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Evaluation of the impact of drought stress levels on yield and morphological traits of borage (*Borago officinalis* L)

Masoud Radmanesh

Department of Agriculture and plant breeding, Mazandaran, **Iran**

***Correspondence:** Masoud_rad0000000@yahoo.com **Received 01-01-2022, Revised: 22-03-2022, Accepted: 24-03-2022 e-Published: 25-03-2022**

Cultivation of medicinal herbs in the country can play an essential part in ensuring public health and employment and preventing the genetic erosion of valuable medicinal plant species due to unprincipled harvesting from their natural habitat as well as non-oil export. Thus, the present experiment was carried out in 2020-2021 on a farm located in Namin city seeking to evaluate the impact of drought stress levels on the yield and morphological traits of borage (Borago officinalis L). The experiment was conducted in completely randomized blocks with three iterations. The treatments included three levels of drought stress including a1: control (no stress), a2: normal stress (irrigation at 70% FC), and a3: intense stress (irrigation at 40% stress). The traits evaluated in the present study included the number of leaves, plant height, the number of flowers, the number of branches, wet yield, dry yield, and dry flower yield. Results indicated that the studied variables differed significantly in terms of all the studied traits at 1% and 5% significance levels. Results revealed that treatment with 40% and 70% FC irrigation resulted in a (15.1 and 24.57%) and (17.16 and 21.12%) decline in wet and dry yields and a (5.12 and 18.71%) and (7.44 and 18.32%) decline in wet and dry flower yields, respectively. in other words, drought stress left a significant impact on the traits, indicating that borage is sensitive to water deficiency and suffers from reduced growth manifested through reduced plant height, wet weight, dry weight, and flower yield under drought conditions.

Keywords: medicinal herbs, borage, drought tension, morphological traits

INTRODUCTION

Borage is an annual plant native to the Mediterranean regions with an extremely growing market over the recent years along with other similar plants (Laurence, 2004, Bi et al. 2021, Gholamin and Khayatnezhad, 2020a, Hou et al. 2021). The vegetative organs of borage contain saponin, tannins, mucilage, considerable amounts of minerals, and small amounts of essential oils (Gholinezhad et al. 2016, Khayatnezhad and Gholamin, 2020b, Sun and Khayatnezhad, 2021, Wang et al. 2022b, Zhu et al. 2021a). Borage is known for its beneficial impacts on the mind, anti-eczema effects, and livelihood effects. Its leaves and stems are diuretic and are used to relieve irritated and damaged tissue. Its medicinal properties include being a milk enhancer adrenal repairer, soothing, sedative, emollient, and mild curing effects (Gholinezhad et al. 2016, Chen et al. 2021, Cheng et al. 2021, Gholamin and Khayatnezhad, 2020b, Sun et al. 2021). Borage is antiperspirant and anti-inflammatory (Abdi, 2021, Alizadeh, 2021a, Karasakal, 2021a, Mohammadzadeh, 2021b). This plant is an annual herbaceous herb from the borage genus with a height of 11-40cm and blue or rarely white and pink flowers. Early spring is the best time to cultivate this plant, but it can be cultivated in fall and later winter as well depending on the environmental conditions (Firuzi et al. 2010, Gholamin and Khayatnezhad, 2020c, Huang et al. 2021, Li et al. 2021, Ma et al. 2021a).

Plant growth is under the influence of various factors. Environmental factors often have a direct impact on the growth of plants. Water is the most significant factor influencing plant growth (Ramezani et al. 2016, Karasakal et al. 2020b, Khayatnezhad and Gholamin, 2021a, Wang et al. 2021, Zhu et al. 2021b). Among various environmental stresses, the damage done to crops by salinity, drought, and temperature stress is more prevalent across the world and has thus been studied more frequently (Alizadeh, 2021b, Karasakal, 2021b, Mohammadzadeh, 2021a, Radmanesh, 2021b). Water deficiency is among the most important crop limitations in semi-arid and arid regions (Gholinezhad et al. 2016). In addition to reducing the water content in plant tissues, drought stress limits growth and changes some of the metabolic and physiological of the plants (Khayatnezhad and Gholamin, 2020a, Zhang et al. 2021, Zheng et al. 2021). on the other hand, the availability of various nutrients are under the significant impact of stress (Wang et al. 2022a). Hence, plant water management under stress conditions is among the important issues in crop production (Xu et al. 2021).

