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Relative water content, cell membrane stability, essential oil and morphology of *Dracocphalum moldavica* L. are influenced by drought stress and manure

Parviz Rahbarian[1], Ali Salehi Sardoei[2*], Somayeh Gholamshahi[1], Ghasem Khorshidi Jor Jandi[1]

[1]*Department of Horticultural Sciences, Islamic Azad University, Jiroft Branch, Iran*

[2]*Young Researchers Club, Jiroft Islamic Azad University, Jiroft Branch, Iran*

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Abstract

In the current study, the effect of water deficit stress or drought on relative water content and cell membrane stability of dragonhead (*Dracocepha lummoldavica*) was studied in a greenhouse experiment carried out at Islamic Azad University, Jiroft branch, in 2009. It was a split plot experimentbased on Randomized Completely Block Design with three replications, in which vertical factor included three levels of drought stress (irrigation when soil moisture reached 75% of field capacity [mild stress], irrigation when soil moisture reached 50% of field capacity [moderate stress] and irrigation when soil moisture reached 25% of field capacity [severe stress]). The results showed that the effect of water deficit stress was significant on relative water content at 5% level and on cell membrane stability at 1% level. Water stress significantly decreased relative water content from 77.69% under mild stress under severe stress. Relative water content increased as manure level increased. considering essential oil content as the main goal of dracocephalum production and concerning the fact that oil yield as 13.10, 9.91, 9.91 and 9.91 kg/ha was achieved by interaction of the two factors in mild stress and 40ton/ha manure, mild stress and 30ton/ha, medium stress and 40ton/ha manure and medium stress and 30ton/ha manure, respectively; the medium drought stress together with application of 30tpn/ha is recommended for Jiroft region as the superior treatment which resulted in production of 9.91kg/ha essential oils.

*** Corresponding Author:** Ali Salehi Sardoei ✉ alisalehisardoei1987@gmail.com

Introduction

Medicinal herbs have been extensively studied in this century mainly because chemical medicines have proved to have side effects and humans tend to use natural products as much as possible (Azizi, 2000). Dragonhead or dragon'shead (*Dracocephalum moldavica*) is herb from mint family (Hussein *et al.,* 2006).

The effective substances of its body are sedative and appetizing. Its essence is antibacterial and is used in curing stomachache and flatulence as well as in food industries, soda manufacturing and health and make-up industries (Omidbeigi, 1997).

Although the effects of drought stress on crops have been extensively studied, the researches on the behavior of medicinal and aromatic herbs under water deficit have not been so extensive (Letchamo and Gosselin, 1996). Nowadays water deficit is known as an important limiting factor of yield increase in arid and semiarid regions and growth decrease is much greater under water deficit than that under other environmental stresses (Rodrigues, 2006). It is more important in regions which experience the problem due to climate change but have not been paid attention (Chaves and Oliveira, 2004) because global environment change programs show the growth of water deficit in future and the recurrence of much more severe events in most parts of the world. Environmental stresses bring about a wide range of responses in plants from genetic changes to the changes in growth speed and yield (Reddy *et al*., 2004). Therefore, in order to understand the conditions for the survival of medicinal herbs in arid regions, their responses to water deficit need to be evaluated and their appropriate growing conditions should be determined (Letchamo and Gosselin, 1994). Levitt (1980) suggests measuring the accumulation of soluble sugars, relative water content (RWC) or leaf water potential (LWP), cell membrane stability (CMS), accumulation of minerals and saturated and unsaturated fatty acid content in various plants under environmental stresses. Blum *et al.*, (1999) reported that plant RWC was the best criterion for measuring plant water status among parameters like plant RWC, plant water potential (PWP) and turgor potential. Several studies have been carried out about measuring RWC and drought resistance in different plants, all of which have shown the decrease in RWC with the increase of water deficit stress [e.g. in wheat (Rezaie and Borzooei, 2006), barley (Dadashi, 2006), rice (Lakshmi *et al*., 2005) and faba bean (Khan *et al*., 2007). In a study on corn under water deficit condition, Valentovic *et al.* (2006) found that electrolyte leakage was used for stabilizing cytoplasm membrane; the stronger the electrolyte leakage, the weaker the membrane stability. They reported that in drought-resistant corn cultivar Nova, electrolyte leakage in leaf was 8% in stress treatment and 5% in no-stress treatment, whereas in drought-sensitive cultivar Ankora, leaf electrolyte leakage in stress and no-stress treatments was 15 and 5%, respectively. Saneoka *et al.* (2004) reported that CMS can be used as a tool in measuring resistance to environmental stresses like drought and that by applying nitrogen fertilizer, drought stress increased CMS in bent compared with no-stress conditions. By applying 45 t manure/ha on soybean, Poor moosavi *et al.* (2007) reported that this crop produced the highest leaf moisture content in response to the fertilizer. At the end of the flowering stage, CMS improved due to intensive moisture stress and increased from 70.14% under optimum irrigation to 76.22% under severe stress. In a study on the effects of water deficit stress on yield and essence of dragonhead, Hassani (2006) reported that the biomass decreased from233 g in no-stress treatment to 112.5 g in severe stress treatment, but essence reached from 0.34 ml of dry matter to 0.35 ml in moderate stress treatment.

