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Water Stress and Natural Zeolite Impacts on Phisiomorphological Characteristics of Moldavian Balm *(Dracocephalum moldavica* L.*)*

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Abstract: There is no doubt that certain plants offer valuable medicinal properties. But there are many challenges that local communities face in the harvesting and cultivation of these plants. Moldavian balm (*Dracocephalum moldavica* L.) is an important medicinal plant and there are three pharmaceutical products that originate from in Iran. Water source limitation is one of the important problems in Iran. The scientists suggest that active substance production is the results of environmental stress on plants. Natural zeolits have some properties such as water absorption and emission and nitrate leaching inhibition, which is useful for soil amendment. A pot experiment was conducted in greenhouse with 12 factorial treatments and three replicates. Four levels of Zeolit and three levels of water stress applied during the plant growth. Growth and development factors such as wet and dry weight, leaf area, chlorophyll content, number of leaves, root length and essential oils content were measured. The results showed that zeolit application had not a significant effect on dry weight. Moreover, there were not a significant effect of water stress on leaf area, number of leaves and root length. There was not an interaction between zeolit and water stress on wet and dry weight and also root length but this interaction was significant on leaf area, chlorophyll content, number of leaves and essential oils content. It concluded that medium level of zeolit with the lowest level of water stress recommended for herb and essential oil production of moldavian balm.

Key words: Dracocephalum moldavica, Moldavian balm, Zeolite, Water stress, Essential oils.

INTRODUCTION

Since May 1978 the World Health Organization (WHO) has been making a study of medicinal plants. This study prompted the initial identification of 20,000 species of medicinal plants and a more detailed investigation of a short list of 200. A great many of these plants have their origins in the world's tropical forests and their present use is largely rooted in traditional medicines which play a major part in maintaining the health and welfare of both rural and city dwellers in developing countries (Mahajan, *et al.*, 2005).

These days, around one third of human needed medicines have herbal origins and scientists, physicians and pharmocologists all try to persuad people toward changing the other two third to medicinal plants too.

Moldavian balm (Dracocephalum moldavica):

Moldavian Balm *(Dracocephalum moldavica)* is an annual herbaceous aromatic plant belonging to family of Lamiaceae (El-Baky and El-Baroty, 2007). It is native to central Asia and in eastern and central Europe (Griffiths, 1994). The oil content and its composition showed great variation due to plant origin (Hussein *et. al*, 2006). Plant of genus *Dracocephalum* is reported to use as a food ingredients, as a tea and as herbal drug and in folk medicine as a painkiller and a tonic for treatment of stomach, kidney and liver disorders, headache, congestion and also Alzheimer disease therapy (Racz *et al.*, 1978). Compost has already been established as a suggested fertilizer for improving the productivity of several medicinal and aromatic plants that can be an environment-friendly way (El-Ashry *et al.*, 1995).

The essential oil of Moldavian Balm *(Dracocephalum moldavica L.)* was characterized by a high containing oxygenated acyclic monoterpenes, having a ketone, aldehyade and alcoholic function groups (El-

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Baky and El-Baroty, 2007). El-Baky and El-Baroty (2007) also mentioned essential oil of this plant has been investigated for their chemical composition, a little is known about the possible the antioxidant and antibacterial and antifungal efficiency in their essential oil against diverse microorganisms.

Water Stress and Zeolite:

Plant growth and productivity can be affected by various abiotic and biotic discharge factors. Plants are frequently exposed to many discharge conditions such as low temperature, salt, drought, flooding, heat, oxidative discharge and heavy metal toxicity (Manivannan *et al.*, 2007) . Water discharge may arise as a result of two conditions, either due to excess of water or water deficit. The more common water discharge encountered is the water deficit discharge known as the drought. The water deficit has impact on ecological and agricultural systems (Shao *et al.*, 2007). The reactions of plants to water discharge differ significantly at various organizational levels depending upon intensity and duration of discharge as well as plant species and its stage of development (Chaves *et al.*, 2003). Understanding plant responses to drought is too important and also has fundamental effectss on manage plant productivity.