Juan et al. (2021) have reported various genotypic changes in different plants in response to drought (Yin et al. 2021). However, the response of aromatic and therapeutic herbs to drought conditions is unknown (Ren and Khayatnezhad, 2021, Si et al. 2020, Tao et al. 2021). An experiment conducted on the two lemongrass genera including C. pendulous and Cymbopogon nardus indicated a significant decline in plant height, leaf area, leaf length, and weight under mild and intense drought treatments (Gholamin and Khayatnezhad, 2020d). In the case of therapeutic herbs that need full reproductive and vegetative growth to produce active contents, drought stress would reduce their active contents and quality (Guo et al. 2021). The present study seeks to evaluate the impact of drought stress levels on the yield and morphological traits of borage (Borago officinalis L).

MATERIALS AND METHODS

The present experiment was carried out in 2020-2021 on a farm located in Namin city seeking to evaluate the impact of drought stress levels on the yield and morphological traits of borage (Borago officinalis L). Namin city is the capital of Namin County, Ardebil province, and is situated at 48 degrees and 29 minutes of longitude, 38 degrees and 25 minutes of latitude, and an altitude of 1,700 meters above the sea level. Namin is a mountainous area, and Namin Chay river passes through the city. Each plot was sampled before cultivation and after harvest to determine the specification of the studied soil. After the initial stages of drying, pounding, and sieving, samples were transferred to the library. Results of chemical soil analysis indicated that the soil at the experiment location was clay, electrically conductive, had a saturation extract of 9.6 dS/m, and a PH of 8.1. Various studies have revealed the suitable PH for horticultural borage to be 4.3-8.3 (Gholinezhad et al. 2016). Table 1 demonstrates the physio-chemical specifications of the soil.

After soil and tillage, one week before cultivation including plowing, discs, etc., the land considered for the cultivation was divided into 2*2m plots based on the cultivation plan. The plots were situated 0.5m away from one another and the blocks had a distance of 8m between them. The amount of each fertilizer was measured before cultivation and the fertilizer was then mixed with the soil. Manual cultivation was eventually carried out on 3/1/2020. The seeds used in the study were acquired from reliable sources in Ardebil. Planting rows were 60cm away from one another and the plants in each row had a distance of 30cm between them. The experiment was conducted on completely randomized blocks with three iterations. The treatment included three drought stress levels of a1: control (no stress), a2: normal stress (irrigation at 70% field capacity), and a3: severe stress (irrigation at 40% field capacity). The maintenance after cultivation included weeding, irrigating, and tillage. Superficial irrigation was performed daily and regularly until germination. The seeds started germination around 20 days after cultivation. Given that the early growth of borage plants is slow, manual weeding was sufficed to at the early stages. The plants were pruned at the four-leaf stage. The drought treatment was applied after complete plant establishment and pruning. A TDR (Time Domain Reflectometer) device was used to measure soil moisture content and plot irrigation would be performed whenever the soil moisture dropped to the specific amounts. Irrigation for each stress level was performed every four days, so the control treatment would be irrigated every four days, the 70%FC treatment would be irrigated every eight days, and the 40%FC treatment would be irrigated every 12 days. The present study then evaluated the qualitative traits and concentration of high-consumption nutrients in plants. To measure proline, 0.4g of wet leaves were pounded with 12cc 3% sulfosalicylic acid. 2cc of the result was then mixed with 2cc ninhydrin acid and 2cc glycolic acetic acid and stored in a bain-marie bath for an hour. 4cc of C- toluene was then added and the result was read in a spectrophotometer at nm. The proline content was then obtained in micromoles per gram of wet weight from the standard table (Bates et al. 1973). Carbohydrate content was measured using the method proposed by Keles and Oncle (2004) and obtained from the standard table in micromoles per gram of wet weight. A Kjeldahl device was used to measure protein and nitrogen contents. To measure protein, total nitrogen was multiplied by 6.25 (Kejeldahl, 1998). Dry incineration was used to measure potassium, sodium, and phosphorus contents (Imami, 1996). SPSS-22 and MSTAT-C statistical software were used to conduct the statistical analysis and Excel software was used to draw the diagrams. The mean comparison was carried out using the Least Significant Difference (LSD) test.