In a study on the effects of drought stress on yield and morphological traits of dragonhead, Safikhani *et al.* (2007) found that the yield of branches with flowers decreased from about 4126 kg/ha in FC 100% treatment to 2477 kg/ha in FC 40% treatment. Anomalous application of chemical fertilizers brings about environmental problems, too. Application of organic fertilizers such as manure along with chemical fertilizers can improve crop yield sustainability in addition to decreasing chemical fertilizer application (Khajuei nejhad *et al.*, 2004). Manure application is a method for avoiding soil moisture decrease. Despite the popularity of herb cultivation in different parts of Iran, little information is available about their responses to stresses.

Therefore, the current study was carried out to investigate the effects of drought stress and manure on relative water content, cell membrane stability, essential oil and morphology.

Materials and methods

Cultivation Conditions

To study the effects of manure application on dragonhead and to evaluate its resistance to drought stress as well as to study dry herb yield and essential oil of drought resistance in the crop, a strip plot experiment was carried out based on a Randomized Completely Block Design with three replications as a pot experiment in the greenhouse of Islamic Azad University, Jiroft Branch, Iran in 2009.

Firstly, the soil was sampled and its physical and chemical parameters were measured (Table 1). The pots were 23 cm high with the diameter of 30 cm. Each one was filled with about 10 kg soil on average. Ten pots received enough water to become saturated. They were covered by plastic sheet and after 24 hours when the redundant water leaked from the bottom hole due to gravity, their soils were sampled and dried in oven for 24 hours at 105°C. Then, the field capacity of the pots was determined. Manure application level was determined according to pot level. After weighing, cattle manure was used in fertilizer treatments. After preparing the pots, the seeds were planted with the rate of 15 seeds/pot at the depth of 0.5-1 cm. After emergence, the plants were thinned twice a month. Finally, four plants were left in each pot.

Treatments

In this study, low irrigation by applying water stress in three levels – mild stress (irrigation at field capacity of 75%), moderate stress (irrigation at field capacity of 50%) and severe stress (irrigation at field capacity of 25%) – constituted the vertical factor and manure application in five levels of 0, 10, 20, 30 and 40 t/ha constituted the horizontal factor.

Statistical Analysis

Analysis of variance was performed using standard techniques and differences between the means were compared through LSD Significant Difference test [$P < 0.05$] using MSTAT-C software package.

Results and discussion

Soil analysis showed that it was loam-sandy, alkaline and had no limitation from salinity and minerals viewpoint. It was poor in nitrogen and good in absorbable phosphorous and potassium (Table 1).

Table 1. Results of the analysis of soil used in experimental pots.

Depth (cm)	PH	EC (ds.m-1)	SP (%)	Total N (%)	AWP (%)	AWK (%)	Texture
0-30	8.1	0.89	25	0.03	12	220	Loamy sand

According to Table (2), ANOVA results considering drought stress and manure treatments effect on dry body yield f aerial part and essential oil yield in hectare and vase suggest that effect of drought stress on vegetative organ yield showed significant difference ($p<0.05$) and the effect on the two other traits was very significant ($p<0.01$). Significant difference was observed about effect of manure and its interaction with drought on vegetative part yield, essential oil amount and oil amount per pot ($p<0.01$). results showed that the difference was due to application of experimental treatments.

