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The Study on the Effect of Different Levels of Zeolit and Water Stress on Growth, Development and Essential Oil Content of Moldavian Balm (*Dracocephalum moldavica* L.)

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Abstract: Problem statement: In order to meet the ever increasing demand of medicinal plants, for the indigenous systems of medicine as well as for the pharmaceutical industry, some medicinal plants need to be cultivated commercially and moldavian balm (*Dracocephalum moldavica*) is one of them. **Approach:** To determine the effects of zeolite and water stress on aforementioned plant's phisiomorphological characteristics, an experiment was conducted with applying four zeolite levels and three water discharge levels during plant growth. Some growth and development parameters were measured. **Results:** Summarized that, zeolite did not have any significant effect on plant height and number of internodes but had, on the other traits. There were not a significant effect of water discharge on plant height, number of flowers and number of internodes but its effect on the flowering time and essential oil content was significant. Moreover, there was not an interaction between zeolite and water stress on number of flowers and number of internodes but this interaction was significant on the other parameters. **Conclusion:** Finally, 2 g zeolite with 50% water losses was recommended to obtain higher essential oil. Additional researches are needed to confirm the results for data from other fields and crops.

Key words: Dracocephalum moldavica , moldavian balm, zeolite, water discharge, essential oils

INTRODUCTION

Moldavian balm (*Dracocephalum moldavica* L.) is a hardy annual plant (2 ft) with aromatic, balm-scented, green foliage and belongs to family Lamiaceae.

The oil content and its composition showed great variation due to plant origin. In Rumania, the percentage of essential oil ranged from 0.2-0.62 (Racz *et al*., 1978). However, in Hungary, Halasz-Zelnik *et al*. (1988) and Hornok *et al*. (1990) reported that the essential oil at flowering stage reached 0.741 and citral was the major component of the oil (30-45%). In Finland, Holm *et al*. (1988) stated that the maximum percentage of oil was 0.62% during the flowering stage and the oil contained 90% of oxygenated acyclic monoterpenes, i.e., geraniol, geranial, neral, nerol and geranyl acetate (Holm *et al*., 1988). In Egypt, El-Gengaihi *et al*. (1995) found that the oil was composed of acyclic oxygenated monoterpenes which reached to 93% of the oil. Moreover, Aziz and El-Sherbeny (2003) stated that the essential oil of moldavian balm plants was characterized by a high percentage of oxygenated monoterpenes (81.4-96.05%) and the major components were geranial (22.82-55.8%), geranyl acetate (9.75-31.48%), neral (16.08-22.02%) and geraniol (0.42-16.59%).

Moldavian balm is widely used in folk medicine as a painkiller and for the treatment of kidney problems (Hussein *et al*., 2006). Extracts of the plant are used against toothache and colds as a poultice against rheumatism (Racz *et al*., 1978); also, this extract acts as stimulated evolution in female rats and rabbits (Boikova and Akulova, 1995) as it is used as antitumor (Chachoyan and Oganesyan, 1996).

The compost must be added to conventional NPK fertilizer to improve soil structure, making the soil easier to cultivate, encouraging root development, providing plant nutrients and enabling their increased

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uptake by plants. Moreover, compost aids water absorption and retention by the soil, reducing erosion and run-off and thereby protecting surface waters from sedimentation, help binding agricultural chemicals, keeping them out of water ways and protecting ground water from contamination (leaMaster *et al*., 1998).

Compost has already been established as a recommended fertilizer for improving the productivity of several medicinal and aromatic plants, as amaryllis (El-Ashry *et al*., 1995), peppermint (O'Brien and Barker, 1996) and Tagetes erecta (Khalil *et al*., 2002).

Water stress is a major environmental problem which affects agricultural land in Iran. The effect of water stress on the growth, yield and active ingredients of medicinal and aromatic plants was studied by many investigators: Denys *et al*. (1993) on *Artemisia annua* L., Misra and Srivastava (2000) on *Mentha arvensis* L., Koocheki *et al*. (2008) on *Zataria multiflora*, *Ziziphora clinopodioides*, *Thymus vulgaris* and *Teucrium polium*.

