



Original article

Application of zeolite improves water and nitrogen use efficiency while increasing essential oil yield and quality of *Salvia officinalis* under water-deficit stress



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ARTICLE INFO

Article history:

Received 27 August 2021

Revised 16 September 2021

Accepted 19 October 2021

Available online 25 October 2021

Keyword:

Essential oil

Nitrogen use efficiency

S. officinalis

Zeolite

Water use efficiency

ABSTRACT

Soil moisture and nitrogen (N) are two of the most important factors affecting the production of medicinal plants. So, the management strategy of these factors is critical and to be identified. In order to study the application of zeolite (Z) (0 and 10 ton ha⁻¹) in *S. officinalis* culture medium under different irrigation regimes (30 % depletion of available soil water (ASW)) and 60 % depletion of ASW) and N (0, 75 and 150 kg N ha⁻¹) a split-factorial experiment was carried out with three replicates in 2018. The highest fresh and dry weight were achieved at irrigation after 30 % depletion of ASW while using 150 kg N ha⁻¹ and 10 ton Z ha⁻¹. Maximum water use efficiency (WUE) (22.10 g.L⁻¹) was obtained after 60 % depletion of ASW and 150 kg N ha⁻¹ and 10 ton Z ha⁻¹. Besides, the maximum nitrogen use efficiency (NUE) was obtained after 60 % depletion of ASW and 75 kg N ha⁻¹ and 10 ton Z ha⁻¹ (14.25 kg kg⁻¹N). Maximum essential oil (EO) content (1.06%) and *cis*-Thujone were obtained from plants subjected to 60 % depletion of ASW and, application of 75 kg N ha⁻¹ and 10 ton Z ha⁻¹. Applying Z with N, in different irrigation regimes did improve soil conditions for achieving higher, WUE and NUE, increased the EO content and yield while decreasing the negative effects from water-deficit stress and has provided a direction towards a stable system.

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1. Introduction

Salvia officinalis L. (Sage), is one of the most important and perennial medicinal plants in the world belongs to the Lamiaceae family (Ghorbani and Esmaeilzadeh 2017). Sage plants has many uses in different industries such as cosmetics, health, medicinal, painting, herbal teas, supplements, pesticides and fungicides, fragrances and flavoring liquids (Said-Al Ahl et al. 2015; Jakovljević et al. 2019; Kulak et al. 2020). The aerial parts of sage plants have a long history of application in cooking and traditional medicine. Because of its seasoning properties and flavoring, this medicinal plant has been extensively used to prepare of various foods. Sage plants have a history of application in folk medicine to treat some

diseases such as rheumatism, inflammation, diarrhea, and etc. (Garcia et al. 2016; Ghorbani and Esmaeilzadeh 2017).

The main compounds of sage essential oil (EO) consist of α -Pinene, α -Thujone, cineole, camphene, β -Thujone and camphor (Kulak et al. 2020). The synthesis and production of these compounds usually are changed under different conditions of fertility and moisture (Govahi et al. 2015; Kulak et al. 2019). Water deficit, among the various environmental constraints, is the main limiting issue in agricultural practices. Also, due to increasing population, decreasing water resources and less access to water needed by the agricultural sector in the future, methods that can reduce evaporation from soil and decrease the loses of used water and nutrients and would lead to increased water and nutrient use efficiency are very important (Cosgrove and Loucks 2015).

Various techniques have been evaluated to reduce the effects of drought and leaching of nutrients of which zeolite (Z) application in the soil/culture media is one of the most important. Z is an eco-friendly material that has been widely stated to improve soil properties, which due to its unique properties can be used effectively in agriculture (Hazrati et al. 2017; Sun et al. 2020).

Natural Z, an inorganic protector of soil, is widely used to enhance the growth and yielding of the crop (Mehrab et al. 2016;

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Peer review under responsibility of King Saud University.



Wu et al. 2019). Z has a due to its unique characteristics of high cation exchange capacity, uniform particle size distribution, and large internal porosity and preserves water and nutrients under drought in the root zone of the plant and reduces the effect of stress on the crop (Aghaalikhani et al. 2012; Hazrati et al. 2017). It has been shown that adding enriched Z with nutrients to the soil leads to increase in water content of the grown plants in that soil, and improved crop yield in various soil types and various crops, i.e. rice, *Aloe vera*, amaranth, sunflower and dragonhead (Sepaskhah and Barzegar 2010; Hazrati et al. 2017; Asl and Hatami 2019; Karami et al. 2020; Guaya et al. 2020).

Among nutrients, N is one of the basic yield-generating elements worldwide and the demand for it to improve agricultural farms for growth and yield is increasing (Mokhtassi-Bidgoli et al. 2013). N consumption is high in Iran and the rate of losses is high due to improper use. On the other hand, since water and soil resources are limited in Iran best use of these resources are a need to provide asustainable and balanced agriculture. The use of this mineral fertilizer in the last few decades led to increased production while a large part of it is lost by the leaching process from the soil. In addition to the negative effects on the environment, especially the threat to human health, and the imposition of economic problems due to the sharp increase in the cost of chemical fertilizers to farmers, N leaching has reduced the efficiency of this valuable input due to the declining efficiency (Good and Beatty 2011).

N fertilizer management is a challenging task and so far scientists have used several techniques to increase N use efficiency (NUE), effectiveness and to reduce leaching, but the use efficiency has not yet improved significantly (Sharma and Bali 2018). On the other hand, the availability and uptake of N in the soil can be significantly affected by several factors such as soil properties, soil N content and soil moisture (Asibi et al. 2019). Therefore, it is believed that soil condition has a significant impact on N uptake and its efficiency. In order to increase N uptake and reduce leaching, several technologies have been reported by researchers (Sharma and Bali 2018). Application of Z in the soil not only has no harm to the natural environment but also could alleviate non-point pollution by reducing nitrate leaching (Faccini et al. 2018). Results from other studies indicate that N uptake and use efficiency decrease in dry soils with high leaching (Nakhli et al. 2017; Jalil Sheshbahreh et al. 2019). Nevertheless the application of Z improves the physical properties of soil, increases the volume of available moisture and thus increasing N uptake and NUE (Sarkar and Naidu 2015). In a study on rice, the use of Z resulted in an increase in total N absorption (Zheng et al. 2018). Another study on rice showed that application of 8 ton Z ha⁻¹ and 80 kg N ha⁻¹ leads to improve in the NUE factor (Sepaskhah and Barzegar 2010). Another study on rice application of Z has increased the storage of ammonium in the soil surface and prevented nitrate leaching to the lower layers of the soil (Zheng et al. 2019). Application of 7.5 ton ha⁻¹ Z with 200 kg N ha⁻¹ on corn significantly improved uptake of N compared to control (Ravali et al. 2019).

