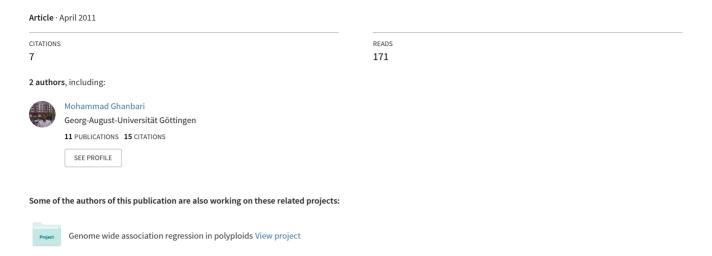
# The Effects of Water Deficit and Zeolite Application on Growth Traits and Oil Yield of Medicinal Peppermint (Mentha piperita L)



## The Effects of Water Deficit and Zeolite Application on Growth Traits and Oil Yield of Medicinal Peppermint (Mentha piperita L)

### Mohammad GHANBARI<sup>1\*</sup>, Siavash ARIAFAR<sup>2</sup>

<sup>1</sup>Department of Plant Breeding, Faculty of Agriculture Research, University of Zabol, Iran. P.O. Box: 98169-

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**Abstract:** In order to study the effect of different rates of zeolite and water deficit in Peppermint (Mentha piperita L), an experiment was conducted in factorial based on a randomized complete block design with three replications in institute of agriculture research in university of Zabol, Iran. Factors were considered as three levels of drought stress (70% fc, 50% fc, 30% fc) and four levels of zeolite (0, 1.5, 2, 2.5 g/1kgsoil). Analyze of variance showed that all of growth parameters and essential oil vield were affected by drought stress and zeolite application. Drought stress motivated a significant reduction in all growth parameter except oil percentage. At mainly zeolite application increased the mean of all traits. Results for interaction effects showed that zeolite application in drought stress increased the means of all traits. Analysis of variance for essential yield revealed that the highest oil yield belonged to drought stress 50% fc and 2.5gr zeolite. It seems that zeolite application in dry lands are exposed to drought stress be helpful for growth parameters and oil yield improvement and prevention of decreased oil yield.

**Keywords**: Essential oil yield; Peppermint; Water deficit; Zeolite.

#### Introduction

Drought is one of the most important environment factor that influence on production of crop plants in arid and semi-arid regions (Blum, 1988). Drought stress causes different physiological effects on plant growth (Blum, 1988) so, Irrigation treatments should be used effectively to increases the sustainability of production in agriculture systems (Geertans and Reas, 2009). There are many ways for drought tolerance that is used by plants such as rapid growth in different stage of plant, adaptation with tropical regions, relative resistance against dryness, high content of protein in leaves and high leaf area index (LAI), also high potential for enough production and high water use efficiency are suitable mechanism for drought tolerance (Geertans and Reas, 2009). Medicinal and aromatic plants have received much attention in several fields as agro aliment, perfume, pharmaceutical industries and natural cosmetic products (Olfa et al., 2099). Secondary metabolites in the medicinal and aromatic plants are conventionally affected

by both gene types, and environmental factors (Yazdani et al., 2002; Gholizade et al., 2010). It means growth parameter, essential oil yield and constituents of oil is impressed by biotic and environmental factors (Clark abiotic Menary, 2008 Aziz et al., 2008). Drought stress has mainly effected on yield and yield component in medicinal plants (Heidari et al., 2008). Peppermint (Mentha pipertial) is an herbaceous and perennial plant belongs to family lamiacea and is considered as a medicinal and aromatic plant and extensively using for the medical and food product industries (Yazdani et al., 2002 Grieve, 1991). Components of Peppermint esinclude sential oils menthol, menthone, methylacetal, menthofuran and palegone (Tabatabaeie and Zari, 2007 Mahmoud and Croteaa, 2003). These components are affected by stress environment such as drought and salinity. Razmjoo et al., (2008) found that drought stress in chamomile reduced some growth parameters and essential oil yield. However in other studies, it was shown that some growth parameters in basil were increased in drought

<sup>&</sup>lt;sup>2</sup>Student of Agroecology, Faculty of Agriculture, University of Zabol, Iran

<sup>\*</sup>Corresponding Author: Phone: +98-542-2232961-2, Fax: +98 - 542-2226765.

stress (Grieve, 1999). Researchers and farmers use some methods to reduce injurious effects of water deficiency; zeolite application is one of the possible approaches to reduce effect of water deficit on plant production. Zeolite consists, alkali and alkaline materials and crystalline alumino silicate ' that have internal surface area for keeping water in drought durations (Silberbush et al., 1993). The aim of this study was the evaluation different traits of Peppermint to different level of water deficit under zeolite application in green house condition.

