

Original Article

The effect of ascorbic acid and bio fertilizers on basil under drought stress

O efeito do ácido ascórbico e biofertilizantes em manjeriço sob estresse hídrico

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Abstract

Evaluate the effect of ascorbic acid application and coexistence of Mycorrhiza fungus and Azospirillum on basil (*Ocimum basilicum* L.) under drought stress. This experiment was performed as a split factorial in a randomized complete block design with three replications in the crop year 2017-2018 in Shahriar, Iran. In this experiment, irrigation was the main factor in three levels, including drought stress based on 40-70-100 mm from the evaporation pan of class A. Biofertilizer including growth-promoting bacteria (Azospirillum) and mycorrhiza fungus in four levels, including a(Non-consumption) B (Seeds of growth-promoting bacteria (Azospirillum)) C (Consumption of mycorrhiza fungus as seeds) D (Concomitant use of growth-promoting bacteria Azospirillum with mycorrhiza fungi as seeds) and ascorbic acid in two levels of foliar application, including A (Absence Application of ascorbic acid) and B (Application of ascorbic acid (two days after irrigation treatment)) was considered as a factorial factor. The results showed that the highest biological yield was obtained in drought stress of 40 mm and application of biological fertilizers in the form of mycorrhiza application with an average of 3307.1 kg/ha, which was about 70% more than 100 mm evaporation stress and no application of biological fertilizer. The use of ascorbic acid under drought stress conditions improved by 10%, the essential oil using ascorbic acid evaporated under drought stress conditions of 100 mm. As a general conclusion, the use of ascorbic acid and Mycorrhiza + Azospirillum biological fertilizer improved the quantitative and qualitative characteristics of basil under drought stress.

Keywords: ascorbic acid, biological fertilizers, basil, drought stress, biological yield, essential oil percentage.

Resumo

Avaliar o efeito da aplicação de ácido ascórbico e coexistência do fungo Mycorrhiza e Azospirillum em manjeriço (*Ocimum basilicum* L.) sob estresse hídrico. Este experimento foi realizado como um fatorial dividido em um delineamento de blocos completos casualizados com três repetições no ano-safra de 2017-2018 em Shahriar, Irã. Neste experimento, a irrigação foi o fator principal em três níveis, incluindo o estresse hídrico baseado em 40-70-100 mm do tanque de evaporação da classe A. Biofertilizante incluindo bactérias promotoras de crescimento (Azospirillum) e fungo Mycorrhiza em quatro níveis, incluindo um (Não consumo), B (Sementes de bactérias promotoras de crescimento (Azospirillum)), C (Consumo de fungos micorrízicos como sementes), D (Uso concomitante de bactérias promotoras de crescimento Azospirillum com fungos micorrízicos como sementes) e ácido ascórbico em dois níveis de foliar aplicação, incluindo A (Ausência de aplicação de ácido ascórbico) e B (Aplicação de ácido ascórbico (dois dias após o tratamento de irrigação)) foi considerado como fator fatorial. Os resultados mostraram que o maior rendimento biológico foi obtido no estresse hídrico de 40 mm e aplicação de fertilizantes biológicos na forma de aplicação de micorrizas com média de 3.307,1 kg/ha, que foi cerca de 70% superior ao estresse evaporativo de 100 mm e sem aplicação de fertilizante biológico. O uso de ácido ascórbico em condições de estresse hídrico melhorou em 10%, o óleo essencial usando ácido ascórbico evaporou em condições de estresse hídrico de 100 mm. Como conclusão geral, o uso de ácido ascórbico e fertilizante biológico Mycorrhiza + Azospirillum melhorou as características quantitativas e qualitativas do manjeriço sob estresse hídrico.

Palavras-chave: ácido ascórbico, fertilizantes biológicos, manjeriço, estresse hídrico, rendimento biológico, porcentagem de óleo essencial.

Introduction

The medicinal plant Basil (*Ocimum basilicum* L.) from the mint family is an annual and herbaceous plant used to treat bloating, some heart diseases, enlarged spleen

and help digestion (Trehan et al., 2019). Basil essential oil has antifungal and antibacterial properties and was used in the cosmetics industry (Mohammadkhani and