Therefore, water and N management together play an important role in plant growth, development and ultimately yield in semiarid areas. Considering the effects of N and irrigation, improving water volume and N applied on medicinal plants are important for the management of sustainable agricultural (Mahajan et al. 2012), because of lack of sufficient water for irrigation, high irrigation costs and fertilizer expenses, and environmental concerns such as pollution due to extreme use of N applied (Dai et al. 2019).

Therefore, because improving water use efficiency (WUE) and NUE is one of the most important goals in sustainable agriculture, and so far no study has been done on the simultaneous use of N and Z under different irrigation regimes on sage, the purpose of

the current study is to evaluate the use of Z and N under water stress on the yield, quality and WUE, and NUE of sage.

2. Materials and methods

2.1. Site description and climatic characteristics

The experiment was carried out at research farm of the Azarbaijan Shahid Madani University, Tabriz, Iran (35° 84' N, 51° 81' E and 1215 m above sea level) in 2018. This region in Iran is mainly characterized as a semi-arid area (mean annual precipitation = 298 mm, and mean monthly maximum and minimum temperatures = 27.4 and -1.2 °C, respectively).

Before starting the experiment, soil samples were taken at 0–30 cm depth to determine their properties. The results of the soil test are shown in Table 1. Zeolite (Z) was sourced from a quarry in the city of Mianeh in northwest Iran and composed of about 90% clinoptilolite. Moreover, hydrophilic polymers named hydrogel 200 A, with density: 1.4 g cm³, size: 50–150 μm. Also, the results of physical and chemical analysis of Z are shown in Table 2

2.2. Land preparation and the applied treatments

Land preparation operations included plowing, two discs perpendicular to each other, creating streams and plotting. The study was designed in a split factorial based on randomized complete block design in three replications. The main factors were irrigation regime in two levels: (I₁) the normal irrigation (irrigation after 30% depletion of available soil water (ASW), (I₂) water deficit (after 60% depletion of ASW) in the depth of root development. The subplots derived from the factorial combination of two different factors, including N with three levels (46% from urea source) (0 (N₁), 75 (N₂) and 150 (N₃) kg N ha⁻¹), and Z at two levels (no application (Z₀) and 10 (Z₁₀) ton ha⁻¹). Considering the levels of each of the studied factors and the number of replications, the experiment had 12 treatments and consisted of 36 experimental units. Each plot had four planting rows with a row spacing of 0.6 m, a distance between each plant of 0.3 m and a length of three m. One day before planting, after creating the furrow and ridges, the Z was distributed on the soil surface and mixed at a depth of 15 cm. To apply N treatment for the respective plots according to the fertilizer needs for sage (75 and 150 kg N ha⁻¹), which was calculated and applied in three stages (10, 50 and 80 days) after planting.

2.3. Irrigation treatment

The irrigation schedule of experimental units was performed based on the method of changing the volume percentage of soil moisture in the depth of root development. Then when the plants were about 20 cm tall (i.e. 30 days after planting), irrigation regimes were applied and continued until plants reached 50% flowering stage. The total water volume in each treatment were I₁ × N₁ × Z₀ (805), I₁ × N₁ × Z₁ (653), I₁ × N₂ × Z₀ (719), I₁ × N₂ × Z₁ (841), I₁ × N₃ × Z₀ (959), I₁ × N₃ × Z₁ (816), I₂ × N₁ × Z₀ (362), I₂ × N₁ × Z₁ (456), I₂ × N₂ × Z₀ (470), I₂ × N₂ × Z₁ (423), I₂ × N₃ × Z₀ (518), I₂ × N₃ × Z₁ (461). The irrigation was done when 30 and 60% of available soil water at a depth of 0 to 30 cm were discharged by the plant or due to evaporation from the soil surface. Irrigation regimes were applied based on the maximum allowable depletion (MAD) percentage of ASW between field capacity to permanent wilting point. The predefined applied treatments were 30 and 60% MAD of ASW. To control soil moisture at a depth of root development, a time domain reflectometry (T.D.R) machine (made in Taiwan) at a depth of 0 to 30 cm of the plot was used. It is worth mentioning that before performing the experiment, by sampling

Table 1
Physicochemical properties of soil.

Soil texture	EC (dS. m ⁻¹)	Soil acidity	Organic matter %	Total N	Available P mg kg ⁻¹	Available K	Available Zn	A Available Cu	Available P Fe	Available P Mn
loam -sandy	5.55	7.97	1.15	0.11	28.5	820	0.52	0.54	1.2	3

Table 2
Physical and chemical properties of zeolite.

TiO ₂ %	MnO	Fe ₂ O ₃	Cl	SO ₃	P ₂ O ₅	SiO ₂	Al ₂ O ₃	K ₂ O	Na ₂ O	MgO	CaO
0.03	0.04	1.5	–	–	0.01	65	12.02	3	1.08	0.1	2.3
CEC = 200 meq/100 g											

from different soil depths at different times, the volumetric percentage of soil moisture was determined by weighing method and then the volumetric percentage of soil moisture in these areas was measured by T.D.R and then a regression equation was calculated between two sets of data which was used to calibrate the T.D.R device. T.D.R data were recorded daily during the plant growth period. At each re-irrigation stage, the plots were irrigated evenly (by counter reading) by leakage method up to soil saturation capacity.