The typical first response of all plants to water deficit is osmotic adjustment. Compatible solutes accumulation in the cytoplasm is cnsidered as a mechanism to contribute discharge tolerance (Hare *et al.*, 1998). To counter with abiotic dischargees, plants increase the osmotic potential of their cells by synthesizing and accumulating compatible osmolyte such as proline (PRO) and glycine betaine (GB) that participates in the osmotic adjustment (Kavikishore *et al.*, 2005). PRO and GB are thought to function as osmoprotectants for proteins (Bohnert *et al.*, 1996).

Chemical treatment and agronomical crop management practices have been tried to reduce the water deficit effects (Manivannan *et al.,* 2007), but the application of zeolite to dischargeed plants attracted little attention. One possible approach for reducing the effect of water deficit on plant productivity is through the addition of zeolite to soil.

Zeolite is a group of natural minerals with physical and physicochemical properties that can be utilized in various areas as construction and agriculture that can absorb and hold potentially harmful or toxic substances. It is also capable of absorbing part of the excess nutrients and also water, resulting in more balanced macronutrient cation ratios in the root environment and can keep water in root zone (Savvas *et al.*, 2004).

Based on Abdul Jaleel et al. (2007) because of the ever increasing demand of medicinal plants, some medicinal plants need to be cultivated commercially, but the soil water shortage causes serious threats to plant production. However, little information is gained about the relationship between water discharge and secondary metabolite content in medicinal plants, it seems necessary to do research related to the correlation between medicinal plants and water deficit for the increasing need of medicinal plants.

Objectives:

The main purpose of this study is to determine whether zeolite increases Moldavian Balm *(Dracocephalum moldavica L.)* drought tolerance and if such tolerance is correlated with its growth, development and essential oil content. It also palns to introduce zeolite as an absorbent of toxic and harmful substances from soil, the excess nutrient and also water. Due to this purpose, the objectives of the study can be set as follow: 1- Determine the effects of various amount of zeolite on some phisiomorphological characteristics (fresh and dry weight, leaf area, chlorophyll content, number of leaves, root length and essential oils content) of moldavian balm. 2- Determine the effects of different levels of water discharge on some phisiomorphological characteristics (fresh and dry weight, leaf area, chlorophyll content, number of leaves, root length and essential oils content) of moldavian balm. 3- Estimate the interaction of different levels of zeolite and water discharge on some phisiomorphological characteristics (fresh and dry weight, leaf area, chlorophyll content, number of leaves, root length and essential oils content) of moldavian balm.

MATERIALS AND METHODS

2.1. Study area:

A pot experiment was carried out in 2004 at the Greenhouse Complex of Astane Ghodse Razavi, Mashhad in the central part of Khorasan province, Iran. The pots' soil was sandy loam with pH 6.8, contains OC (60%), total P (0.93 ppm) and total K (1.47 ppm) with an EC of 12 mmohs cm^{-1}.

2.2. Experiment design:

A factorial experiment based on a randomized complete design with 12 treatments and 3 replications was used totally in 36 pots. Four zeolite levels (Zero, 1.7, 2 and 2.5gr/kg pot soil) with N:P:K (150:96:52) fertilizers, saturated irrigation during rapid growth stage and then 3 soil water evacuation levels (50, 60 and 70%) during growth to harvesting stages were done. A gypsum block was installed in each pot to estimate soil water content. For zeolite recommendation refered to Tso (1990) and also Urotadze et al. (2002).

Other agricultural operations were the same as the other medicinal plants. In flowering stage number of leaves, leaf area and chlorophyll content were determined. Fresh and dry weight were also obtained. Moreover, the essential oil content was extracted from aerial parts of the collected samples of each treatment by hydrodistillation for 3h, using a Clevenger-type apparatus.

The effects of all 12 treatments on phisiomorphological characteristics and essential oil content of Moldavian Balm *(Dracocephalum moldavica* L.*)* were studied. The data were analyzed by one-way Analysis Of Variance (ANOVA) using the Statistical Analysis System (SAS) and means were compared by LSMEAN test at 1% probability level.