#### Material and methods

This experiment carried out as a factorial experiment based in a randomized complete block design within three repetitions in 60 pots. Two factors were investigated: 1-zeolite application in four levels (zero, 1.5, 2, 2.5 g/1kg pot soil) and 2- water deficit in three irrigation treatments (70% fc, 50% fc and 30% fc). Peppermint plants for this experiment were originated from land race that belongs to Sistan region in east south of Iran, than these plants were initiated from rhizome cutting (10 cm long) and according to the method described by Farahani et al., (2009) seventy five samples were transferred into pots (30cm diameter and 50cm height) for zeolite application referred to Tso (1999) and Urotadze et al., (2002). In order to determine soil moisture used TDR (devise measures volumetric moisture) and according to soil moisture curve water given to target pots in three levels of water deficit factor (70% fc, 50% fc and v30%fc) and water amount was measured by counter. Other agricultural operations were the same as the other medicinal plants, at the end of the growing season, fresh leaf weight, dry leaf weight, stem wet weight, stem dry weight, root wet weight, root dry weight, leaf number, leaf area, shoot dry weight, essential oil percentage, essential oil yield were measured, and water use efficiency (WUE) was measured too. Essential oil percentage was extracted from shoot by hydro distillation methods in modified Clevenger apparatus (Ozturk et al., 2004). And essential oil yield was calculated by essential oil content and shoot dry yield.(Khorasanenjad et al., 2011)

The data were transformed in m<sup>2</sup> (square meter) and were analyzed by variances analysis (ANOVA), using the SAS GLM procedure (SAS institute 2002). Means were compared by Duncan test at 5% probability level.

#### Result

Fresh and dry leaf weight

The variance analysis in table 1 showed that the effect of water deficit was significant on both fresh and dry leaf weight, by increasing the discharge of soil humidity from 70% fc to 30% fc fresh and dry leaf weight had significant decrease. The effect of zeolite application was significant for fresh and dry leaf weight, and highest mean on both fresh and dry leaf weight was observed in 2.5g zeolite. The mean fresh and dry leaf weight for zeolite application in different water deficit levels has been shown in table 3. Results showed that drought intensity was decreased by increasing the zeolite application. In 30% fc with increasing in zeolite the mean of dry leaf weight reached from 18.54gr to 32.76g and fresh leaf weight reached from 41.7 to 67.14. The same result was obtained in 50% fc and 60% fc. It seems that the zeolite application significantly reduced the effects of drought on leaf growth.

**Table 1:** Variance analysis of main and interaction effects on different traits of Peppermint.

		Means of Squares											
Source of variation	df	Leaf dry weight	Leaf wet weight	Stem dry weight	Stem wet weight	Root dry weight	Root wet weight	Leaf number	Leaf area	Shoot dry weight	Wue	Oil per- centage	Oil yield
Rep	4	6199.72	7504.54	7916.58	13018.13	988.08	4326.29	978885	1403954	25839.02	53.81	0.41	23.99
Zeolite	2	**7805.68	**30340.3	**38960.5	**82839.1	**5702	**13217	**4857730	**9714132	**108210.7 5	**378.3 9	**33.97	**188.56
Water deficit	3	**6530.91	**110435	**161344	**353709	**71592.3	**137401	**37100876	**63693454	**455144.5 2	**1836. 77	**94.4	**644.09
Zeo- lite×Water deficit	6	**3809.59	*5501.24	*6725.24	*15722.9	<sup>n.s</sup> 662.19	<sup>n.s</sup> 1593.9	**2326859.5	**1228268. 25	**19445.8	*49.63	**17.51	**72.70
Error	44	1473.02	1903.71	2618.8	3564.77	706.27	952.58	645313	441246.2	5838.8	14.8	0.41	13.12

<sup>\*, \*\*:</sup> significant at 5 % and 1% level of probability respectively.

