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Research Article

Changes in Content of Chlorophyll, Carotenoids, Phosphorus and Relative Water Content of Medicinal Plant of Borage (*Borago officinalis* L.) under the Influence of Mycorrhizal Fungi and Water Stress

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Abstract

Background and Objective: Drought is one of the factors that affect medicinal plants and on the other hand, the role of mycorrhizal fungi was effective in improving the performance of some medicinal plants exposed to water stress. For this purpose, an experiment was conducted as split-plot in randomized complete block design with 3 replications in the Boyerahmad region at years 2015 and 2016. **Materials and Methods:** The experiment factors were considered of irrigation levels (main-plot) as irrigation after $S_1 = 30$, $S_2 = 60$, $S_3 = 90$, $S_4 = 120$ and $S_5 = 150$ mm water evaporation from evaporation pan class A and mycorrhiza fungi (sub-plot) were considered at the levels of non application (NM), application with mycorrhiza fungi species of *Glomus mosseae* (GM) and *Glomus intraradices* (GI). **Results:** Results showed that the effect of year on the studied traits of borage was not significant but the interaction of water stress and mycorrhizal fungi on chlorophyll a, chlorophyll b, total chlorophyll, carotenoids, flowers phosphorus, grain phosphorous and leaf relative water content of borage were significant. Irrigation after 30 and 60 mm evaporation from pan evaporation+without and the use of mycorrhizal fungi of water *Glomus mosseae* and *Glomus intraradices* proportion to irrigation after 150 mm evaporation from pan evaporation+without application and the use of mycorrhizal fungi increased studied characteristic of borage. **Conclusion:** The results of this study was showing to decrease in the properties of borage medicinal plant as a result of drought stress but the application of mycorrhizal fungi could increase borage traits in drought stress conditions in the study and decrease of the negative effects of drought stress.

Key words: Water stress, phosphorus, carotenoids, chlorophyll, borage, mycorrhiza

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Borage (*Borago officinalis* L.) is an annual plant that has multiple properties of medicinal, industrial and due to the many different compounds is used in traditional medicine¹. Among the environmental factors hindering the growth and yield of plants of agronomic, horticultural and medicinal, drought considered the most important factor of production decline, especially in arid and semi arid areas². In stress condition, mycorrhiza fungi causes accounted more carbon for root plant and therefore, increase root growth and expansion of its length due to the increase in surface area of root with soil and thus increase absorb of water and nutrients in the root discharge area³.

Chlorophyll is one of the major components of chloroplasts for photosynthesis. Reduction in the chlorophyll content under drought stress is intended as a clear indication of light oxidation of pigment and decomposition of chlorophyll⁴. The severity of drought stress, mycorrhizal fungi by increasing the percentage of colonization and photosynthetic pigments can be effective in reducing the effects of drought and increased grain yield in plant fenugreek⁵. Drought stress had significant effect on chlorophyll basil (*Ocimum basilicum* L.), so that by reducing soil moisture content, chlorophyll content decreased. The effect of fungi *G. mosseae* in reducing the impact of drought was more than *G. intraradices*⁶.

Stress condition reduces uptake of element especially phosphorus and decreases transfer of this material to the organs and vessels (seed) and thus reduces the photosynthetic material⁷. Mycorrhizal fungi hyphae can penetrate to tiny pores that even the hairs not able to enter to tiny pores and are increasing the amount of water absorption⁸. However, the results of one study showed that the effect of water stress and mycorrhizal fungus on Relative Water Content (RWC) of plant pumpkin was significant and increasing of drought stress was decreased relative water content but mycorrhizal fungi increase the plant's RWC⁹.

Borage is a medicinal plant that because of its health benefits and good taste greatly used among people of many different countries. The most important challenge in producing this plant is lack of awareness of how cultivate, also the effect of important environmental parameters on this plant. Since, drought is one of the factors affecting on agricultural development of Kohgiluyeh and Boyerahmad region and also because of the effective role of mycorrhizal fungi in improving the yield of some plants under water stress was conducted this study in the Boyerahmad region.