RESULTS AND DISCUSSION

3.1. Fresh and Dry Weight:

The variance analysis of measured characteristics has been presented in table 1. As it is observed, the effect of zeolite treatment on the fresh weight was significant while was not significant on dry weight ($p< 0.01$). The effect of water discharge was signisicant on both fresh and dry weight ($p< 0.05$). There was not any interaction between zeolit and water discharge with regard to the impact on the fresh and dry weight of Moldavian Balm *(Dracocephalum moldavica L.).*

Table 1: Analysis of variance

Variables	jp	Mean Square						
		Fresh weight	Dry weight	Leaf area	Chlorophyll content	Leavesnumber	Root length	Essential Oil Content
Treatment	11	0.19^{**}	0.09^{*}	97.73^{**}	260.38^{**}	23^{**}	$11.05^{n.s}$	2.53^{**}
Zeolite	3	0.33^{**}	$0.06^{n.s}$	376.45^{**}	294.74^{**}	48^{**}	29.92*	4.78^{**}
Water Discharge	2	0.24^{*}	0.14^{*}	$73.63^{n.s}$	84.5^{**}	$7^{n.s}$	$10.13^{n.s}$	2.13^{**}
Zeolite x Discharge	6	$0.11^{n.s}$	$0.1^{n.s}$	149.74^{*}	301.82^{**}	16^{**}	$11.09^{n.s}$	1.54^{**}

The average fresh and dry weight of Moldavian Balm *(Dracocephalum moldavica L.)* for zeolite in different water discharge levels has been shown in table 2. As it can be seen, the maximum fresh and dry weight pertained to 25gr zeolite and 50% of water discharge. In addition the minimum fresh weight pertained to non-used zeolit pots and 60% water discharge. While the minimum dry weight could be observed in 20gr zeolite and 70% water discharge. The results those have been presented in table 2 show that in the absence of zeolite, both fresh and dry weight decreased as the water discharge increased from 50% to 70%, in 25gr of zeolite , the same result was obtained as the water discharge increased from 50% to 70%. In 20gr zeolite by increasing water discharge from 50% to 70%, fresh weight did not show any significant difference while the dry weight decreased significantly. The table 2 also shows that in 30gr of zeolite by increasing the water discharge from 50% to 70%, the fresh weight decreased, while the dry weight increased.

Table 2: Effects of zeolite and water discharge on phisiomorphological characteristics

Zeolite	Water Discharge	Fresh weight	Dry weight	Leaf area	Chlorophyll content	Leaves' number	Root length	Essential Oil Content
0	50	2.03^{abc}	1.07^{ab}	65.73^{abcd}	40.9^{ab}	22^{bcd}	12.74^{ab}	1.7^{b}
	60	0.67^{d}	0.77^{bcd}	46.4^{f}	25.2^{g}	16^{g}	5.91^{c}	0.9^{f}
	70	0.8^{d}	0.7^{cd}	55.1^{def}	13.1^{i}	19^{ef}	10.71^{abc}	0.9^{f}
20	50	1.43^{bcd}	0.83^{abcd}	60.9^{bcd}	33.1^{dc}	21^{cde}	12.19^{ab}	1.4^{cd}
	60	1.63^{bc}	1.03^{abc}	66.7^{abc}	41.7^{a}	23^{bc}	10.74^{abc}	1.5^{c}
	70	1.33^{bcd}	0.57^{d}	63.8^{bcd}	38.3^{bc}	22^{bcd}	12.59^{ab}	1.4^{cd}
25	50	2.77^{a}	1.17^{a}	75.4^{a}	43.47^{a}	26^{a}	13.79^{a}	2^{a}
	60	1.37^{bcd}	0.87^{abcd}	66.83^{abc}	25.87^{g}	23^{bc}	13.19^{ab}	1.4^{cd}
	70	2.23^{abc}	0.77^{bcd}	69.6^{ab}	35.7^{cd}	24^{ab}	12.41^{ab}	1.8^{b}
30	50	1.17^{cd}	0.6^{d}	58^{cde}	19.8^{h}	20^{def}	8.55^{bc}	1.1^{c}
	60	1.27^{bcd}	0.8^{bcd}	66.67^{abc}	31.7^{ef}	23^{bc}	10.16^{abc}	1.3^{d}
	70	0.8^{d}	0.8^{bcd}	52.2^{ef}	29.1^{f}	18^{fg}	8.81^{bc}	0.9^{f}

In general, it could be said that zeolite and water discharge affected on fresh and dry weight independently (Table 3 and 4), while there was no interaction between zeolite and water discharge on the average fresh and dry weight; additionally, the impact of zeolite did not depend on soil humidity discharge. These results corresponds to Ranjbar et al. (2004) report, which was based on the quality of tobacco in different amounts of zeolite and water discharge.

