioned compounds. Therefore, the use of nitrogen-containing nutrition sources increases the accumulation of EOs. In this respect, it has been proven that different fertilizer applications increase the EO content in the present study. Similarly, the application of different fertilizers such as biological, chemical, and hydrogel in the planting bed increased the EO content of Lippia citriodora and *Zingiber officinale* (Kumar et al., 2018; Shahhoseini et al., 2018).

In the present study, the different water regimes and fertilizers altered the content of some EO components. α-Thujone was a dominant compound followed by camphor, 1.8-cineole, and β-thujone. Previous studies have reported similar results on *S. officinalis* (Bettaieb et al., 2009; Rioba et al., 2015; Govahi et al., 2015; Bahtiyarca Bagdat et al., 2017; Sonmez and Bayram, 2017). Some reports explained the interpret of the irrigation restriction's effects on the EO changes as difficult and have stated that there is no accurate information on the effects of irrigation stress on the EO components (Azizi et al., 2009; Bettaieb et al., 2009; Sonmez and Bayram, 2017). Data about fertilizer treatments showed that the maximum value of the α-thujone, camphor, 1.8-cineole, and β-thujone varied among the treatments. Ozguven et al. (2008) have suggested that various fertilizers, soil types, climatic conditions, irrigation models, and growing techniques may affect the composition of secondary metabolites. On the other hand, Bradley (2006) determined the main compounds of the *S. officinalis* as follows: α-thujone (from 10 to 60%), β-thujone (from 4 to 36%), camphor (from 5 to 20%), and 1,8-cineole (from 1 to 15%). Also, *cis*-thujone (from 18 to 43%), *trans*-thujone (from 3 to 8.5%), camphor (from 4.5 to 24.5%), 1,8-cineole (from 5.5 to 13%), a-humulene (from 0 to 12%), a-pinene (from 1 to 6.5%), camphene (from 1.5 to 7%), limonene (from 0.5 to 3%), linalool, and bornyl acetate (2.5% maximum) compounds have been suggested as major *S. officinalis* compounds (ISO 9909). The amounts of the major constituents in the present study were largely within the range of the proposed standards.

5. Conclusions

In this study, the effects of water deficiency were evident on the yield and yield components of *S. officinalis*. By increasing drought stress, the yield decreased significantly. Among the nutrient sources, NPK fertilizer was more effective on the agronomic productivity and chemical characteristics. The highest EO content was observed in moderate followed by severe drought stress. α-Thujone was identified as the most major compound, and its biosynthesis was more elevated in the 2nd cutting. The highest accumulation of α-thujone was determined in moderate drought stress. Oxygenated monoterpenes were found as the greatest chemical class of EO. Therefore, it can be suggested that the cultivation of *S. officinalis* with regular irrigation intervals by the use of NPK fertilizers will be good practice for achieving optimal production.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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