MATERIALS AND METHODS

Method of testing, type of plan and time of testing: The experiment conducted as split-plot in randomized complete block design with 3 replications in the Boyerahmad region at years 2015 and 2016. The experiment factors were considered of irrigation levels (main-plot) as irrigation after $S_1 = 30$, $S_2 = 60$, $S_3 = 90$, $S_4 = 120$ and $S_5 = 150$ mm water evaporation from evaporation pan class A and mycorrhiza fungi (sub-plot) were considered at the levels of non application (NM), application with mycorrhiza fungi species of *Glomus mosseae* (GM) and *Glomus intraradices* (GI). After plowing and seedbed preparation, plots were obtained with dimensions of 3×5 m. The distance between the experimental main plots of 3 m, subplots 1 m and distance between repetitions 3 m were considered. Before sowing the seeds of borage, done test soil (Table 1) and about 7 g of inoculants containing mycorrhizal fungal spores (clinic production of organic plant protection of Asadabad in Hamadan with registration number 27.1554) were cast in each hole of borage planting¹⁰.

Borage seed were planting in the first half of April and with plant spacing between row and on row 50×30 cm¹¹. Planting operations were performed manually and as furrow and stack. Until the emergence, irrigation was performed once in every 2 days. After germination and seedling establishment (trifoliolate stage), thinning and weed control operations were carried out. Then the irrigation treatments were applied.

Measurement of chlorophyll and carotenoids: The content of chlorophyll a, chlorophyll b, total chlorophyll and carotenoid (at 50% flowering time) were measured from leaves near flowering shoot of borage. For this purpose, (1) The value of half a gram of fresh material was poured in a porcelain mortar, then split using 95% ethanol and was crushed as well. (2) 20 mL acetone 80% added to the sample, then was placed in centrifuge set (6,000 rpm) for 10 min. The

Table 1: Soil test results

Characteristics	Characteristics	Characteristics	Characteristics
Depth (cm)	0-30	Clay (%)	26
Electrical conduction (dS m ⁻¹)	0.4	Sand (%)	28
Total saturation acidity (pH)	7.9	Soil texture	Loam
Neutralized (%)	47	Cu able absorption (ppm)	2
Materials TNV (%)		Mn able absorption (ppm)	14.4
Organic carbon (%)	0.9	Fe able absorption (ppm)	13
Total nitrogen (%)	0/09	Zn able absorption (ppm)	2.7
P able absorption (ppm)	10		
K able absorption (ppm)	206		

upper isolated extract from the centrifuge was transferred to a glass balloons. (3) The number of samples inside the balloon was poured in a cuvette spectrophotometer and then separately absorbance rate was read at wavelengths of 663 nm for chlorophyll a and 645 nm for chlorophyll b and 470 nm for carotenoids by spectrophotometric model UV/VIS 911. (4) Finally, using the following formula chlorophyll a, b, total chlorophyll and carotenoids according milligrams per gram of samples fresh weight were obtained¹²:

$$\text{Chlorophyll a} = (19.3 \times A_{663} - 0.86 \times A_{645}) V / 100W$$

$$\text{Chlorophyll b} = (19.3 \times A_{645} - 3.6 \times A_{663}) V / 100W$$

$$\text{Carotenoides} = 100 (A_{470} - 3.27 (\text{mg chl. a}) - 104 (\text{mg chl. b}) / 227$$

$$\text{Total chlorophyll} = \text{Chlorophyll a} + \text{Chlorophyll b}$$

Where:

V = Volume of filtrate (upper solution of centrifuges)

A = Absorption of light at wavelengths of 663, 645 and 470 nm

W = Sample fresh weight in grams

Measurement of Relative Water Content (RWC): Measuring the relative water content (RWC) was performed by Ritchie *et al.*¹³:

$$\text{RWC} = \frac{F_w - D_w}{S_w - D_w} \times 100$$

Where:

F_w = Fresh weight immediately after sampling

D_w = Leaf dry weight after being in the oven

S_w = Saturated weight of leaf after being indistilled water

Measurement of phosphorus: For measurement phosphorus content of flowers at 100% flowering time and phosphorus content of seed (at the end of plant growth) was used to plant ash. To conduct this study, in first a few drops of distilled water was poured on the ashes of one gram of dry matter into the Chinese jar. Then in order to solve the plant ash was added to it 2 mL nitric acid 1:2 and was worn with a nice glassy rods. The resulting solution was flat through a filter paper into a balloon 100 mL inside the jars washed with a few milliliter of distilled water and the solution was poured on filter paper and was added to the acidic solution of ash inside the balloons. The resulting solution was neutral with ammonia 1:1, then 5 mL nitric acid 1:2 and 15 mL represents vanadate molybdate were added to it and the final volume of solution

was brought to 100 mL with distilled water. The above solution absorption was measured at wavelength 450 nm by a spectrophotometer model UV/VIS 911. Also, standard solutions absorption was measured at wavelength 450 nm. Control for sample solution of ash and standard solution is standard solution 0 ppm. For draw a standard curve, obtained numbers from absorbance of standard solution was used on y-axis and also obtained numbers from absorbance of the standard solution concentration was used on the x-axis and standard curves were plotted, then with the help of standard curve was determined the amount of plant samples phosphorus in according to parts per million (ppm)¹⁴.

Data analysis: Data analysis using the software MSTATC and comparison of means by Duncan's multiple range test was performed.

RESULTS

Chlorophyll a, chlorophyll b, total chlorophyll and carotenoids: Results showed that the effect of year on the studied traits of borage (chlorophyll a, chlorophyll b, total chlorophyll and carotenoids). The interaction of water stress and mycorrhizal fungi on chlorophyll a of borage also was significant in probability level 1% (Table 2). Irrigation after 30 and 60 mm evaporation from pan evaporation+without and the use of mycorrhizal fungi of water *Glomus mosseae* (GM) and *Glomus intraradices* (GI) proportion to irrigation after 150 mm evaporation from pan evaporation+without application and the use of mycorrhizal fungi increased chlorophyll a about 58-57% (Table 3). The interaction of water stress and mycorrhizal fungi on chlorophyll b of borage also was significant in probability level 1% (Table 2). chlorophyll b increased about 73-75% by treatments of irrigation after 30 and 60 mm evaporation from pan evaporation+without application and the use of mycorrhizal fungi of water *Glomus mosseae* (GM) and *Glomus intraradices* (GI) proportion to irrigation after 150 mm evaporation from pan evaporation+without application and the use of mycorrhizal fungi (Table 3). The interaction of water stress and mycorrhizal fungi on total chlorophyll of borage also was significant in probability level 1% (Table 2). Irrigation after 30 and 60 mm evaporation from pan evaporation+without and the use of mycorrhizal fungi of water *Glomus mosseae* (GM) and *Glomus intraradices* (GI) proportion to irrigation after 150 mm evaporation from pan evaporation+without application and the use of mycorrhizal fungi increased total chlorophyll about 63-64% (Table 3). The interaction of water stress and

Table 2: Statistical results of the effect of water stress and mycorrhizal fungi on chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of borage

Source of variations	Mean squares				
	Degree of freedom	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids
Year	1	1.518 ^{ns}	0.500 ^{ns}	4.087 ^{ns}	0.335 ^{ns}
Year × replication	2	1.404 ^{ns}	0.678 ^{ns}	3.960 ^{ns}	0.122 ^{ns}
Water stress	3	72.562**	23.254**	171.451**	4.161**
Water stress × year	4	0.041 ^{ns}	0.049 ^{ns}	0.064 ^{ns}	0.017 ^{ns}
Error	16	0.447	0.078	0.539	0.028
Mycorrhizae fungi	2	24.397**	6.781**	57.298**	1.077**
Year × mycorrhizae fungi	2	0.108 ^{ns}	0.046 ^{ns}	0.331 ^{ns}	0.019 ^{ns}
Water stress × mycorrhizae fungi	8	3.718**	1.357**	6.232**	0.388**
Year × water stress × mycorrhizae fungi	8	0.037 ^{ns}	0.022 ^{ns}	0.029 ^{ns}	0.015 ^{ns}
Error	16	0.547	0.259	1.372	0.068
CV (%)		10.050	13.790	1.620	16.270

