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Research article

EFFECTS OF DROUGHT STRESS AND MANURE ON PLANT GROWTH PROMOTING STABILITY IN DRAGONHEAD (*DRACOCEPHALUM MOLDAVICA*)

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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, Jiroftbranch, in 2009. It was a split plot experiment based on Randomized Complete 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 same trend was observed in dry weight of vegetative body meaning that by increase in drought stress, dry weight of vegetative body was decreased but the difference of mild (FC=75%) and medium (FC=5%) stress on dry vegetative body yield was or non significant. correlation coefficients among the measured traits show that there is strong positive correlation between dry weight of vegetative body of Dracocephalum with vegetative body fresh weight, stem number per plant, plant height, shoot dry weight and leaf dry weight which constitute its components ($p<0.01$), but dry weight of vegetative body of Dracocephalum was negatively correlated with inter node length and there was or non significant correlation between of dry weight of vegetative body and stem diameter. Leaf dry weight had significant and positive correlation with fresh weight of vegetative body, dry weight of vegetative body and plant height and stem number but was or non significantly correlated with diameter and length of stem inter node.
Keywords: Dragonhead, Drought Stress, Manure, shoot dry weight, yield.

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 [1]. Dragon head or dragon's head (*Dracocephalum moldavica*) is herb from mint family [7]. 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 [14]. 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 [9]. 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 [16]. It is more important in regions which experience the problem due to climate change but have not been paid attention [2] 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 [15]. 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 [10].

MATERIALS AND METHODS

To study the effects of manure application on dragonhead and to evaluate its resistance to drought stress as well as to study cell water relations and the physiology 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 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. 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.

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

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). According to Table (2), 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 inter node 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 or non significant on inter node 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 or non significant on stem number and inter node length. It sounds that the changes generated in the tested traits were due to experimental treatments ($p<0.01$ and $p<0.05$).

Table 2: 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 ^{ns}	190237.62 ^{ns}	5.089 ^{ns}	34.73 ^{ns}	1.20 ^{ns}	0.022 ^{ns}	50263.45 ^{ns}	226848.93 ^{ns}
Drought Stress	2	23475660.15*	9838642.95*	5.75 ^{ns}	327.60*	0.52 ^{ns}	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 ^{ns}	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 ^{ns}	88.64*	0.471 ^{ns}	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.

Investigating effect of manure (compost) on Dracocephalum, Husseini et al [7] reported that effect of compost on plant height, stem number and dry and fresh weight was significant, which is in agreement with the results of the present study. Studying effect of drought stress on dracocephalum, Safikhani et al [17] reported that the stress had no significant effect on plant height but had significant effect on leaf and stem yield, which is in accordance with our results. According to Table (3), vegetative part weight is reduced as drought stress increases so that vegetative body yield is 16553kg in mild stress while it is 12425kg in sever stress showing significant difference between the two stress, the difference was about 7128kg. The same trend was observed in dry weight of vegetative body meaning that by increase in drought stress, dry weight of vegetative body was decreased but the difference of mild (FC=75%) and medium (FC=5%) stress on dry vegetative body yield was or non significant. The reduction of vegetative body yield by increase in stress is due to reduction of stem and leaf dry weight, the plant reduces photosynthetic organs in response to dehydration for reducing evaporation which results in reduction of yield under enhanced stress.

Closure of stomata during drought stress hinders CO2 absorption so the photosynthetic materials and thereby yield is reduced. In accordance with our results, Gholizadeh et al [3] investigated effect of drought stress on dracocephalum and reported that by increase in stress level from mild to severe, plant fresh weight was decreased from 2.033gr/plant to 0.8gr/plant, but considering aerial part dry weight there was or non significant difference between sever and medium stress.

Safikhani et al [17] investigated effects of drought stress on yield and morphological traits of dracocephalum and obtained results similar to those achieved in the present study. The authors maintained that by increase in stress level from FC=100% to FC=40%, flowering shoot yield decreased from 4126kg/ha to 2477kg/ha. Flowering shoot yield in medium stress was 3479kg/ha and there was no significant between mild and severe drought concerning flowering shoot yield. In a similar experiment, Hassani [5] reported that fresh material yield among drought treatments was significant and dry material yield of drought stress at FC85%, FC70% and FC50% showed no significant difference in dracocephalum, which accords to our results.