2.4. Planting, growing and harvesting operations

Sage seedlings (provided by Zarrin Giah Urmia Company) were planted in the field on May 6th 2018. In order to fully establish the seedlings after planting, irrigation was performed equally for all treatments for one month according to the control treatment (irrigation after 30 % depletion of ASW).

The flowering process started almost 95 days after planting (i.e. mid-august). The plants were finally harvested at 50–60% flowering stage (i.e., about 120 days after planting or late September) in the experiment. To determine the effect of the studied treatments on the fresh and dry yield, content and yield of EO for each plot in two rows next to each other and 0.5 m from the beginning and end of 4 rows of planting in each experimental unit were left as a margin. Also, sage bushes were harvested in the flowering stage on September 11 and dried in the shade for two weeks.

2.5. Studied traits and methods of measuring them

In this study, traits such as fresh and dry shoot yield, content and yield of EO, main constituents of EO, WUE and NUE and as well as N concentration, uptake of total N (kg N ha⁻¹) and soil residual N after harvesting the plants were examined.

2.6. Determination of fresh and dry yield of sage

To measure the yield of dry and fresh matter, two m² were cut from the soil surface, they were placed in plastics and transferred to the laboratory. After weighing the fresh yield, the samples were dried in the shade and the open air under shade conditions and the dry matter yield was measured.

2.7. Calculation of Water-use efficiency

In order to determine the WUE, the amount of supplied water in each plot was first determined using a Counter. The amount of WUE was calculated through the amount of dry matter yield (biological yield) obtained from the amount of supplied water (Hazrati et al., 2017).

WUE was calculated according to the following formula:

$$\text{WUE} = \text{dry matter yield (kg)} / \text{total water consumed (liter)}$$

2.8. Nitrogen content in plant and soil

To measuring of the total N in soil and plant samples were randomly prepared from each plot. After drying, the samples were finally prepared by digestion with sulfuric acid, salicylic acid, and oxygenated water of their extracts and used to measure N and the amount of total N using titration after Distillation was measured using a Kjeldahl device (Novozamsky et al. 1974).

2.9. Nitrogen-use efficiency

NUE was estimated by the defined method by Chen et al. (2010):

$$\text{NUE (g fgn}^{-1}) = \text{LYL}_{\text{Df}} / \text{LN}_{\text{Yf}}$$

NUE: N use efficiency (kg/kg N)

LYL_{Df}: Difference in crop yield in the fertilized plot

LN_{Yf}: The amount of N used per hectare

2.10. Essential oil yield and content

To extract the EO, (50 g, three replicates for each treatment) of dried sample (aerial parts) was extracted by the hydrodistillation technique for 2.5 h using a Clevenger-type apparatus based on the procedure recommended in British Pharmacopoeia (British Pharmacopoeia 1993). The obtained EO drying by sodium sulfate was carefully weighed and at the end, the percentage of EO was measured. The EO was kept at 4 °C until further analysis. The EO yield was calculated based on the multiplication of the product's EO content and shoot's dry matter yield.

2.11. Separation and identification of essential oil composition

Identification of EOs composition using gas chromatography device (Agilent Technologies-7890 A) gas chromatography-mass spectrometry device (GC-MS) was done. So that the amount of 0.20 of EO was removed by 10 µl syringe and injected into the GC device. The percentage of constituents of each EO was calculated after separation with inhibition index. Also, 1 µl of each EO was diluted in 2 mL of dichloromethane and then injected into a chromatographic-mass spectrometer device and the mass spectra related to the EO compounds were obtained for qualitative evaluation (identification). Finally, the compounds in each EO were identified using the Retention Index and the recommendations of the gas chromatography-mass spectrometer database and compared with the standard compounds.

2.12. Gas chromatography (GC)

Chromatographic gas model Agilent Technologies-7890A system that fitted with a fused silica HP-5MS (30 m × 0.25 mm (ID) × 0.25 μm (FT)), was used. The column temperature program starts at 60° C and gradually increases at a rate of 3° C per minute to 250° C and stops for 8.5 min at the final temperature. The injection chamber temperature was 250° C. N gas was used as the carrier gas with a flow rate, 1 mL/min. The detector used in the GC device was FID type with a temperature of 280° C and a gain ratio of 1 to 100.

2.13. GC-MS chromatography

Chromatographic gas model Agilent Technologies-7890A was equipped with HP-5 column with (30 m × 0.32 mm (ID) × 0.25 μm (FT), was used. The temperature of the oven was planned as follows: from 60 (held isothermally for 2 min) to 210° C with ramp: 3° C/min, then increased to 240° C with ramp 20° C/min and the final temperature kept for 8.5 min. Run time: 60 min. Electron ionization energy was 70 eV in the electronic ionization (EI) mode, ion-source: 230° C, Detector: 290° C MS, Interface line temperature: 280° C, Injector: 280° C, Split ratio: 1:50, EO was diluted with hexane (1:100 ratio) and helium was used as carrier gas (flow rate, 1 mL/min), mass range: 50–480 *m/z*, and the injection volume of samples were 1.0 μL. The compounds were detected and identified by retention indices (RI), Wiley MS data system library (Wiley, Chichester, UK) and previous literature. Finally, the percentage of constituents was measured based on the peak areas obtained by the response factor of the detector (Adams 2007).

2.14. Data analysis

SAS statistical program was used to analyze all the data. Before analyzing the data, they were tested for normality and after ensuring the normal distribution, they were analyzed. A split factorial experiment was used to analyze harvest stage data. The LSMEANS command was used to compare means, at a $p < 0.05$ probability.

3. Results

All measured traits of sage such as fresh and dry weight, NUE, N content, total uptake of N (kg N ha⁻¹) and soil nitrogen (N residue), EO content, EO yield, and constituents of EOs were significantly affected by treatments (Zeolite (Z), nitrogen (N) and soil water content). Furthermore, ANOVA analysis showed that effects of both main and interaction effects were significant.

3.1. Fresh and dry weight

Three-way interaction effects of irrigation, N and Z decreased fresh and dry yield in response to water deficit condition; however, applying N fertilizer and Z to counteract this response in both irrigation regimes. The highest fresh and dry weights were 13226.2 and 3309.2 kg ha⁻¹ after 30 % depletion of ASW and 150 kg N and 10 ton Z ha⁻¹ compared with the irrigating after 60 % depletion of ASW N₁ and Z₀ were 72 and 73% higher, respectively (Table 3).