*, **Significant level at 0.5 and 0.1% and ns: No significant

Table 3: Comparison of the effect of the interaction between water stress and mycorrhizal fungi on chlorophyll a, chlorophyll b, total chlorophyll and carotenoids of borage

Treatments of water stress+mycorrhizae fungi	Chlorophyll a	Chlorophyll b	Total chlorophyll	Carotenoids
	(mg g ⁻¹)			
S ₃₀ NG	9.166 ^a	4.721 ^a	13.83 ^{ab}	2.019 ^{ab}
S ₃₀ GM	9.476 ^a	4.842 ^a	14.30 ^a	2.133 ^a
S ₃₀ GI	9.347 ^a	4.833 ^a	14.15 ^a	2.016 ^{ab}
S ₆₀ NG	8.580 ^{ab}	4.884 ^a	13.42 ^{abc}	2.241 ^a
S ₆₀ GM	9.412 ^a	5.050 ^a	14.45 ^a	2.158 ^a
S ₆₀ GI	9.211 ^a	4.936 ^a	14.10 ^a	2.016 ^{ab}
S ₉₀ NG	5.430 ^c	3.028 ^b	8.512 ^e	1.455 ^c
S ₉₀ GM	8.450 ^{ab}	3.950 ^b	12.02 ^{bcd}	1.581 ^{bc}
S ₉₀ GI	8.335 ^{ab}	3.554 ^b	11.87 ^{cd}	1.589 ^{bc}
S ₁₂₀ NG	4.507 ^{cd}	1.788 ^c	6.267 ^{fg}	0.717 ^d
S ₁₂₀ GM	7.610 ^b	3.363 ^b	10.96 ^d	1.469 ^c
S ₁₂₀ GI	7.609 ^b	3.202 ^b	10.80 ^d	1.405 ^c
S ₁₅₀ NG	3.908 ^d	1.237 ^c	5.147 ^g	0.5267 ^d
S ₁₅₀ GM	4.747 ^{cd}	3.223 ^b	7.970 ^{ef}	1.433 ^c
S ₁₅₀ GI	4.570 ^{cd}	3.0167 ^b	7.585 ^{ef}	1.307 ^c

S: Water stress, GM: *G. mosseae*, GI: *G. intraradices*, NG: Non glomus

Table 4: Statistical results of the effect of water stress and mycorrhizal fungi on flowers phosphorus, grain phosphorus and relative water content of borage

Source of variations	Mean squares			
	Degree of freedom	Flowers phosphorus	Grain phosphorus	Leaf relative water content
Year	1	6246.023 ^{ns}	1275.205 ^{ns}	113.075 ^{ns}
Year × replication	2	26766.116 ^{ns}	25732.270 ^{ns}	25.988 ^{ns}
Water stress	3	140691.705**	133149.725**	2951.033**
Water stress × year	4	46.929 ^{nsns}	861.051	7.459 ^{ns}
Error	16	3216.837	13715.161	39.603
Mycorrhizae fungi	2	31844.486**	37341.439*	898.026**
Year × mycorrhizae fungi	2	1356.055 ^{ns}	3633.815 ^{ns}	33.055 ^{ns}
Water stress × mycorrhizae fungi	8	1689.167**	2467.791**	39.301**
Year × water stress × mycorrhizae fungi	8	22.148 ^{ns}	243.936 ^{ns}	2.504 ^{ns}
Error	16	3293.461	9826.825	62.720
CV (%)		30.18	43.49	12.04

*, **Significant level at 0.5 and 0.1% and ns: No significant

mycorrhizal fungi on carotenoids of borage also was significant in probability level 1% (Table 2). The carotenoids increased about 73-76% by treatments of irrigation after 30 and 60 mm evaporation from pan evaporation+without and the use of mycorrhizal fungi of water *Glomus mosseae* (GM) and *Glomus intraradices* (GI) proportion to irrigation

after 150 mm evaporation from pan evaporation+without application and the use of mycorrhizal fungi (Table 3).