The results of analysis of variance of the effects of drought stress and manure on cell RWC and cell membrane stability in dragonhead (Table 2) showed that the effect of manure, drought stress and manure × drought stress interaction on RWC was significant at 5% statistical level. It seems that the difference of this parameter was caused by treatments with the probability of 95%. Moreover, the effects of manure and water deficit stress and their interactions were significant on cell membrane stability at 1% level, and the differences of cell membrane stability were brought about by treatments with the probability of 99% (Table 2).

Table 2. Analysis of variance for the effects of manure and water deficit stress on Dragonhead plant.

S.O.V	MS				
	df	Dry herb yield (kg/ha)	Essential oil yield (kg/ha)	Relative water content	Membrane stability
Replication		83975.48 ^{ns}	6.937 ^{ns}	63.85 ^{ns}	4656.62 ^{ns}
Drought Stress	2	10182108.02*	87.087	563.24*	48040.02**
Error A	2	1592662.22	1.618	64.62	1381.29
manure	4	25831360.63**	87.760**	218.19*	62294.22**
Error B	4	1365132.93	1.729	60.05	4923.87
Drought Stress × manure	8	4271322.13**	19.468**	175.85*	38432.94**
Error C	8	682346.70	1.959	61.16	1841.41
CV%	16	21.05	24.2	10.91	22.13

^{ns} Non Significant at 0.05 probability level and *, ** Significant at 0.05 and 0.01 probability levels, respectively.

According to Table (3), ANOVA results about effects of drought stress and manure on vegetative yield suggest that drought stress had significant influence on all the measured traits except for average internode length and lateral shoot number ($p<0.05$). Effect of manure was significant on dry and fresh weight of vegetative body and dry weight of stem and leaf ($p<0.01$) and stem number, plant height and stem diameter ($p<0.05$) but was not significant on internode length. Interaction of manure and drought stress was significant on dry and fresh weight (vegetative body) and stem diameter and leaf dry weight ($p<0.01$) and plant height and stem dry weight ($p<0.01$) but not significant on stem number and internode length. It sounds that the changes generated in the tested traits were due to experimental treatments ($p<0.01$ and $p<0.05$).

Table 3. Analysis of variance for the effects of manure and water deficit stress on Dragonhead plant.

S.O.V	df	MS							
		Shoot fresh weight (g)	Shoot dry weight (g)	No. shoot	Plant Height (cm)	Inter node Length (cm)	Stem Diameter (cm)	leaf fresh weight (g)	leaf dry weight (g)
Replication	2	4084856.82	190237.62	5.089	34.73	1.20	0.022	50263.45	226848.93
Drought Stress	2	23475660.15	9838642.95	5.75	327.60	0.52	11.35	724805.66	2909252.29
Error A	4	2869528.18	1598461.15	4.089	56.29	1.031	1.33	95170.61	386615.72
manure	4	487793252.86	26127715.38	28.74	293.97	1.276	1.73	1046068.16	6919216.68
Error B	8	19721286.51	1180085.95	2.22	36.37	0.651	0.35	45522.56	1606408.51
Drought Stress × manure	8	63975329.51	4005581.95	2.47	88.64	0.471	4	165976.14	1066408.51
Error C	16	13538660	606607.19	3.81	35.19	0.305	0.308	25830.77	192324.73
CV%		24.43	19.73	16.39	14.36	14.73	13.07	18.55	21.07

^{ns} Non Significant at 0.05 probability level and *, ** Significant at 0.05 and 0.01 probability levels, respectively.

Relative water content (RWC)

As can be seen in Fig. 1, the highest RWC (85.88%) was obtained under mild stress by applying 30 t manure/ha. The lowest RWC (about 47.73%) was obtained under severe stress with manure application. The point is that the mild stress with the application of 30 t manure/ha, severe drought stress with the application of 20 t manure/ha, moderate stress with the application40 t manure/ha and moderate stress with the application of 20 ad 30 t manure/ha did not show significant differences in RWC. As mentioned, manure helps in preserving water in plant. The results are consistent with Poor moosavi *et al.* (2007).

Table 4. Interaction of Different Drought Stress Levels on Growth Indexing in *Dracocephalum moldavica*.