3.2. Water use efficiency

The result showed the interaction of irrigation, N and Z had a significant effect on WUE; the highest WUE was 22.01 g L⁻¹ at the irrigation after 60 % depletion of ASW and 150 kg N and 10 ton Z ha⁻¹ was obtained that which in comparison to irrigation

after 30% depletion of ASW and non-application of N and Z was 72% higher (Fig. 1).

3.3. Nitrogen use efficiency, nitrogen concentration, total N uptake and soil residual N

The result of our study on NUE indicated that N, Z and irrigation interaction has a significant effect on NUE. The highest NUE was 14.25 kg.kg⁻¹N in irrigation after 60% depletion of ASW, application of 75 kg N ha⁻¹ and 10 tons Z ha⁻¹. Moreover, the lowest NUE (4.75 kg.kg⁻¹N) was obtained in the water regime of irrigation after 30% depletion of ASW, application of 150 kg N ha⁻¹ without Z (Fig. 2). The application of Z could significantly increase the NUE at both irrigation levels compared to the treatments with no application of Z.

Results indicated that due to the interaction of irrigation, N and Z, the highest and lowest of plant N content were 2.85 and 2.37 %, respectively. The maximum N content was obtained in irrigation after 60 % depletion of ASW, application of 150 kg N ha⁻¹ and 10 tons Z ha⁻¹ while, the lowest N content was observed after 30% depletion of ASW without N and Z application (Table 4). According to the soil analysis, the soil N before planting was 0.11% (Table 1) which was used to determine the amount of required N for plant considering the application of Z and fertilizer requirements of sage in the desired condition (80 kg N ha⁻¹) (Omid Beigi, 2005).

The mean comparison showed that of the interaction of irrigation, N and Z, the highest and lowest percentages of soil residual N reached to 0.14 and 0.10%, respectively. The highest N residual in the soil was reported in irrigation after 30% depletion of ASW and application of 150 kg N ha⁻¹ and 10 ton Z ha⁻¹, and in irrigation after 30% depletion of ASW and without application Z and N, respectively (Table 4). Z application increased the N concentrations at 0–30 cm soil depth. Our results showed that interaction of irrigation, N and Z resulted in the highest N uptake which was 92.48 kg N ha⁻¹ in irrigation after 30% moisture depleting, and the application of 150 kg N ha⁻¹ and 10 ton Z ha⁻¹ (Table 4).

3.4. Essential oil content and yield

The result showed that the interaction of irrigation, N and Z were significant on EO yield and content. The highest EO content was 1.06% in the irrigation after 60% depletion of ASW and 75 kg N ha⁻¹ and 10 ton ha⁻¹ Z (Fig. 3). The highest and lowest EO yield, 21.34 and 5.68 kg ha⁻¹ were obtained after of 30% depletion of ASW and application of 150 kg N ha⁻¹ and 10 ton.ha⁻¹ of Z, and irrigation after 60% depletion of ASW without application N and Z, respectively (Fig. 4).

3.5. Constituents of essential oils

The results of EO analysis showed that there are 56 compounds in sage. The main constituents of EO were α-Pinene, camphene, β-Pinene, cineole, *cis*-Thujone, *trans*-Thujone, α-Campholenal, camphor, α-Humulene and Viridiflorol. The mean comparison indicated that the interaction effect of irrigation, N and Z have a significant effect on some compounds such as α-Pinene, camphene, β-Pinene, *cis*-Thujone, *trans*-Thujone, α-Campholenal and camphor were significant (Table 5).

The highest *cis*-Thujone compounds was obtained after 30% depletion of ASW and application of 75 kg N ha⁻¹ and 10 ton Z ha⁻¹(45.77%) compared to 60% depletion of ASW and 150 kg N ha⁻¹ without Z that was reported as 32% higher. The highest *trans*-Thujone compound was recorded after 30% depletion of ASW and application of 150 kg N ha⁻¹ without Z (20.19%) in which compared to depletion after 30% of ASW and 75 kg N ha⁻¹ without Z application and it was approximately 66% higher. The highest

Table 3
Influence of different irrigation regimes, nitrogen and zeolite on fresh and dry weight of sage.

Nitrogen (kg N ha ⁻¹)	Zeolite (ton ha ⁻¹)	Irrigation after 30% depletion of available soil water		Irrigation after 60% depletion of available soil water	
		Shoot fresh weight	Shoot dry weight	Shoot fresh weight	Shoot dry weight
0	0	7151.2 ^d	2074.4 ^d	3717.4 ^c	899.7 ^d
0	10	8813.3 ^c	2478.6 ^c	5423.9 ^b	1624.1 ^c
75	0	9932.8 ^{bc}	2566.2 ^{bc}	6135.9 ^b	1749.3 ^c
75	10	9742.2 ^c	2739.8 ^{bc}	8333.4 ^a	2330.2 ^b
150	0	1132.8 ^b	2787.3 ^b	8405.9 ^a	2779.2 ^a
150	10	13226.2 ^a	3309.2 ^a	9603.8 ^a	2811.6 ^a

Means with similar letters in each column indicate no significant difference at the 5% level.

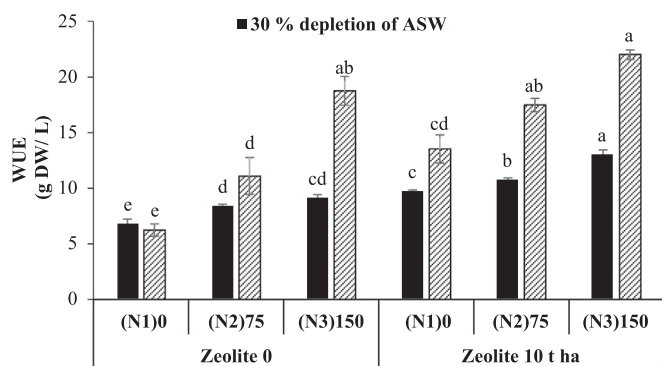


Fig. 1. WUE was affected by interactive effect of different levels of irrigation (irrigation after 30 (I₁) and 60 (I₂) depletion of soil available water), zeolite levels (0 and 10 ton ha⁻¹) and N fertilizer (0 (N₁), 75 (N₂), and 150 (N₃) kg N ha⁻¹) in Sage. Bars are the means ± standard error of the mean (n = 3). Columns with different letters are significantly different at the 5% level of probability according to the protected LSD test.