Flowers phosphorus and grain phosphorus: Results showed that the effect of year on the studied traits of flowers phosphorus and grain phosphorus was not significant

Table 5: Comparison of the effect of the interaction between water stress and mycorrhizal fungi on flowers phosphorus, grain phosphorous and relative water content of borage

Treatments of water stress+mycorrhizae fungi	Flowers phosphorus (ppm)	Grain phosphorous (ppm)	Leaf relative water content (%)
S ₃₀ NG	268.7 ^{ab}	305.1 ^a	78.40 ^a
S ₃₀ GM	298.8 ^a	334.9 ^a	81.08 ^a
S ₃₀ GI	284.7 ^{ab}	320.4 ^a	80.15 ^a
S ₆₀ NG	255.4 ^{abc}	290.3 ^a	68.65 ^{ab}
S ₆₀ GM	294.2 ^a	328.9 ^a	80.52 ^a
S ₆₀ GI	278.6 ^{ab}	313.6 ^a	78.15 ^a
S ₉₀ NG	145.7 ^{de}	182.7 ^{abc}	60.14 ^{bc}
S ₉₀ GM	206.1 ^{abcd}	254.7 ^{ab}	70.22 ^{ab}
S ₉₀ GI	187.6 ^{bcd}	217.6 ^{abc}	68.33 ^{ab}
S ₁₂₀ NG	63.68 ^{ef}	93.04 ^{bc}	49.97 ^{cd}
S ₁₂₀ GM	163.7 ^{cd}	200.3 ^{abc}	63.35 ^{bc}
S ₁₂₀ GI	139.7 ^{de}	183.6 ^{abc}	61.69 ^{bc}
S ₁₅₀ NG	35.30 ^f	72.21 ^c	40.20 ^d
S ₁₅₀ GM	120.5 ^{def}	166.3 ^{abc}	53.80 ^c
S ₁₅₀ GI	109.3 ^{def}	155.2 ^{abc}	51.53 ^{cd}

S: Water stress, GM: *G. mosseae*, GI: *G. intraradices*, NG: Non glomus

(Table 4). The interaction of water stress and mycorrhizal fungi on flowers phosphorus of borage also was significant in probability level 1% (Table 4). Irrigation after 30 and 60 mm evaporation from pan evaporation+without and the use of mycorrhizal fungi of water *Glomus mosseae* (GM) and *Glomus intraradices* (GI) proportion to irrigation after 150 mm evaporation from pan evaporation+application of mycorrhizal fungi increased flowers phosphorus about 87-88% (Table 5). The interaction of water stress and mycorrhizal fungi on grain phosphorous of borage also was significant in probability level 1% (Table 4). Grain phosphorous increased about 75-78% by treatments of irrigation after 30 and 60 mm evaporation from pan evaporation+without and the use of mycorrhizal fungi of water *Glomus mosseae* (GM) and *Glomus intraradices* (GI) proportion to irrigation after 150 mm evaporation from pan evaporation+without application and the use of mycorrhizal fungi (Table 5).

Relative water content: Results showed that the effect of year on the relative water content of leaf was not significant (Table 4). The interaction of water stress and mycorrhizal fungi on relative water content of borage leaf also was significant in probability level 1% (Table 4). Irrigation after 30 and 60 mm evaporation from pan evaporation+without and the use of mycorrhizal fungi of water *Glomus mosseae* (GM) and *Glomus intraradices* (GI) proportion to irrigation after 150 mm evaporation from pan evaporation+application of mycorrhizal fungi increased relative water content about 55% (Table 5).