**Table 2**: Mean comparison of water deficit and zeolite on different traits of Peppermint.

	Leaf dry weight (g/m²)	Leaf wet weight (g/m²)	Stem dry weight (g/m²)	Stem wet weight (g/m²)	Root dry weight (g/m²)	Root wet weight (g/m²)	Leaf number	Leaf area	Shoot dry weight (g/m²)	Wue	Oil per- centage (%)	Oil yield (mlg/m²)
Water deficit (%fc)												
70	149.2 <sup>a</sup>	203.6°	240.1 <sup>a</sup>	494.4 <sup>a</sup>	145.8 <sup>a</sup>	215.4 <sup>a</sup>	4284a	5472a	5259 <sup>a</sup>	2.7 <sup>a</sup>	0.43°	11.28 <sup>b</sup>
50	90.49 <sup>b</sup>	135.4 <sup>b</sup>	190.3 <sup>b</sup>	315.4 <sup>b</sup>	117.5 <sup>b</sup>	185.7 <sup>b</sup>	2979b	3717b	4292.1 <sup>b</sup>	1.9 <sup>b</sup>	$0.59^{b}$	17.5 <sup>a</sup>
30	25.38°	55.17 <sup>c</sup>	65.7°	134.9°	$31.0^{c}$	59.3°	1431.c	1908c	5259.6°	$0.8^{\rm c}$	$0.62^{a}$	$6.22^{c}$
Zeolite (g/kg soil)												
0	52.86°	86.04°	108.5 <sup>d</sup>	187.4°	75°	119.04 <sup>c</sup>	3222ª	2696.4°	2617 <sup>c</sup>	1.34 <sup>c</sup>	0.69 <sup>a</sup>	10.11 <sup>c</sup>
1	75.84 <sup>bc</sup>	112.3 <sup>bc</sup>	142.7°	267.6 <sup>b</sup>	94.3 <sup>b</sup>	142.2 <sup>b</sup>	2547 <sup>b</sup>	3445.2 <sup>b</sup>	3129°	1.66 <sup>b</sup>	$0.61^{a}$	9.11 <sup>b</sup>
2	89.88 <sup>b</sup>	135.6 <sup>b</sup>	184.08b	$306.7^{\rm b}$	94.3 <sup>b</sup>	164.4 <sup>b</sup>	3564 <sup>a</sup>	4164 <sup>a</sup>	3871 <sup>b</sup>	$2.2^{a}$	$0.54^{b}$	10.59 <sup>b</sup>
2.5	134.6 <sup>a</sup>	191.6 <sup>a</sup>	226.2a	364.5 <sup>a</sup>	122.3 <sup>a</sup>	188.2 <sup>a</sup>	3159 <sup>a</sup>	4509 <sup>a</sup>	4693 <sup>a</sup>	2.54 <sup>a</sup>	0.51 <sup>b</sup>	16.9 <sup>a</sup>

Means followed by the same letter(s) within a column are not statistically different at the p=5% level