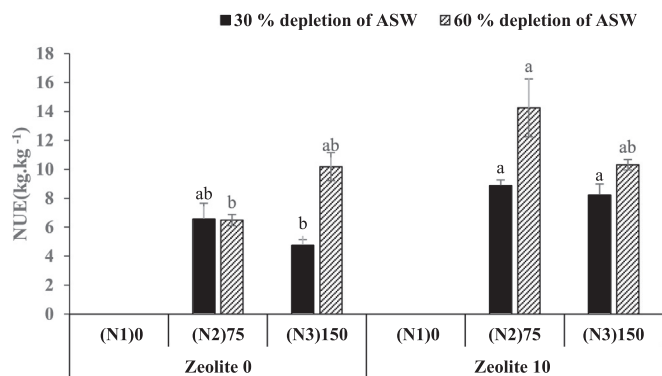


Fig. 2. NUE was affected by interactive effect of different levels of irrigation (irrigation after 30 (I₁) and 60 (I₂) depletion of soil available water), zeolite levels (0 and 10 ton ha⁻¹) and N fertilizer (0 (N₁), 75 (N₂), and 150 (N₃) kg N ha⁻¹) in Sage. Bars are the means ± standard error of the mean (n = 3). Columns with different letters are significantly different at the 5% level of probability according to the protected LSD test.

amount of α-Campholenal compound obtained after 60% depletion of ASW and without application of N and Z which was 18.90%. This compound in both irrigation regimes in two treatments of non-application of N and Z and application treatment 150 kg N ha⁻¹ and application of 10 ton.ha⁻¹ of Z was significant, but its amount was either not observed in the other treatments or its amount was not significant (Table 5). The highest amount of camphor was obtained after 30% depletion of ASW and 75 kg N ha⁻¹ and no application of Z (16.64%), which was higher than irrigation after

30% depletion of ASW and 75 kg N ha⁻¹ and 10 ton.ha⁻¹ Z (9.79%). The highest and lowest amount of α-Pinene was obtained after 30% depletion of ASW and application of 150 kg.ha⁻¹ and 10 ton.ha⁻¹ of Z (4.80%) while its amount in irrigation after 60% depletion of ASW, 75 kg N ha⁻¹ and no application of Z was 3.25%. The lowest camphene compound was obtained in irrigation after 30% depletion of ASW and application of 75 kg N ha⁻¹ and 10 ton Z ha⁻¹ that increased by 43% in irrigation after 30% depletion of ASW and 75 kg N ha⁻¹ and non-application of Z (Table 5).

4. Discussion

N nutrition is one of the most key factors in crop production (Jalil Sheshbahreh et al. 2019). The mixture of Z and N, which is comparable to slow-release fertilizers, plays an important role in yield improvement under different irrigation regimes (Sepaskhah and Barzegar 2010). The results showed that water stress significantly reduced yield while the application of Z was able to reduce the effects of water stress. Water stress is one of the most impacting factors which alter seriously the plant physiology, finally leading to the decline of the crop productivity (Bodner et al. 2015). Cell inflammation as one of the consequences of water stress, leads to disruption of physiological processes, prohibition of leaf growth, reduction of photosynthesis and finally plant's death (Petridis et al. 2012). Photosynthesis is a major factor for plant growth and yield, and the plants' ability to maintain it in environmental stresses conditions is vital to provide yield stability. Decreased crop growth is often related to restricted photosynthesis capacity in plants (Ashraf and Harris 2004; Allen et al. 2020). Water stress can destroy lipid bilayer structure of the plasma membrane and change the activity of enzymes attached to the membrane which then results in closing the pores, reducing the activity of enzymes effective in photosynthesis, and dampening the torsion of cells (Cruz de Carvalho 2008). Consequently, preventing cell growth reduces yield, and these influential factors even with N application in severe water stress condition cannot be improved (Hu and Schmidhalter 2005; Allen et al. 2020). However, we showed when no or limited water stress is present, N consumption increases the dry and fresh weight of the aerial parts. It can be concluded that the application of Z with retention of water and N and their gradual release during the growing season of sage might leads to increased yields (Polat et al. 2004; De Smedt et al. 2017). Sun et al. 2020 reported that the application of Z (0, 5 and 10 tons ha⁻¹) improves the dry weight of rice aerial parts. Simultaneous application of different irrigation regimes with the Z application resulted in an increase in the yield of *Aloe vera* in all levels of irrigation (Hazrati et al. 2017). In a study on sunflower, the application of nutrient-enriched Z in soil improved plant biomass (Guaya et al. 2020). In a study on rice, it has been found that the application of Z under water stress has increased yield and improved grain quality (Zheng et al. 2018). In another study on rice, urea fertilizer with

Table 4
Comparison of the average efficiency and nitrogen uptake of sage under the influence of different irrigation regimes, nitrogen and zeolite.

Nitrogen (kg ha ⁻¹)	Zeolite (ton ha ⁻¹)	Irrigation after 30% depletion of available soil water			Irrigation after 60% depletion of available soil water		
		Content of plant nitrogen (%)	Soil N (%)	Nitrogen uptake or accumulation (kg ha ⁻¹)	Content of plant nitrogen (%)	Soil residual N (%)	Nitrogen uptake or accumulation (kg ha ⁻¹)
0	0	2.37 ^d	0.10 ^d	49.06 ^d	2.52 ^e	0.12 ^{cd}	22.68 ^d
	10	2.48 ^{bc}	0.11 ^{cd}	61.56 ^c	2.70 ^{cd}	0.12 ^d	43.91 ^c
75	0	2.43 ^{cd}	0.12 ^{bc}	62.35 ^c	2.62 ^d	0.14 ^{ab}	45.86 ^c
	10	2.68 ^a	0.12 ^{bc}	71.14 ^b	2.73 ^{bc}	0.15 ^a	63.52 ^b
150	0	2.56 ^b	0.13 ^b	73.71 ^b	2.80 ^{ab}	0.13 ^{bc}	77.70 ^a
	10	2.80 ^a	0.14 ^a	92.48 ^a	2.85 ^a	0.14 ^{ab}	80.12 ^a

Means with similar letters in each column indicate no significant difference at the 5% level.