Drought Stress	Manure (ton/ha)	Cow Shoot fresh weight (g)	Shoot dry weight (g)	No. shoot	Plant Height (cm)	Inter node Length (cm)	Stem Diameter (cm)	leaf fresh weight (g)	leaf dry weight (g)
Fc 75%	0	4060	1131	8.66	24.50	3.4	6	622	326
	10	12805	3405	12.33	44.6	5	3.3	1882	781
	20	29428	7275	13.67	46.6	3.3	3.6	3470	1554
	30	20666	4956	14.33	38.5	3.5	5.5	2725	1091
	40	30806	7574	14	50.8	3	6.8	4165	1614
Fc 50%	0	5040	1854	9.33	38	4	3.3	1176	522
	10	10885	3018	12.67	45.6	4	4.8	1604	767
	20	11705	3294	11	42.1	4	4.5	1737	804
	30	17655	4601	13.33	52.6	3.6	4.6	2428	1099
	40	19792	5353	12.33	52.1	4	4.3	2784	1257
Fc 25%	0	3620	1287	9	32.3	3.6	2.5	394	246
	10	9304	2820	11	35	4	3.6	1910	520
	20	11720	3204	12	38.1	3.5	4.1	1602	605
	30	20353	5197	14	41.8	3.8	2.5	2598	1010
	40	17126	4231	11	36.6	3.1	3.8	2115	797
LSD		6369	1857	3.397	10.27	0.95	1.32	759.1	383.3

*Means separated by LSD multiple ranges test at the $P < 0.05$ level.

EC

As can be seen in Fig. 2, moderate stress treatment with the application of 40 t manure/ha had the highest electrolyte leakage in terms of EC measurement of 443.0 $dS.m^{-1}$. It or non show statistically significant difference with moderate stress treatment with the application of 30 t manure/ ha whose EC was 422.7 $dS.m^{-1}$. But it showed significant differences with other treatments. The lowest electrolyte leakage, i.e. the highest membrane stability, was resulted from the interaction between severe drought stress and the application of 10 t manure/ha with the EC of46.67 $dS.m^{-1}$.

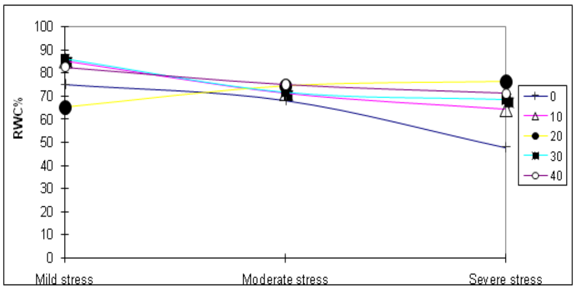

Fig. 1. Means comparison of effect of the interaction between drought stress and manure on leaf relative water content in dragonhead.

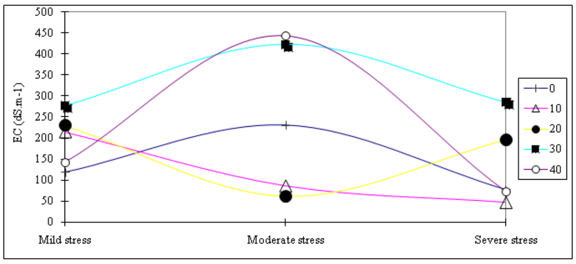

Fig. 2. Means comparison of effect of interaction between manure and water deficit stress on cell membrane stability in dragonhead.

Dry herb yield

According to (fig 3), considering interaction of the treatments, the highest yield of vegetative body was achieved by application of 40ton/ha of manure and 30ton/ha of manure in mild stress as 7573.7 and 7274.7kg/ha, respectively. Application of 30ton/ha of manure in mild stress was or non significantly different from 40ton/ha of manure in medium stress with 5354.3kg but showed significant difference from other treatments.

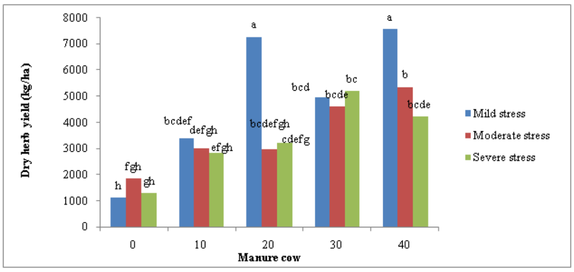

Fig. 3. Means comparison of effect of interaction between manure and water deficit stress on dry herb yield in dragonhead.