Reduced yield has been reported by Letchamo et al [10] in thyme, Misra and Srivastava [13] in minth, Lebaschi and Sharifi [11] in plantago, yarrow, salvia, chamomile and marigold. Khazaei et al (2008) investigated the effect of irrigation regime as one time per 7, 14 and 21 days on hyssop and thyme and reported that shoot yield of thyme in 7 days irrigation as 1156gr/m2 which was or non significantly different from that of 14 days as 1157gr/m2, but was superior to 21 days irrigation regime (726gr/m2). Hyssop yield in 7 and 14 days irrigation regime were 550.2 and 320.3gr/m2 respectively which, in accordance to our results, were not significantly different from each other.

Table 3: Mean Comparison of Different Manure Cow and Drought Stress on *Dracocephalum moldavica*

	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)
Drought Stress								
Fc 75%	19553a	4868a	12.60a	41.03ab	3.65a	5.06a	1073.6a	2573.4a
Fc 50%	13015b	3624ab	11.73a	46.13a	3.96a	4.33ab	890.2ab	1946.2ab
Fc 25%	12425b	3348b	11.40a	36.80b	3.63a	3.33a	635.9b	1724.2b
LSD	5431	1282	2.05	7.607	1.029	1.173	312.8	630.2
Manure Cow (ton/ha)								
0	4240c	1424c	9c	31.61b	3.70ab	3.94b	365.1c	731.3d
10	10998bc	3081bc	12b	41.77a	4.38a	3.94b	689.5bc	1799.1c
20	17617ab	4591ab	12.22ab	42.33a	3.61ab	4.11b	988ab	2269.9b
30	19558a	4918a	13.88a	44.33a	3.66ab	4.22b	1066.9a	2584.3b
40	22575a	5719a	12.44ab	46.55a	3.38b	5a	1223.3a	3021.7a
LSD	7.24	1718	1.623	6.557	0.81	0.64	337.5	435.1

Effect of drought stress on stem number of dracocephalum was or non significant and as can be seen from Table (3), by increase in drought stress the number of side shoot was reduced so that the highest number of lateral shoot as 12.6 was achieved in mild stress and the lowest number as 11.40 was obtained by severe drought stress.

By increase in drought stress severity, plant height was decreased but there was or non significant difference between mild and medium stress. Drought stress showed or non significant difference in inter node length but the highest inter node length as 3.96cm was achieved in medium stress. The highest stem diameter was obtained by mild stress as 5.06mm which was or non significantly different from 4.33mm obtained by medium stress. By increase in drought stress, leaf weight was reduced from 1073.6kg in mild stress to 635.9kg in sever stress, but dry weight yield in mild stress was not significantly different from that of medium stress which was about 890.2kg/ha.
This was the case for stem dry weight which was reduced as drought stress was increased. The highest stem dry weight obtained in mild stress as 2573.4kg/ha was or non significantly different from that achieved in medium stress as 946kg/ha but was significantly different from stem dry weight in severe stress. One of the first signs of water shortage is reduced turgescence and thereby decreased growth and development of cells especially in stem and leaves. By decrease in cell volume, organ size is limited so the first tangible effect of dehydration can be seen by smaller size of leaves and reduced height of plants. Furthermore, under dehydration condition absorption of nutrients is decreased and hence leaf growth and development is constrained. As a result of reduced leaf area, light absorption and total photosynthetic capacity of plants is reduced. Obviously by limitation of photosynthetic products under water shortage, plant growth and consequently plant yield is reduced. In a similar experiment, Hassani [5] reported reduction of side shoots from 22.47 in non-stress condition to 14.42 in severe stress and reduction of plant height and leaf and stem weight in Dracocephalum which accords to our results. The author maintained that reduced height and stem number is an adaptation mechanism of dracocephalum under water shortage condition. Reduced yield of leaf and stem and diameter and height of plant in dracocephalum under drought condition has also been reported by Safikhani et al [17].
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.