Table 1: the physio-chemical specifications of the soil at a depth of 0-30cm

Soil Texture	Sand	Clay	Silt	So	Po	Ph	Ni	Organic matter	Apparent weight	PH	Ec
	%	%	%	ppm	ppm	ppm	%	%	g/cm^3		Ds/m
Clay	31.9	47	19.8	422	8.5	9.22	0.04	0.78	1.28	8.1	1.4

RESULTS AND DISCUSSION

Results of variance analysis on the studied traits (Table 2) revealed that the studied treatments were significantly different at 1% and 5% significance levels in terms of all traits. In other words, drought stress impacted the studied traits significantly.

Plant height is a genetic trait influenced by the environment (Peng et al. 2021). Results of comparing plant height for the drought treatment (Figure 1) indicated that the highest mean plant height was observed in the control treatment at 33.33cm, while the lowest plant height was observed in the 40%FC treatment at 21.2cm. Results revealed that the 40% and 70%FC treatments led to a 20.75% and 36.39% decline in plant height compared to the control, respectively (Table 2).

A study of the impacts of various irrigation regimes in Plantago ovata revealed that plant height declined significantly as the intervals between irrigation increased (Gholamin and Khayatnezhad, 2021). A study of the influence of various irrigation levels on rosemary and mint indicated that plant height would decline as a result of increased drought stress (Jia et al. 2020). Davis et al (1983) demonstrated that plant height in anise declined significantly as the water available to the plant was reduced (Davies and Van Volkenburgh, 1983). Comparison of the mean number of leaves for drought stress treatments (Figure 2) revealed that the largest number of leaves was observed in the control treatment at 55.8, while the smallest number of leaves were observed in the 40%FC treatment at 44.97. Results suggested that the 40% and 70% FC treatments led to a 13.82% and 19.41% decline in the number of leaves, respectively.

Table 2: results of variance analysis of the studied traits

S.O.V	df	Mean of Square							
		Plant height	Number of leaves	Number of branches	Number of flowers	Wet yield	Dry yield	Wet flower yield	Dry flower yield
Rep	2	3.498	13.69*	0.506	5.512	110.2**	0.287	35921*	200.4*
Drought stress levels	2	111.08**	93.28**	1.723**	50.15**	522.23**	5.863*	264297.9**	5936.8**
Error	4	19.57	5083.58	0.337	2.683	2.439	0.091	0.955	1.413
C.V.%		16.36	14.5	5.58	2.22	1.69	2.27	1.03	0.26

*, ** Significant at the level of five and one percent probability

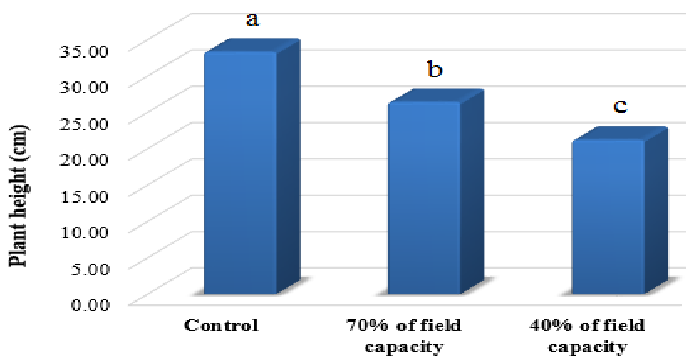

Figure 1: mean impact of drought stress on plant height in borage medicinal herb

The presence of different letters indicates a significant difference by Duncan test at the level of 5% probability