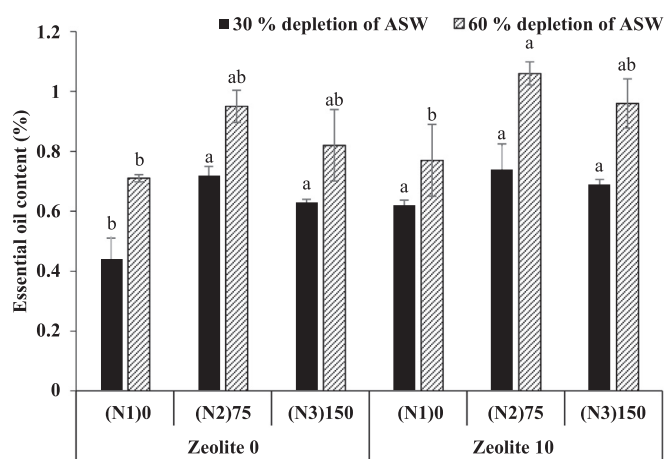


Fig. 3. Essential oil content was affected by interactive effect of different levels of irrigation (irrigation after 30 (I₁) and 60 (I₂) depletion of soil available water), zeolite levels (0 and 10 ton ha⁻¹) and N fertilizer (0 (N₁), 75 (N₂), and 150 (N₃) kg N ha⁻¹) in Sage. Bars are the means ± standard error of the mean (n = 3). Columns with different letters are significantly different at the 5% level of probability according to the protected LSD test.

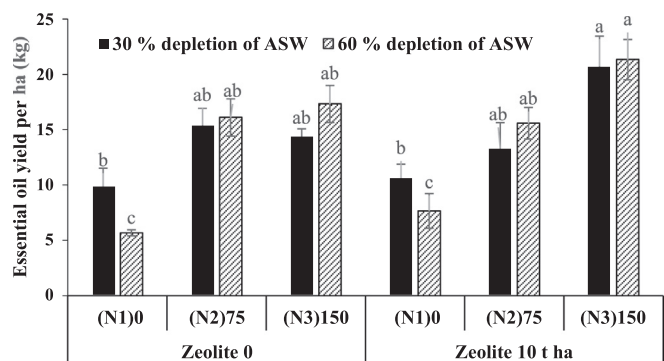


Fig. 4. Essential oil yield was affected by interactive effect of different levels of irrigation (irrigation after 30 (I₁) and 60 (I₂) depletion of soil available water), zeolite levels (0 and 10 ton ha⁻¹) and N fertilizer (0 (N₁), 75 (N₂), and 150 (N₃) kg N ha⁻¹) in Sage. Bars are the means ± standard error of the mean (n = 3). Columns with different letters are significantly different at the 5% level of probability according to the protected LSD test.

three stage installment of conventional plus application of Z (10 tons ha⁻¹) and urea treatment as starter fertilizer plus Z were significantly improved weight gain of 1000 grains by 8.5 and 10.7%, respectively compared with N fertilizer and non-application of Z (Wu et al. 2019). In general, it can be concluded that the selective absorption and controlled release of nutrients from Z can improve

plant growth by increasing the long-term accessibility of nutrients and water (Ahmed et al. 2010). On the other hand, the use of Z, in addition to improving water balance in the soil, plays a role in optimizing N consumption and reducing the use of N in agriculture production. The reduction of N leaching is also a beneficial management strategy for conserving the ecosystem (Nur Aainaa et al. 2018; Kennedy, 2020; De Smedt et al. 2017) which can be achieved by the proper application of Z to the soil.

The decrease in WUE under water stress is due to more significant reduction in photosynthesis compared to respiration result from damaged leaf mesophyll due to stress (Allen et al. 2020). The proper ability of Z to absorb water make this compound to be known as a water regulator, which can be used to improve the water balance in the soil under water stress condition. It has been shown that the application of Z to ensure the stability of water reservoir in the zone of root during drought and facilitates the horizontal diffusion of water into the soil (Polat et al. 2004; De Smedt et al. 2017; Kennedy, 2020). Qiang et al (2019) indicated that the application of N in corn crop had increased WUE. Other study exhibited, the interaction of water stress and N on *Echinacea*, the highest WUE in N application and after depleting 50% of soil moisture content was obtained (Jalil Sheshbahreh et al. 2019). In another study on wheat, moderate water stress with the application of 120 kg N ha⁻¹ increased WUE (Rathore et al. 2017).

Karami et al. (2020) showed that the use of Z (even with reduction of available water) has increased the WUE on the *Amaranthus hypochondriacus*. The study on *Aloe vera* showed that different irrigation regimes and Z had a significant effect on WUE and interaction of water stress and application of Z has led to increased WUE in this plant (Hazrati et al. 2017). A study on rice plants has shown that the application of Z under low water stress increased WUE (Zheng et al. 2018). Under water stress, the application of Z could increase the WUE with the application of 150 kg N ha⁻¹. It can be concluded that since Z is among the group of natural porous minerals with their crystalline structure (in such a way that they act as molecular sieves) has can to open channels in their network, and allow some ions to pass through and block the passage of some other ions. Z is able to absorb water up to 70% of its volume, which is due to its high porosity that originates from its crystal structure (De Smedt et al. 2017; Kennedy, 2020). We assume that Z acted as a water regulator since one of the main features of Z is its ability to absorb and return water. This ability of Z can improve the water balance in the soil under water stress conditions, especially during insensitive growth stages. The application of Z leads to the water reservoir which remained stable in the root zone during the dry season and contributed to the horizontal diffusion of water into the soil (Jakkula and Wani 2018). Therefore, by preventing leaching of elements, Z was able to increase the amount of N uptake by the plant and lead to improved WUE and on the other hand, Z in the soil maintain moisture and improvement in soil moisture can

Table 5

Major constituents of sage essential oil under the influence of different irrigation regimes, nitrogen and zeolite.