**Table 3**: Mean comparison of interaction effect on different traits of Peppermint.

Water deficit (%fc)	Zeolite (g/kg soil)	Leaf dry weight (g/m2)	Leaf wet Weight (g/m2)	Stem dry Weight (g/m²)	weight (g/m²)	Root dry weight (g/m²)	Root wet weight (g/m²)	Leaf number	Leaf area	Shoot dry weight (g/m²)	Wue	Oil percentage (%)	Oil yield (mlg/m²)
70	0	86.58 <sup>c</sup>	129.6°	153.9 <sup>d</sup>	235.8 <sup>cd</sup>	76.5a	130.8 <sup>a</sup>	2432 <sup>bc</sup>	3847 <sup>cd</sup>	393.8 <sup>de</sup>	1.67 <sup>de</sup>	$0.216^{\rm h}$	5.11 <sup>e</sup>
70	1.5	128.16 <sup>bc</sup>	170.4 <sup>b</sup>	199.2 <sup>bc</sup>	359.6 <sup>bc</sup>	119.5 <sup>a</sup>	173.5 <sup>a</sup>	2716 <sup>b</sup>	5409 <sup>ab</sup>	446.4 <sup>bc</sup>	$2.35^{bc}$	$0.258^{gh}$	8.30 <sup>de</sup>
70	2	149.58 <sup>b</sup>	208.44 <sup>ab</sup>	256.5 <sup>b</sup>	417.7 <sup>b</sup>	$130.6^{a}$	$209.7^{a}$	3713 <sup>a</sup>	6282 <sup>a</sup>	576.76 <sup>b</sup>	$2.74^{b}$	$0.324^{g}$	12.7 <sup>cd</sup>
70	2.5	231.84 <sup>a</sup>	306 <sup>a</sup>	$350.8^{a}$	546.3 <sup>a</sup>	143.4 <sup>a</sup>	228.7 <sup>a</sup>	3164 <sup>a</sup>	6379 <sup>a</sup>	690.2 <sup>a</sup>	$3.81^{a}$	$0.324^{g}$	19.03 <sup>b</sup>
50	0	53.46 <sup>de</sup>	86.7 <sup>def</sup>	122.9 <sup>efg</sup>	194.2 <sup>ef</sup>	130.3 <sup>a</sup>	178.7 <sup>a</sup>	1381 <sup>d</sup>	2696 <sup>e</sup>	322.72 <sup>e</sup>	1.59 <sup>e</sup>	$0.972^{b}$	17.13 <sup>bc</sup>
50	1.5	82.08 <sup>c</sup>	111.24 <sup>de</sup>	168.4 <sup>cd</sup>	309.6°	135.7 <sup>a</sup>	199.2 <sup>a</sup>	1341°	3312 <sup>de</sup>	$386.28^{cd}$	1.92 <sup>cd</sup>	$0.532^{\rm f}$	13.05 <sup>dc</sup>
50	2	87.12 <sup>c</sup>	141.84 <sup>cd</sup>	$228.4^{bc}$	367.5 <sup>bc</sup>	142.3 <sup>a</sup>	221.04 <sup>a</sup>	1822 <sup>b</sup>	4518 <sup>c</sup>	$457.92^{bc}$	$2.39^{bc}$	0.484 <sup>ef</sup>	14.98 <sup>bc</sup>
50	2.5	139.32 <sup>b</sup>	$201.7^{b}$	241.3 <sup>b</sup>	$390.2^{bc}$	174.9 <sup>a</sup>	262.8 <sup>a</sup>	1186 <sup>bc</sup>	4361 <sup>cd</sup>	550.6 <sup>b</sup>	$2.92^{b}$	$0.66^{d}$	25.04 <sup>a</sup>
30	0	18.54 <sup>e</sup>	41.7 <sup>f</sup>	$48.7^{\rm h}$	114.3 <sup>g</sup>	18.18 <sup>a</sup>	47.5°	1595 <sup>bc</sup>	1546 <sup>f</sup>	69.5 <sup>e</sup>	$0.406^{e}$	1.20 <sup>a</sup>	$8.09^{de}$
30	1.5	17.58 <sup>e</sup>	55.26 <sup>ef</sup>	60.4 <sup>g</sup>	133.74 <sup>fg</sup>	27.72 <sup>a</sup>	54 <sup>a</sup>	1164 <sup>b</sup>	1615 <sup>f</sup>	105.48 <sup>e</sup>	$0.537^{e}$	$0.752^{c}$	5.99 <sup>e</sup>
30	2	32.94 <sup>de</sup>	56.5 <sup>ef</sup>	67.3 <sup>g</sup>	134.8 <sup>fg</sup>	29.7 <sup>a</sup>	62.6 <sup>a</sup>	1137°	2729 <sup>e</sup>	129.96 <sup>e</sup>	$0.678^{e}$	$0.416^{\rm f}$	4.1 <sup>e</sup>
30	2.5	$32.76^{de}$	67.14 <sup>ef</sup>	$86.4^{fg}$	156.9 <sup>fg</sup>	48.42 <sup>a</sup>	73.2 <sup>a</sup>	1693 <sup>bc</sup>	1751 <sup>f</sup>	167.58 <sup>e</sup>	$0.883^{e}$	0.54 <sup>e</sup>	6.7 <sup>e</sup>

Means followed by the same letter(s) within a column are not statistically different at the p=5% level

#### Fresh and dry stem weight

The results showed that water deficit and zeolite application had significantly effect on fresh and dry stem weight (Table 1). The highest means of fresh and dry stem weight was observed in 70% of field capacity and the highest means of fresh and dry weight was obtained in 2.5 g zeolite. Variance analysis of interaction effect had a significant different in 1%. In all drought levels with increasing amount of zeolite the mean of fresh and dry stem weight was increased, the highest mean in treatments belonged to 70% fc in 2.5 g zeolite and the lowest mean of fresh and dry weight was observed in absent of zeolite and 30% fc.