In regions where water scarcity is the principal limiting factor for cultivation, farmers are interested in using some methods to deduce injurious effects of water deficiency. 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 to reducing the effect of water deficit on plant productivity is through the addition of zeolite to soil. Zeolite is a group of naturally occurring minerals with physical and physicochemical properties that can be used in such diverse areas as construction and agriculture that can absorb and hold potentially harmful or toxic substances. It also is capable of absorbing part of the excess nutrients and also water, resulting in more balanced macronutrient cation ratios in the root environment and also can keep water in root zone (Sawas *et al*., 2004).

In order to meet the ever increasing demand of medicinal plants, for the indigenous systems of medicine as well as for the pharmaceutical industry, some medicinal plants need to be cultivated commercially, but the soil water deficit pose serious threats to plant production (Abdul Jaleel *et al*., 2007). 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.

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. In contrast is to introduce zeolite as an absorbent of toxic and harmful substances from soil, the excess nutrient and also water.

MAERIALS AND METHODS

A pot experiment carried out in 2004 at the Greenhouse Complex of Astane Ghodse Razavi, Mashhad in the central part of Khorasan province, Iran (Latitude: 36°17'45 N, Longitude: 59°36'43 E). 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}.

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 2.5 g kg^{-1} 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 referred 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, number of flowers and number of internodes were determined. Fresh and dry weight and also flowering time were 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

Flowering time: Table 1 shows that the effect of zeolite, water discharge and their interaction on flowering time was significant ($p<0.01$).

With regard to Table 2, it can be seen that in non-using zeolite situation, by increasing humidity discharge from 50-70%, the flowering time postponed and it reached from 154-160 days, it also reached from 154-157 days when we utilized 20 g zeolite and the soil humidity discharge increased from 50-70%, which in both case the difference was significant. In presence of

25 g zeolite, by increasing the soil humidity discharge from 50-60% the flowering time also increasing from 150-156 days but it reached to 153 days in 70% water loss, all these changes ware significant too. When the zeolite usage was 30 g, with the changes in the humidity discharge from 50-70%, the time of flowering was postponed which this change was also significant. In this report the most delayed flowering time was observed in the the absence of zeolite and also 30 g zeolite with 70% soil humidity discharge (160 days), while the earliest flowering was in 25 g zeolite and 50% of soil humidity discharge (150 days).

Number of flowers: As it can be observed in Table 2, in all amounts of zeolite with increasing of soil water discharge, the number of flowers decreased non-significantly. The variance analysis of measured characteristics in Table 1 shows that the effect of zeolite on flower number had been significant ($p<0.01$). Table 2 also indicates that the effect of water discharge on number of flowers had been non-significant. In general, flower number were affected by the parameters those were effective on leaf number.

Number of internodes: As vividly depicted in Table 1, the effect of zeolite, water discharge and their intraction on internodes' number were non-significant.

Plant height: Based on Table 1, the effect of zeolite treatment and water stress on the height of Moldavian Balm (*Dracocephalum moldavica* L.) was not significant. It can be seen that the interaction of zeolite and water stress on the height was significant ($p<0.01$).

According to Table 2, it is observed that in the absence of zeolite, increasing the discharge of humidity from 50-70% leads to decrease in the plant height which this difference was not significant. When the usage of zeolite was 20 g, a significant difference was not observed between the effect of 50 and 70% of humidity discharge on plant height, while, these amounts showed significant effect on plant height when the humidity discharge was 60%. In 25 g zeolite, none of the humidity discharge amounts showed any significant difference with each other and finally in 30 g zeolite just the difference between 60 and 70% of humidity discharge on plant height was significant. Furthermore, the maximum plant height was observed in the absence of zeolite and 50% of humidty discharge and the minimum height also were observed in the absence of zeolite and 60% of humidty discharge.

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.).