Table 3: Effects of zeolite on phisiomorphological characteristics

Zeolite	Fresh weight	Dry weight	Leaf area	Chlorophyll content	Leaves' number	Root length	Essential Oil Content
0	1.17[b]	0.84[a]	55.74[b]	26.4[c]	23[**]	9.79[ab]	1.17[c]
20	1.47[ab]	0.81[a]	63.8[ab]	37.7[a]	48[**]	11.84[a]	1.43[b]
25	2.12[a]	0.93[a]	70.61[a]	35.01[b]	7[n.s]	13.13[a]	1.73[a]
30	1.08[b]	0.73[a]	58.95[b]	26.87[c]	16[**]	9.19[b]	1.09[c]

Table 4: Effects of water discharge on phisiomorphological characteristics

Water Discharge	Fresh weight	Dry weight	Leaf area	Chlorophyll content	Leaves' number	Root length	Essential Oil Content
50	1.85[a]	0.91[a]	65.01[a]	34.32[a]	22[a]	11.82[a]	1.55[a]
60	1.23[a]	0.87[a]	61.65[a]	31.12[b]	21[a]	10[a]	1.28[b]
70	1.29[a]	0.71[a]	60.18[a]	29.05[c]	21[a]	11.14[a]	1.24[b]

3.2. Leaf area:

As it can be seen in table 1, zeolite had significant effect on the leaf area ($p< 0.01$) which corresponds to Ranjbar et al. (2004) results on Tobacco. The effect of water stress on leaf area was not significant. This result also corresponds to Ranjbar et al. (2004) report on tobacco, Sarma (1985) on mustard and also Flent et al. (1996) on sunflower. There was a significant effect ($p< 0.05$) between zeolite and water discharge with regard to the impact on the leaf area but because of the non-significant simple effect of water stress, it can be assumed that in the leaf aera increasing, the role of zeolite was more effective than the role of irrigation factor.

It is obvious from table 2 that in the absence of zeolite, by increasing the discharge of soil humidity from 50% to 60%, the leaf area had significant decrease, while there wasn't any significant difference between 60% and 70% of soil humidity discharge. Increasing the discharge of humidity in 20 and 25gr zeolite did not have any significant effect on the leaf area. Moreover, the increasing of soil water discharge from 50% to 60% in the presence of 30gr zeolite did not have any significant effect on the leaf area while more humidity discharge up to 70% leads to significant decrease in this parameter. The highest amount of Moldavian Balm *(Dracocephalum moldavica L.)* leaf area was observed in the presence of 25gr zeolite and 50% of water discharge but the lowest amount of it could be seen in the absence of zeolite and 60% water discharge.

3.3. Chlorophyll Content:

The information that is presented in the table 1 shows that the effect of zeolite has been significant ($p< 0.01$) on chlorophyll content which these results did not correspond to Ranjbar et al. (2004) report. In addition, the amount of soil humidity discharge had significant ($p< 0.01$) effect on Moldavian Balm *(Dracocephalum moldavica L.)* chlorophyll content. This result was also different from Ranjbar et al. (2004) result, while, it corresponds to Nielson and Orcutt (1996) results who proclaimed that decreasing in soil humidity would lead to decline of chlorophyll content in wheat. Furthermore, in this study the singnificant ($p< 0.01$) interaction between zeolite and water discharge on chlorophyll content could be observed which is rather in contradiction with Ranjbar et al. (2004) result.

According to table 2, it is assumed that the highest chlorophyll content pertained to the 25gr zeolite and 50% of soil humidity discharge while the lowest amount in this experiment related to the absence of zeolite and 70% of humidity discharge. The results which were presented in this table, also shows that in the absence of zeolite the chlorophyll content declined by increasing the humidity discharge from 50% to 70% and this difference was significant.