DISCUSSION

Drought is one of the factors influencing medicinal plants and on the other hand, the role of mycorrhizal fungi in improving the yield of some medicinal plants exposed to

water stress was effective. As ecological and physiological studies also have demonstrated that mycorrhizal symbiosis preserves plants in contrast of stress by avoiding of drought stress. Chlorophyll is one of the major components of chloroplasts for photosynthesis. Reduction in the chlorophyll content under drought stress is intended as a clear indication of light oxidation of pigment and decomposition of chlorophyll⁴. The severity of drought stress, mycorrhizal fungi by increasing the percentage of colonization and photosynthetic pigments can be effective in reducing the effects of drought and increased grain yield in plant fenugreek⁵. Also in this study mycorrhizal fungi increased chlorophyll of borage in the condition of water stress. In another study, drought stress had significant effect on chlorophyll basil (*Ocimum basilicum* L.) so that by reducing soil moisture content, chlorophyll content decreased. The effect of fungi *G. mosseae* in reducing the impact of drought was more than *G. intraradices*⁶. It was shown that corn plants were inoculated with the fungus *G. mosseae* compared with non-inoculated plants with fungi, increased chlorophyll content in normal conditions and water shortages¹⁵. In another report, effect mycorrhizal fungi on chlorophyll a, chlorophyll b and carotenoids fennel (*Foeniculum vulgare* Mill.), was significant in probability level 1% and cause improves these traits¹⁶.

Stress condition reduces uptake of element especially phosphorus and decreases transfer of this material to the organs and vessels (seed) and thus reduces the photosynthetic material¹⁵. Likely the use of bio-fertilizers had to the exacerbate effects on soil microbial activity and subsequently with increasing readily available of element phosphorus in the soil for plants as well as creation balance between these elements with physical and chemical phase of soil had improved yield of medicinal plants of *Calendula*¹⁷.

Also in present study with increasing drought stress reduced flowers phosphorus and seeds phosphorus of borage and application of the mycorrhizal fungi decreased severe of water stress.

Mycorrhizal fungi hyphae can be penetrate to tiny pores that even the hairs not able to enter to tiny pores and are increasing the amount of water absorption⁸. Also in this study, mycorrhizal fungi increased Relative Water Content (RWC) of borage in the condition of water stress. In a study, water stress decreased the relative water content (*Origanum vulgare* L.), while inoculation with mycorrhizal fungi increases the relative water content¹⁸. However, the results of one study showed that the effect of water stress and mycorrhizal fungus on Relative Water Content (RWC) of plant pumpkin was significant and increasing of drought stress was decreased relative water content but mycorrhizal fungi increase this plant's RWC⁹. In this study, the interaction of water stress and mycorrhizal fungi created significant changes in chlorophyll a, chlorophyll b, total chlorophyll and carotenoid, relative water content, flowers and seeds phosphorus of borage and with increase of water stress decreased this traits but the application of mycorrhizal fungi could increase studied characteristics of borage in drought stress conditions and reduce the negative effects of stress in this study.

CONCLUSION

Drought is one of the factors influencing medicinal plants and on the other hand, the role of mycorrhizal fungi in improving the yield of some medicinal plants exposed to water stress was effective. As ecological and physiological studies also have demonstrated that mycorrhizal symbiosis preserves plants in contrast of stress by avoiding of drought stress. Of course in this study, the interaction of water stress and mycorrhizal fungi created significant changes in chlorophyll a, chlorophyll b, total chlorophyll and carotenoid, relative water content, flowers and seeds phosphorus of borage and with increase of water stress decreased this traits but the application of mycorrhizal fungi reduce the negative effects of water stress but the application of mycorrhizal fungi could increase studied characteristics of borage in drought stress conditions and reduce the negative effects of stress in this study.

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