		Irrigation after 30% depletion of available soil water						
		α -Pinene	Camphene	β -Pinene	<i>cis</i> -Thujone	<i>trans</i> -Thujone	α -Campholenal	Camphor
Nitrogen (kg ha ⁻¹)	Zeolite (ton ha ⁻¹)							
0	0	3.51 ^{ab}	3.28 ^{abc}	2.10 ^{ab}	42.23 ^{ab}	10.00 ^b	6.77 ^a	11.82 ^{bc}
	10	3.44 ^b	3.49 ^{ab}	2.49 ^a	39.11 ^{ab}	8.47 ^{bc}	0.04 ^b	14.13 ^{ab}
75	0	4.26 ^{ab}	4.43 ^a	2.60 ^a	36.41 ^{bc}	6.74 ^c	0.03 ^b	16.64 ^a
	10	3.28 ^b	2.04 ^c	1.78 ^b	45.77 ^a	9.38 ^{bc}	0.04 ^b	9.79 ^c
150	0	3.26 ^b	3.09 ^{bc}	2.28 ^{ab}	31.28 ^c	20.19 ^a	0.04 ^b	12.25 ^{bc}
	10	4.80 ^a	3.25 ^{abc}	1.85 ^b	42.09 ^{ab}	10.76 ^b	6.17 ^a	11.35 ^c
		Irrigation after 60% depletion of available soil water						
		α -Pinene	Camphene	β -Pinene	<i>cis</i> -Thujone	<i>trans</i> -Thujone	α -Campholenal	Camphor
Nitrogen (kg ha ⁻¹)	Zeolite (ton ha ⁻¹)							
0	0	4.09 ^a	4.35 ^a	2.55 ^{ab}	33.30 ^b	9.84 ^c	18.90 ^a	14.52 ^a
	10	3.27 ^a	3.21 ^{bc}	2.25 ^{bc}	39.55 ^{ab}	10.80 ^{bc}	0.00 ^c	13.77 ^a
75	0	3.24 ^a	2.53 ^c	2.17 ^c	39.96 ^a	13.81 ^{ab}	0.00 ^c	11.69 ^a
	10	4.31 ^a	3.65 ^{ab}	2.60 ^a	35.43 ^{ab}	14.84 ^a	0.00 ^c	11.78 ^a
150	0	4.30 ^a	3.93 ^{ab}	2.61 ^a	36.21 ^{ab}	9.79 ^c	0.04 ^c	14.74 ^a
	10	3.22 ^a	2.44 ^c	2.14 ^c	41.23 ^a	12.59 ^{abc}	13.06 ^b	11.43 ^a

Means with similar letters in each column indicate no significant difference at the 5% level.

reduce the negative effects of stress on plant and also can improve the absorption of water and elements by roots and ultimately enhanced in increase the WUE.

The NUE factor in Iran is often very low, which has led to some concerns that excessive N in the field may adversely influence the quality of the environment through leaching and volatilization (Malekian et al. 2011). Increasing NUE in crops shows how these plants effectively convert available N into economic yield. To investigate the effects of agricultural practices on these indicators, assessment of components including absorption efficiency or recycling (N uptake to N ratio) and physiological efficiency or internally (relative economic yield to absorbed N) can be useful. NUE in plants may change with any factor affecting production (Salvagiotti et al. 2009). NUE depends on water and N availability in soil (Moll et al. 1982; Lian et al. 2020). The efficiency of N productivity is indicated as the ratio of dry matter yield to the amount of N absorbed. In other words, the amount of N has been absorbed, used, and converted into yield and product. Z used with N can increase the efficiency of this source by improving the N use through the control of retention of NH₄, contributing to increased N uptake and crop yields.

Previous studies stated that if the soil is rich in organic N and microbial biomass, high yield will be achieved without the use of N, or with a small amount of N fertilizer and the high NUE will be expected. In this case, more application of N fertilizer will reduce the amount of NUE. Selective uptake and controlled release of nutrients from Z results in increased long-term availability of water and nutrients if Z source is selected properly and added to the soil as a modifier and improve plant growth (Polat et al. 2004; De Smedt et al. 2017; Jakkula and Wani 2018; Kennedy 2020).

In another study, application of Z under water stress conditions significantly increased plant drought resistance, N uptake and NUE (Wu et al. 2019). The main reasons for the increased N uptake in response to may be due to (1) N absorption by Z which decrease N losses when N is available, and (2) slow release of N in contrast to N management without Z (Aghaalikhani et al. 2012; Malekian et al. 2011). A larger average content of available N released by Z that corresponded to the N requirement of the sage plant in surface soil resulted in a higher N uptake.

Increasing evidence shows that Z mixed with mineral fertilizer applied to soil enhances N uptake in plants (Kavoosi 2007; Karami et al. 2020). Furthermore, the Z amendment could also significantly

increase the N availability for crops similar to the effects of the slow-release fertilizer (Aghaalikhani et al. 2012). In another study, the application of Z in acidic soils could improve the physical properties of the soil and increase the NUE of the corn plant (Nur Aainaa et al. 2018). Another study on corn showed that N application increased N uptake and leaf N concentration at the harvest time but decreased NUE (Qiang et al. 2019). A study has shown that the application of different levels of N has increased the N concentration in the aerial parts of *Echinacea* at all levels of irrigation (Jalil Sheshbahreh et al. 2019). The highest NUE has been obtained when 40 kg of N per hectare was applied, a study on sage showed that the highest levels of N in leaf was observed in stress-free treatment. Also, vermicompost + bacterial treatment had the highest soil N compared to the control (Govahi et al. 2015). In the study on maize, NUE decreases with increasing N levels (Qiang et al. 2019). A study on wheat indicated that the effects of N and irrigation had a significant effect on NUE. NUE increased with increasing irrigation (Rathore et al. 2017). Studies on corn have shown that using N and water has a positive effect on the WUE in the plants, but this positive effect disappears when their amount is not optimal (Li et al., 2019). Due to the interaction of water stress and N on *Echinacea*, the highest NUE in N application and irrigation was obtained after depleting 50% of soil moisture (Jalil Sheshbahreh et al. 2019). Another study in the rice plant showed that a combination of urea, Z and fresh straw with nutrients increased the cation exchange capacity while there was no significant difference between them. On the other hand, it has a significant influence on number of tillers, the height of plant, and dry matter of the plant. The use of urea and Z fertilizer together has a greater impact than urea fertilizer without Z (Wulandari et al. 2019). In a study on rice, the application of Z in the soil led to N retention and increased N efficiency (Sepaskhah and Barzegar 2010).