3.4. Number of leaves:

The information that was presented in the table 2 shows that the highest number of leaves (26) obtained in 25gr zeolite and 50% water discharge, while; the lowest leaves (16) was observed in the absence of zeolite and the middle amount of soil water discharge (60%). This table also demonstrates that when no zeolite was used, by increasing the soil humidity discharge from 50% to 70% number of leaves decreased from 22 to 19 which was significant, whereas; in 20gr zeolite, no significant change in the number of leaves could be seen with increasing of the soil water loses. The non-significant effect of zeolite on leaves'number was also observed in 25 and 30gr zeolite.

According to table 1, the effect of zeolite and its intraction with water stress has been significant ($p< 0.01$) on the number of leaves which these results corresponded to Ukrainian Zeolite Information website report which claimed that usage of natural zeolite improves plant leaf number and biomass. In all the measured parameters the highest amount was related to 25gr zeolite usage. The reason for this, is zeolite ability to conserve water and nutrition. In table 1 also it is clear that water stress did not have any significant effect on leaf number that is similar to results of Nakhjavani Moghaddam and Ghahraman (2005). While, Koocheki *et al*, (2008) mentioned that water stress has significant effect on the leaves' number of majority of plants.

3.5. Root length:

As vividly depicted in table 1, the effect of zeolite in root length has become significant in this experiment ($p< 0.05$); whereas, the irrigation treatment effect and also its interaction with zeolite did not have any significant effect on root length.

The average root length for zeolite in different water discharge levels (table 2) shows that in the absence of zeolite the root length did not have significant difference by increasing the soil humidity discharge, this also was true in other levels of zeolite which means that by increasing the amount of humidity discharge, root length didn't have any significant diffence even the zeolite level had been higher. The only significant difference occurred when the humidity discharge reached from 50% to 60% and also zeolite was not used. The maximum root length pertained to 25gr zeolite and 50% of soil humidity discharge because of the availability of enough water and the minimum root length could be experienced in 60% of soil humidity discharge and zero gr zeolite, it seems the reason is insufficient water availability for plant and also the zeolite absence in order to maintain and preserve soil humidity.

3.6. Essential Oil Content:

Table 1 indicates that the treatments of zeolite, water discharge and also their interaction had significant effect ($p< 0.01$) on the essential oil content of Moldavian Balm *(Dracocephalum moldavica L.)*. Based on Ranjbar et al. (2004), zeolite did not have any significant effect on the amount of tobacco nicotine which did not correspond to the results of this experiment but again in his experiment it is observed that treatment of water stress and its interaction with zeolite had significant effect on nicotine amount which was similar to the results of this study. The achieved results also did not correspond to Tso's (1990) results which claimed that the irrigated tobacco had less nicotine and alcaloide, but it corresponded to the results of Jangir and Rajender (1996) which were done on cumin. Laythen and Nielsen (1999) and Franz (1983) claimed that the sufficient soil humidity can alter the physical and chemical characteristics of leaf and it is expected that the irrigated medicinal plant has more essential oil than the one which is raised in dry condition.

The average of measured characteristics in table 2 shows that in the absence of zeolite with increasing the soil humidity discharge from 50% to 70%, essential oil content was decreased which this reduction was significant. Moreover, it can be seen that by increasing the zeolite level up to 20gr, more soil humidity discharge, did not show any significant effect on essential oil content. While in 25gr zeolite, less soil water caused less essential oil content same result obtained in 30gr zeolite. In addition, the maximum essential oil content was available in 25gr zeolite and 50% humidity discharge; whereas, the minimum content of this important trait pertained to 30gr zeolite and 70% soil humidity discharge.

4. Conclusion:

This study was conducted to introduce zeolite as an environmet-friendly mineral for increasing plant critical factors. It has also designed to determine whether zeolite increases moldavian balm *(Dracocephalum moldavica* L.*)* drought tolerance and if this introduced tolerance is related to level of water stress. Our results depicted that the increasing of zeolite and water stress have a significant effect on most of measured parameters including essential oil content. Finally, it can be mentioned that 2gr zeolite with 50 % water losses are lead to improving higher weight and essential oil and generally the productivity of this plants.

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