The average of measured characteristics in Table 2 shows that in the absence of zeolite with increasing the soil humidity discharge from 50-70%, essential oil content was decreased from 1.7-0.9 which this reduction was significant. Moreover, it can be seen that by increasing the zeolite level up to 20 g, more soil humidity discharge, did not show any significant effect on essential oil content. While in 25 g zeolite, less soil water caused less essential oil content significantly,

Table 1: Analysis of Variance (ANOVA)

		Mean square				
Variables	df	Flowering time	Flowers' number	Internodes' number	Plant height	Essential oil content
Treatment	4	30**	0.41**	$3.45^{n.s}$	25.11**	2.53**
Zeolite	3	47**	0.88**	$8.00^{n.s}$	$16.16^{n.s}$	4.78**
Water discharge	2	42**	$0.21^{n.s}$	$0.25^{n.s}$	$18.03^{n.s}$	2.13**
Zeolite × discharge	6	18**	$0.24^{n.s}$	$2.25^{n.s}$	31.95**	1.54**

**: $p<0.01$; df: Degree of freedom; ns: Non-significant

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

Zeolite	Water discharge	Flowering time	Flowers' number	Internodes' number	Plant height	Essential oil content
0	50	154^{def}	2^{ab}	12^{a}	31.67^{a}	1.7^{b}
	60	159^{ab}	1^{bc}	10^{a}	22.86^{f}	0.9^{f}
	70	160^{a}	1^{bc}	10^{a}	27.27^{abcde}	0.9^{f}
20	50	154^{def}	1^{bc}	11^{a}	26.19^{cdef}	1.4^{cd}
	60	152^{fg}	3^{a}	12^{a}	30.72^{ab}	1.5^{c}
	70	157^{bc}	2^{abc}	11^{a}	24.19^{def}	1.4^{cd}
25	50	150^{g}	3^{a}	13^{a}	30.55^{abc}	2.0^{a}
	60	156^{cd}	3^{a}	12^{a}	28.44^{abcd}	1.4^{cd}
	70	153^{ef}	3^{a}	13^{a}	28.68^{abc}	1.8^{b}
30	50	157^{bc}	1^{c}	10^{a}	26.53^{bcdef}	1.1^{c}
	60	155^{cde}	2^{ab}	11^{a}	23.34^{ef}	1.3^{d}
	70	160^{a}	1^{c}	11^{a}	28.18^{abcd}	0.9^{f}

same result obtained in 30 g zeolite. In addition, the maximum essential oil content was available in 25 g zeolite and 50% humidity discharge; whereas, the minimum content of this important trait pertained to 30 g zeolite and 70% soil humidity discharge.

DISCUSSION

About flowering time this study's results were similar to Ranjbar *et al*. (2004). They also reported that the effect of soil humidity discharge on flowering time has been significant. In addition, these results were corresponded to Tso (1990) who claimed that enough soil humidity provides the earlier flowering in Tobacco (Tso, 1990). While in Ranjbar *et al*. (2004) reported the effect of zeolite and its interaction with water was not significant on flowering time which does not correspond to the current research's results (Ranjbar *et al*., 2004).

Moreover, the variance analysis of flower number corresponded to Chelopecka and Andriano (1997) results. But the research result in terms of zeolite treatment effect on plant height did not correspond to Ranjbar *et al*. (2004) result on Tobacco. Moreover, the impact of water stress on plant height was not significant which corresponded to Nakhjavani Moghaddam and Ghahraman (2005) results, they proclaimed that the water stress does not have any significant effect on the height of wheat. It can be seen that the interaction of zeolite and water stress on the height in this study was the same as Tso (1990) results.

The last but not least, about essential oil content Ranjbar *et al*. (2004) reported that 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 (1990) results which claimed that the irrigated tobacco had less nicotine and alcaloide, but it corresponded to the results Jangir and Singh (1996) which were done on cumin. Davis 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.

CONCLUSION

This study was conducted to introduce zeolite as an environment-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 essential oil content which is the most important parameter in medicinal plants. Finally, it can be mentioned that 2g zeolite with 50 % water losses are lead to improving higher essential oil and generally the productivity of this plants.

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