Essential oil yield

According to (fig 4), representing mean comparison of interaction of drought stress and manure on dracocephalum essential oils, the highest oil yield as 13.10kg/ha is achieved by mild stress which didn't show a significant difference with 9.91kg/ha obtained by application of 30 and 40tpn/ha of manure (irrigation after soil moisture reached to Fc50%) in medium stress. Probably retention of soil moisture by manure has resulted in high essential oil production.

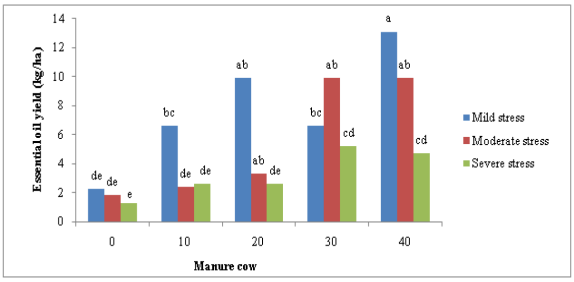

Fig. 4. Means comparison of effect of interaction between manure and water deficit stress on Essential oil yield in dragonhead.

Growth Characteristics

According to Table (4), mean comparisons of interaction between drought and manure on fresh yield of vegetative of Dracocephalum show that the highest vegetative body yield (fresh) as 30806kg/ha was achieved when the plant was cultivated in no-drought condition and treated by 40ton/ha of manure which was not significantly different from the yield obtained by application of 20ton/ha of manure as 29428kg/ha. The lowest vegetative fresh yield as 3620kg/ha was achieved by severe drought stress together with lack of manure application. The highest dry weight of vegetative body of Dracocephalum as 7500kg/ha were achieved by application of 40 ton/ha manure in mild stress. It was not significantly different from that obtained by application of 20ton/ha manure as 7275kg/ha in the same drought stress but these treatments were superior to other treatments. It looks that when there is no limitation in water availability, application of 20 ton/ha manure is suitable treatment for achieving the highest vegetative body yield in Dracocephalum but when the plant is exposed to water shortage, application of 30 to 40ton/ha of animal (cow) manure is recommended because under 50% drought stress, the highest dry weight of vegetative body is obtained by using 40 and 30 ton/ha as 5353 and 4601kg/ha, respectively. Under very severe drought stress, application of 30ton/ha manure is partly superior to 40ton/ha manure. The highest plant height as 52.6 and 52.1cm was achieved in medium stress (FC=50%) by application of 30 and 40ton/ha manure, respectively. The highest internode diameter as 6.8mm was obtained by application of 40ton/ha in mild stress. The highest leaf dry weight as 1614, 1554 and 1257 kg/ha were achieved in mild stress and 40ton/ha manure, mild stress and application of 20ton/ha and medium stress and 40ton/ha (FC=50%), respectively. Investigating effect of irrigation regime and manure application on okra and pepper yield in mix culture, Lawal and Rahman (2007) recommended application of 5 ton/ha manure and irrigation with 10 day interval meaning medium stress. The authors also applied 400kg/ha of fertilizer which is partly similar to our study. Gholizadeh *et al.* (2006) investigated drought stress and zeolite (an alumina-silicate natural substance used for prevention of water loss) on Dracocephalum and reported that concerning dry matter and essential oils of the plant, application of 25gr zeolite (the third level) in 12kg soil together with 50% of moisture depletion resulted in the highest dry matter and 2% essential oils; the results are similar to our result considering similarity between water retention property of both zeolite and manure. Ghanbari *et al.* (2005) investigated irrigation regime and animal manure on *Cumini fructus* and reported that by application of manure, irrigation frequency can be reduced and suitable yield can be achieved which is in agreement with our results.

Conclusions

considering essential oil content as the main goal of dracocephalum production and concerning the fact that oil yield as 13.10, 9.91, 9.91 and 9.91 kg/ha was achieved by interaction of the two factors in mild stress and 40ton/ha manure, mild stress and 30ton/ha, medium stress and 40 ton/ha manure and medium stress and 30ton/ha manure, respectively; the medium drought stress together with application of 30 ton/ha is recommended for Jiroft region as the superior treatment which resulted in production of 9.91kg/ha essential oils.

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