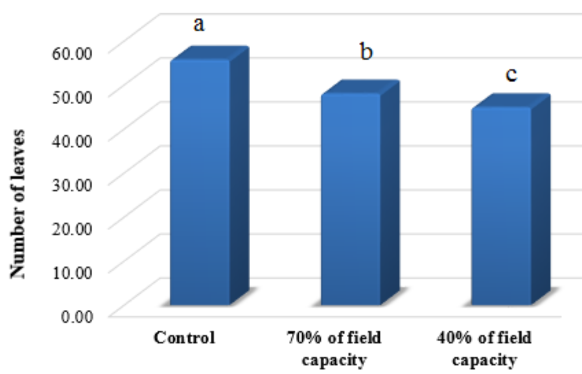

Figure 2: mean impact of drought stress on the number of leaves in borage medicinal herb

The presence of different letters indicates a significant difference by Duncan test at the level of 5% probability

The plant loses less water due to perspiration as a result of the reduced number of leaves depending on the drought severity (Karasakal et al. 2020a). Zehtab Salmasi (2001) demonstrated that the number of leaves declined significantly in anise as the water available to the plant was reduced (Zehtab-Salmasi et al. 2001). A study of the influence of drought levels on the number of branches found that the largest number of branches were observed in the control treatment at 10.98, while the 70%FC treatment had the smallest number of branches (Figure 3). A large number of branches under drought conditions is considered an unfavorable trait since it wastes soil moisture (Khayatnezhad and Gholamin, 2021b). Results indicated that 40% and 70% FC treatments resulted in a 13.2% and 3.13% decline in the number of branches, respectively (Table 2).

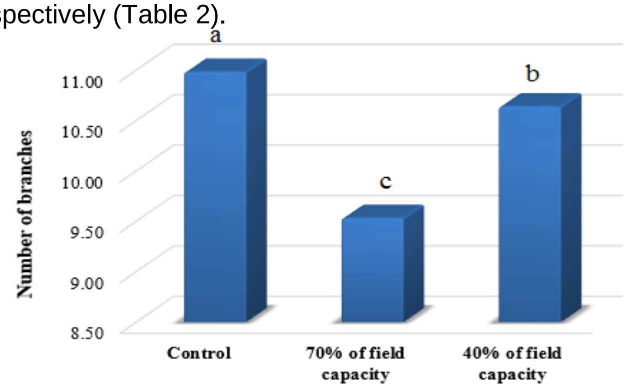

Figure 3: mean impact of drought stress on the number of branches in borage medicinal herb

The presence of different letters indicates a significant difference by Duncan test at the level of 5% probability

Applying drought stress reduced the number of flowers per plant. Comparing the mean number of flowers in various drought stress treatments (Figure 4) revealed that the largest number of flowers was observed in the control treatment at 78.07, while the smallest number was observed in the 40%FC treatment at 70.35. Zehtab Salmasi (2001) demonstrated that the number of flowers declined significantly in anise as the water available to the plant was reduced. Results suggested that 40% and 70% treatments resulted in a 7.95% and 9.88% decline in the number of flowers, respectively (Table 2).

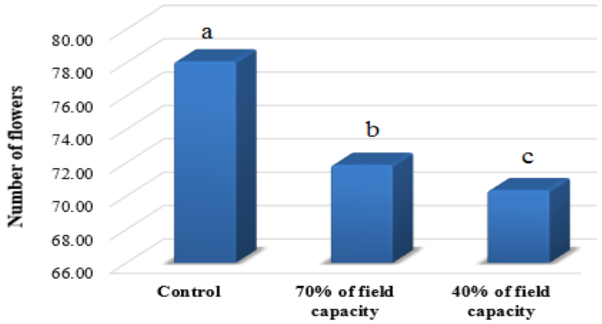

Figure 4: mean impact of drought stress on the number of flowers in borage medicinal herb

The presence of different letters indicates a significant difference by Duncan test at the level of 5% probability

Wet yield reduced as a result of drought stress application. Results of mean wet yield for drought stress treatments (Figure 5) revealed that the highest wet yield was observed in the control treatment at 106.47ton/ha, and the lowest wet yield was observed in the 40%FC treatment at 81.31 ton/ha.