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

Drought Stress	Manure Cow (ton/ha)	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	4060fg	1131h	8.66c	24.50d	3.4bc	6ab	622g	326f
	10	12805cd	3405cdef	12.33abc	44.6ab	5a	3.3de	1882def	781cde
	20	29428a	7275a	13.67a	46.6ab	3.3bc	3.6de	3470ab	1554a
	30	20666b	4956bcd	14.33a	38.5bc	3.5bc	5.5abc	2725bcd	1091bc
	40	30806a	7574a	14a	50.8a	3c	6.8a	4165a	1614a
Fc 50%	0	5040efg	1854fgh	9.33bc	38bc	4abc	3.3de	1176fg	522ef
	10	10885cdef	3018defgh	12.67ab	45.6ab	4abc	4.8bcd	1604ef	767cde
	20	11705cde	3294bcdefg	11abc	42.1abc	4abc	4.5cd	1737ef	804cde
	30	17655bc	4601bcde	13.33a	52.6a	3.6bc	4.6bcd	2428cde	1099bc
	40	19792b	5353b	12.33abc	52.1a	4abc	4.3cd	2784bc	1257ab
Fc 25%	0	3620g	1287gh	9bc	32.3cd	3.6bc	2.5cd	394g	246f
	10	9304defg	2820efgh	11abc	35bcd	4abc	3.6e	1910def	520ef
	20	11720cde	3204cdefg	12abc	38.1bc	3.5bc	4.1cd	1602ef	605def
	30	20353b	5197bc	14a	41.8abc	3.8bc	2.5e	2598cd	1010bcd
	40	17126bc	4231bcde	11abc	36.6bc	3.1bc	3.8de	2115cde	797cde
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

It was or non 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 40 ton/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 inter node 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 [12] 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 [3] 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 [4] 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.
According to Table (5), correlation coefficients among the measured traits show that there is strong positive correlation between dry weight of vegetative body of dracocephalum with vegetative body fresh weight, stem number per plant, plant height, stem dry weight and leaf dry weight which constitute its components ($p<0.01$), but dry weight of vegetative body of dracocephalum was negatively correlated with inter node length and there was or non significant correlation between of dry weight of vegetative body and stem diameter. Leaf dry weight had significant and positive correlation with fresh weight of vegetative body, dry weight of vegetative body and plant height and stem number but was or non significantly correlated with diameter and length of stem inter node.

Table 5: The correlation coefficient for the effects of manure and water deficit stress on Dragonhead plant

	Shoot fresh weight (g)	**No. shoot**	**Plant Height (cm)**	**Inter node Length (cm)**	**Stem Diameter (cm)**	**leaf fresh weight (g)**	**leaf dry weight (g)**	**Shoot dry weight (g)**
Shoot fresh weight (g)	1/0							
No. shoot	0.846^{**}	1.0						
Plant Height (cm)	0.725^{**}	0.722^{**}	1.0					
Inter node Length (cm)	-0.370^{ns}	-0.096^{ns}	0.123^{ns}	1.0				
Stem Diameter (cm)	0.273^{ns}	0.217^{ns}	0.133^{ns}	-0.428^{*}	1.0			
leaf fresh weight (g)	0.978^{**}	0.833^{**}	0.799^{**}	-0.297^{ns}	0.348^{ns}	1.0		
leaf dry weight (g)	0.982^{**}	0.847^{**}	0.727^{**}	-0.225^{ns}	0.345^{ns}	0.965^{*}	1.0	
Shoot dry weight (g)	0.996^{**}	0.849^{**}	0.684^{*}	-0.406^{*}	0.306^{ns}	0.969^{**}	0.974^{*}	1.0

*,** and ns show significance at 5 and 1% and non-significance, respectively.

CONCLUSION

Regarding the importance of dry weight of aerial part (vegetative body) and leaf dry weight of dracocephalum during years with sufficient precipitation or when water is not limiting factor, application of 20ton/ha of animal (cow) manure is recommended for achieving the highest dry yield of vegetative body and dry weight of leaf. But when water is limiting factor, concerning two aforementioned traits, application of 30-40 ton/ha of cow rotten manure is recommended for obtaining acceptable yield. Application of 40ton/ha cow manure in medium drought by providing average dry yield as 1257kg/ha and application of 20ton/ha cow manure in mild stress with average yield of 1554kg/ha was or non significantly different from each other and application of this amount of manure supplies deracocephalum nutrition demands.

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