#### Fresh and dry root weight

Water deficit had a significant effect on the fresh and dry root weight (Table1). Mean comparison showed that the maximum dry root weight (117g) and fresh root weight (180.4g) was obtained in 50%fc. It seems with intensity drought stress from 70%fc to 50% fc plant tries with increasing root length gets to enough mois-

ture so root weight has increased but this reaction is not seen in 30%fc. Zeolite application had a significant effect on fresh and dry root weight. Using 2.5 g zeolite as compared with others level, caused to the highest average for dry root weight (122.3g) and fresh root weight (188.2g) in contrast there was no interaction effect for fresh and dry root weight. It seems treatments had same effects on root growth (Table 1).

#### Leaf number and leaf area

Table 1 indicated that water deficit, zeolite and their interaction had significant different for leaf number and leaf area. With increasing drought intensity from 70% fc to 30% fc the leaf number decreased from 4284 to 1431 leaf in m² and leaf area had significant decreasing from 5472mm² to 1908 mm². Mean comparison of zeolite indicated that the highest means of leaf number and leaf area belonged to 2 g and 2.5 g zeolite. Analysis of interaction effects for leaf number and leaf area showed in 30% fc with increasing zeolite from 0 g to 2.5 g. There was

no significant different among means but in 50% fc and 70% fc, average of leaf number and leaf area with increasing zeolite to 2.5 g in kg soil has increased and interaction effect of 2.5g zeolite and 70% fc had the most effective rather than treatments.

#### Water use efficiency (WUE)

The information presented in the table 1 indicated that the effect of zeolite had been significant on WUE, the highest WUE obtained in 70% fc. The effect of Zeolite had significant effect and the highest WUE obtained in 2.5 gr. Also interaction effect had significant different on WUE. And the highest WUE obtained in 70% fc in 2.5 g zeolite. Table 3 also demonstrates that in 70% fc and 50% fc by increasing the zeolite, amount of WUE increased which was significant. Although the lowest WUE was observed in the absent of zeolite and 30% fc but in 30% fc with increasing zeolite from 0 g to 2.5 g.there was no significant change in the WUE.

#### Shoot dry weight

According to Table 1 water deficit, zeolite and their interaction have significant effect in shoot dry weight. The Highest shoot weight was achieved in 70% fc. Mean comparison of zeolite levels showed that the highest mean was observed in 2.5g and 2 g zeolite. Table 3 indicated that the application of zeolite in drought levels increased the weight of shoot in experimental plots. The highest weight was observed from treatment with 70% fc, Results of zeolite application in 70% fc and 50% fc showed that the increasing of zeolite improved shoot dry weight in plots. But in 30% fc no significant effect was observed among zeolite levels.

#### Oil percentage

In the present study, water stress had significant effect on oil content. The oil percentage was increased by water deficit. With increasing drought intensify from 70% fc to 30% fc oil content was reached from 0.43% to 0.62%. Zeolite levels had significant effect in oil percent-

age. With increasing the amount of zeolite from 0 to 2.5 g, oil content was reduced from 0.69% to 0.51%. Mean comparison of interaction effect indicated that with increasing zeolite level from 0g to 2.5 g in each of water deficit (30%, 50% and 70% fc) amount of oil contents was significantly reduced. The highest oil percentage was observed by 30% fc in absent of zeolite.

#### Essential oil yield

The oil yield response to drought stress of medicinal menthe is given in Table2. The oil yield was affected by water deficit levels. Plant in 50% fc had the highest significantly oil yield and use of 2g zeolite in 1kg soil had more effective than others level of zeolite. According to table 3, the maximum essential oil yield was available in 2.5g zeolite and 50% fc and followed in 50% fc and 2g zeolite. theminimum content of this important trait pertained to 30% fc in 2g zeolite.