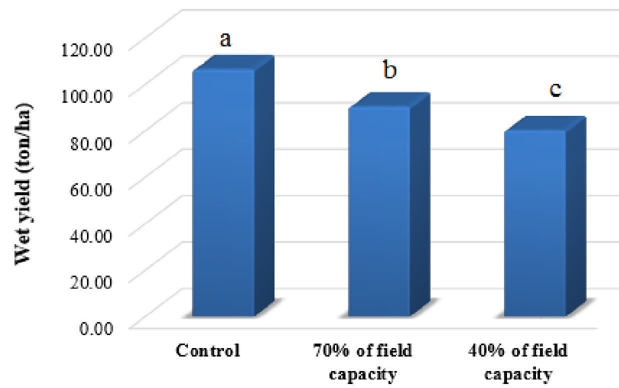

Figure 5: mean impact of drought stress on wet yield in borage medicinal herb.

The presence of different letters indicates a significant difference by Duncan test at the level of 5% probability

Zehtab Salmasi (2001) reported a significant relationship between the water available to plant in anise and harvest index and grain yield. The reduced yield of plants under drought stress can be expressed through the three general mechanisms of reduced active photosynthetic radiation absorption, reduced light consumption efficiency, and reduced carbon dioxide exchange per unit of the absorbed light (Khayatnezhad and Nasehi, 2021). The study of the influence of drought levels on mean dry yield revealed that the control treatment (no stress) produced the greatest dry yield (12.45ton/ha) which was significantly different from the yield obtained from 40% and 70%FC drought treatments. 40% and 70%FC drought treatments had the lowest dry yields of 10.31 and 9.82ton-ha, respectively, which placed them in the same class (Figure 6). Results suggested that 40% and 70% treatments resulted in a (15.1 and 24.57%) and (17.16 and 21.12%) decline in wet and dry yields, respectively (Table 2).

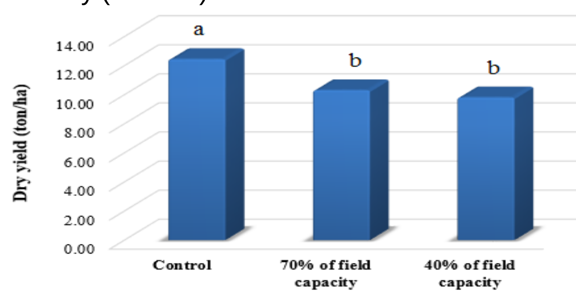

Figure 6: mean impact of drought stress on dry yield in borage medicinal herb

The presence of different letters indicates a significant difference by Duncan test at the level of 5% probability

Overall, irrigation at the 70% field capacity appears suitable in terms of dry yield. Reducing the dry weight in the organs of plants suffering from drought stress can be due to the unavailability of sufficient water for cell turgor. Reduced vegetative area reduced the plant's capability to absorb light and produce photosynthetic materials, resulting in reduced weight of the organs (Li et al. 2022). Results of the present study suggest that the dry matter decreased with the increase in drought stress. Cell wall flexibility declined in growing cells under drought stress, which resulted in reduced cell development and growth (Radmanesh, 2021a). The reduced water content in the environment surrounding the root disrupted the transfer of the nutrients required to grow and prevent the production of new dry matter, resulting in reduced growth. Moreover, lower water uptake through the roots is associated with reduced cell turgor, which reduces cell division and inhibits growth. Drought stress also limits photosynthesis due to the closure of the pores (Ma et al. 2021b).