In fact, in this study, we presume that the use of natural minerals such as Z has improved the chemical and physical traits of the soil which in turn led to increased water and N storage capacity in the soil for a long time and improved N uptake, content and efficiency. As a result, required chemical fertilizers were decreased and we were able to prevent potential environmental pollution (Ippolito et al. 2011; De Smedt et al. 2017 Jakkula and Wani 2018; Kennedy 2020). In the present experiment, it was observed that the application of Z reduced the leaching rate of soil N, which increased the rate of plant uptake and thus increased the efficiency

of N. Z can effectively control the nutrient status in the root zone of plants, so increasing N utilization (Wu et al. 2019).

Biosynthesis of EO in herbs is influenced by several factors such as genetics, physiological variations, conditions of climatic and edaphic, geographic conditions, environmental stresses, and agronomical practices (Keshavarz Afshar et al. 2014; Asl and Hatami 2019). Studies have shown that the EOs in many plants are increased due to water stress, which is a consequence of starch and protein breakdown that stimulate the production of EOs (Kulak, 2020). In this study, EO yield was decreased with an increase in water depletion levels from 30% to 60% of ASW, but the content of EO was increased with increase in drought intensity. It has been known that the yield of EO is often decreased under drought, but the content of EO was improved (Farahani et al. 2009). The EO content and dry matter yield are two prominent subjects in calculation of EO yield and due to the fact that N fertilizer produced higher dry yield at higher levels, we expected to reach this amount of EO content. Based on the results, it can be stated that although the yield of dry matter in the plant decreases with decreasing water availability and consequently drought stress, the use of Z and N, especially at high levels can alleviate this response to water stress conditions. Water stress adverse effects on the production yield of this plant can be reduced to some extent by the positive effect of Z and N in improving plant nutrition situations under water stress. Z plays a key role in increasing N retention and improving the efficiency of this nutrient in photosynthesis and green surface production, which will lead to increased growth and yield (Nakhli et al. 2017; De Smedt et al. 2017; Jakkula and Wani 2018; Kennedy, 2020). Meanwhile, the application of Z has caused the retention of water and N and its gradual release to the plant during the growing season, leads to increasing the dry matter and consequently the EO of the plant (Allen et al. 2020).

The study on peppermint showed that although N is not present in the structure of peppermint EO, it considers as an essential element through the use of photosynthetic materials to increase leaf area and production of more nutrients that can increase the number of compounds present in the essential oil (Marotti et al. 2004). In a study on the mint plant, the yield of EO increased by application of 225 mg.L⁻¹N (Chrysargyris et al. 2017).

It has been shown that the major constituents of EOs are terpenoids based on isoprenoid subunits (C₅ H₈) whose biosynthesis is primarily based on acetyl CoA, nicotinamide adenine dinucleotide phosphate (NADPH) and adenosine triphosphate (ATP). These factors and their amounts are depend on the concentration of mineral nutrients in the plant. Another important point is that secondary metabolites are derived from primary metabolites, so they cannot have involved in the high EO content of the plant unless the plant is growing properly (role of primary metabolites). (Verpoorte and Alfermann 2013).

A study on sage showed that the highest amount of α -Thujone, cineole and camphor was obtained in moderate water stress and decreased with increasing water stress intensity. When plants encounter moderate stress, the EO yield was increased (Bettaieb et al. 2009). There are many reports on the EO composition in different species of sage. In the study on sage, with increasing N levels the content of β -Pinene increased. The interaction between N and phosphorus was significant for both α -Thujones and β -Thujones contents. These compounds were increased due to weekly N and phosphorus application (30 and 60 kg.ha⁻¹, respectively). Camphor is the major component of EO in all treatments and the percentage of this compound in the EO is higher than the threshold suggested by the ISO standard (Rioba et al. 2015). In the study on sage, the highest α -Thujone, cineole and camphor were obtained in the first, second and third harvests, respectively with application of vermicompost + bacteria under moderate water stress (Govahi

et al. 2015). Research has shown that Z increases the amount and quality of EO in *Ginkgo biloba* (Machado and Caldas 2003).

5. Conclusion

The goal of current study was to determine the effect of N and Z application on NUE, WUE, biomass yield and quality of sage under water stress. One of the most important findings was to reduce the amount of N leaching, increase its absorption and also increase the efficiency of N application under different irrigation regimes with application of Z. Also, the application of Z could help the uptake of water and N nutrient by the plant roots by increasing the retention of soil moisture. This led to increased fresh and dry yield and ultimately improved WUE in various treatments. The application of Z with different levels of irrigation and N for fresh and dry yield, improving WUE and NUE, content and composition of EOs depending on the purpose is recommended according to the above results. Generally, the application of Z in combination with N and different irrigation regimes reduced water consumption and N losses and, on the other hand increased plant growth and yield which has many benefits such as reducing economic costs and alleviating environmental risks. These outcomes encourage investigations to determine the efficacy of Z on N usage across multiple plant species, climates, and types of soil under different abiotic stresses. Accordingly, it can be suggested that the application of Z will have great eco-friendly benefits in agricultural crop 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.

Acknowledgement

The authors would like to thank Azarbaijan Shahid Madani University, Tabriz, for conducting and financial support of this research.

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