#### **Discussion**

The results of variance analysis showed that the drought stress significantly affected the growth traits such as leaf dry weight, leaf wet weight, stem wet weight, stem dry weight, root dry weight, root wet weight, leaf number, leaf area, shoot dry weight, WUE, oil percentage and oil yield. Mean comparison for drought levels revealed that the highest mean in all traits except oil percentage belonged to irrigation in 70% fc. In other hand, treatment of 70% fc caused the highest value for all traits. Drought stress reduced the yield of medicinal and aromatic plants by three main mechanisms: first, whole canopy absorption of incident tosynthetic active radiation may be reduced; second drought stress decreased the efficiency with which absorbed photosynthetic active radiation is used by the crop to produce new dry matter. Third drought stress may limit biomass weight or grain yield in medicinal and aromatic plants by reducing the harvest index. Farahani (2009) believed that depending upon the plant species and plant genotypes, drought stress can increase, decrease or have not effect on the levels of metabolites. Khalid (2006) reported that drought reduced fresh and dry weight in two genotypes of Ocimum. Azizi et al (2008) described that increasing drought stresses caused reduction both in fresh and dry weight Moldavian Balm. The results of effect of drought in other medicinal plant indicated that water deficit during the vegetative can result in reduction in water use efficiency, shorter plants, smaller leaf areas and least leaves of Mint (Safikhani et al., 2007., Abbaszadeh et al., 2008), Yarrow (Sharifi Ashoorabadi et al., 2005), Chicory (Taheri et al., 2008 and Rahmani et al., 2008). With increasing drought levels, oil yield was decreased. Reduction in essential oil yield may be due to disturbance in photosynthesis carbohydrate production under stress condition and suppuration of the plant growth (Flexas and Medrano, 2002). Khalid (2006) observed that water stress strongly depressed essential oil yield. In this study almost all traits and essential oil yield were reduced with increasing the drought level from 70% fc to 30% fc but with increasing drought intensity from 70% fc to 30% fc the essential oil percentage was increased. The results of an experiment showed that essential oil yield was reduced under water deficit stress but essential oil percentage was increased under stress (Aliabadi et al., 2009). Sing Sangwan et al., (2006) indicated that the level of essential oil was maintained or enhanced under drought condition. In this experiment the highest value for oil percentage was observed in 30% fc and the highest shoot yield belong to 70% fc but the highest oil yield was observed in 50% fc, it seems that the drought stress increases the essential oil percentage of more medicinal and aromatic plants, because in case of stress, more metabolites are produce in the plants and substances prevent from oxidization in the cell, but essential oil yield reduce under drought stress, because the interaction between the amount of the essential oil percentage and shoot yield its consider important as two components of the essential oil yield an therefore irrigation in 50% fc is suitable point for having balance between oil percentage and shoot yield that lead to the highest essential oil yield. Also results showed that the effect of zeolite levels was significant for all traits and zeolite application has had different effect on growth parameter and oil yield. Positive effects of zeo-

lite were reported by other researchers these effects could be are result of high potentially of zeolite to absorb water and conserve water in the soil and improve Physical properties. (Boman and Evans. 1990). In order to more investigate, interaction effects at zeolite and water deficit were calculated. Result of interaction effects of zeolite×water deficit were similar with the reports of Goksey et al., (2004), Muire et al., (2007), and Afsharmanesh (2009). High content of polymer with water supply caused opening stomata for a long time, sub squinty good fixation of CO2 resulted an increase of dry matter in medicinal plant (Khadem et al., 2010). Similar to our results, incorporation of zeolite into soil has increased wheat biomass (Howard et al., 1999). With regard to table 3 it can be seen that effect of zeolite × water deficit had significant different on all traits except root dry weight and root wet weight, mean comparison showed that in water deficit with increasing amount of zeolite the mean of all trait increased. The highest essential oil vield was observed in 50% fc with 2.5 gr zeolite. Interaction effect between zeolite and water deficit showed that increasing of zeolite caused significant reduction in drought intensity on growth parameter. High level of zeolite application increased the water use efficiency. In other word, zeolite application decreased the adverse effect of stress and caused to increased WUE, effect of water stress on WUE depends on plant species, phonological stage of plant in drought period and stress intensity (Kumari, 1988).

#### Conclusion

Results indicated that the use of zeolite in drought durations has a significant effect on essential oil yield which is the most important parameter in medicinal plants. It can be mentioned that 2.5g of zeolite with 50% of water losses are lead to improving higher essential oil and generally the productivity of this plants. Zeolite application can improve shoot yield and follow oil yield under drought stress conditions and it can persist less damaging of drought stress in medicinal and aromatic plant farming. In addition this may be recommended for soil in arid and semi-arid regions to enhancing drought tolerance in medicinal and aromatically plants.

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