A study of the impacts of drought stress levels on mean wet flower yield (Figure 7) indicated that the highest yield was observed in the control treatment (no stress) at 3069.29kg/ha, and the lowest yield was observed in the 40%FC treatment at the 2494.95kg/ha. The study of the impacts of drought stress levels on mean dry flower yield (Figure 8) indicated that the highest yield was observed in the control treatment (no stress) at 482.7kg/ha, and the lowest yield was observed in the 40%FC treatment at the 394.25kg/ha (Figure 8). Results suggested that 40% and 70%FC treatments led to a (5.12 and 18.71%) and (7.44 and 18.32%) decline in wet and dry flower yield, respectively (Table 2).

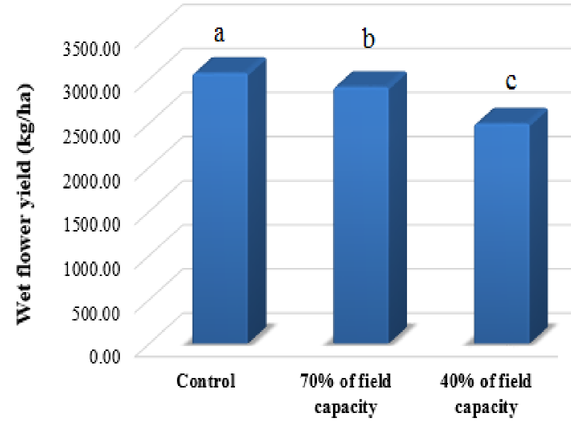

Figure 7: mean impact of drought stress on wet flower yield in borage medicinal herb

The presence of different letters indicates a significant difference by Duncan test at the level of 5% probability

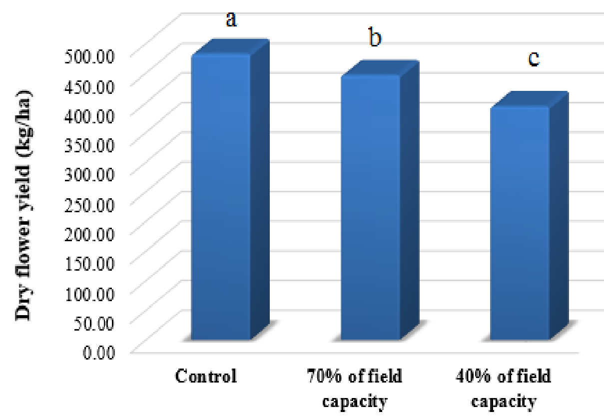

Figure 8: mean impact of drought stress on dry flower yield in borage medicinal herb

The presence of different letters indicates a significant difference by Duncan test at the level of 5% probability

Table 2: the impact of stress on the studied traits of borage medicinal herb

	Plant height	Number of leaves	Number of branches	Number of flowers	Wet yield	Dry yield	Wet flower yield	Dry flower yield
Mean	26.98	49.62	10.39	73.43	92.39	10.86	2825.45	441.24
Percentage reduction of 40% of farm capacity compared to control treatment	20.75	13.82	13.20	7.95	15.10	17.16	5.12	7.44
Percentage reduction of 70% of farm capacity compared to control treatment	36.39	19.41	3.13	9.88	24.57	21.12	18.71	18.32

CONCLUSION

Results of the present study revealed that 40% and 70%FC treatments led to a (15.1 and 24.57%) and (17.16 and 21.12%) decline in wet and dry yields, respectively, and a (5.12 and 18.71%) and (7.44 and 18.32%) decline in wet and dry flower yield, respectively. In other words, drought stress left significant impacts on the studied traits, which indicates that borage is sensitive to drought conditions and will suffer from reduced height, wet, and dry total and flower weights as a result of water deficiency.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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This paper was from my own master thesis.

AUTHOR CONTRIBUTIONS

Masoud Radmanesh conducted, planned, Analyzed the data, wrote manuscript and interpreted the results and involved in manuscript preparation. All authors read and approved the final version..

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