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Heidari, 2007). Drought stress occurs in plants when the amount of water intake is less than its losses; this may be due to excessive water loss or reduced absorption, or the presence of both (Afkari, 2014). Drought stress affects photosynthetic pigments (Rezaei and Pazoki, 2015). Low concentrations of photosynthetic pigments can directly reduce photosynthetic capacity and limit primary production (Dashti et al., 2015). During drought stress, plants store osmotic regulators such as amino acids, sugars, some from mineral ions, hormones, and proteins, try to cope with stress. Among organic compounds, proline is one of the most important osmotic regulators (Prasad et al., 2004). One of the most important negative effects of drought stress is reduced access and absorption of various nutrients for the plant (Mohammadi et al., 2016). It has been reported that drought stress plays an important role in changes in the active ingredient of medicinal plants (Li et al., 2020). In the study of the effect of drought stress on yield and composition of savoury essential oil (*Satureja hortensis* L.), it was reported that the accumulation of essential oil in drought conditions increased significantly (Oliveira et al., 2020). One of the issues that are considered essential for basil, like other medicinal and crop plants, the need for proper cultivation and supply of nutrients. Biological fertilizers or microbial fertilizers had contained substances (solid, liquid or semi-solid) containing one or more species of specific microorganisms that a group with the secretion of plant growth hormones causes more and better expansion of the root system and better absorption of elements. As a result, the plant grows more and increases the yield by increasing the quality and quantity of plant yield components (Daei et al., 2009). Today, the use of microorganisms that coexist with plants as biofertilizers to provide nutrients and to use the potential of organisms and organic matter in the soil to increase the quantity and quality and maintain environmental safety (Sirjani et al., 2011). Biofertilizers are currently a suitable alternative to chemical fertilizers to increase soil fertility and crop production (Megersa et al., 2018). These include Plant Growth Promoting Rhizobacteria (PGPR) (Vessey, 2003). This group of bacteria affects the yield of crops through bio-stabilization of nitrogen, dissolution of phosphorus and potassium, increasing bioavailability of soil minerals, inhibition of pathogens, and production of growth-regulating hormones. These bacteria are called plant growth-promoting bacteria (PGPR) because they increase the growth and development of crops (Barassi et al., 2007). *Azospirillum* spp. (*Azospirillum* spp.) is one of the most important active bacteria promoting plant growth in the root environment (rhizosphere) which, in addition to bio-stabilization of nitrogen and solubilization of soil phosphorus, produces significant amounts of growth stimulants, especially growth stimulants? Gibberellins and cytokinins affect the growth, development and yield of crops (Hasanabadi et al., 2019). Bacteria of the genus *Azospirillum* have a cooperative relationship with the host plant (Fukami et al., 2018). A group of these biological fertilizers are mycorrhiza fungi. Mycorrhiza fungi have a symbiotic relationship with the roots of most crops. Increasing the absorption of nutrients such as phosphorus and some trace elements increases water absorption,

reduces the negative impact of environmental stresses and increases resistance. Against pathogens, it improves the growth and yield of host plants in sustainable agricultural systems (Sadhana, 2014). On the other hand, oxidative damage is one of the effects of dehydration stress caused by oxygen free radicals such as superoxide radicals, hydrogen peroxide and hydroxyl radicals (Kirova et al., 2021). The plant is equipped with a sweeper system to fight against these oxygen-free radicals. This defence system is called the antioxidant defence system (Jamshidi-kia et al., 2020). One way to increase resistance is to increase the level of substrates of antioxidant enzymes and intracellular antioxidants such as ascorbic acid (Paciolla et al., 2019). Ascorbic acid is a small water-soluble molecule with high antioxidant properties and acts as a primary substrate in cyclic pathways to detoxify and neutralize single superoxide and oxygen radicals (Alves et al., 2021). External use of ascorbic acid increases resistance to drought stress and reduces the harmful effects of oxidative stress (Khazaei and Estaji, 2020). Therefore, considering the development of drought and macro-policies of agriculture to achieve an organic solution to drought, this study aims to investigate the possibility of increasing drought stress resistance and preventing the improvement of the quantitative and qualitative yield of basil (*Ocimum basilicum* L.) It is done using biological fertilizers and ascorbic acid under drought-stress conditions.

Materials and Methods

This experiment was performed to investigate the effect of biological fertilizers and ascorbic acid on the yield and yield of basil (*Ocimum basilicum* L.) under drought stress in the crop year 2017-2018 Shahriar region. The test site was located at latitude 35.21E and longitude: 51.38 N at an altitude of 927 above sea level. The climate of this region is arid and semi-arid, and the average rainfall of the last 38 years has been 251 mm, the average annual air temperature is 16.5 C, the average soil temperature is 15.5 c, and the evaporation rate from the pan is 2607 mm, respectively (Figure 1).

Before conducting field experiments to determine the physical and chemical properties of the soil, sampling was done from a depth of 0-30 cm (Table 1). The experiment was performed as a split factorial in a randomized complete

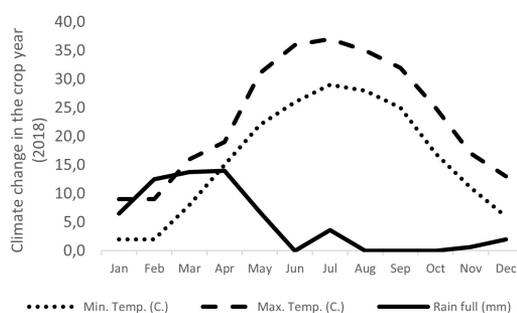


Figure 1. Graph of climate change during the planting period of 2018.

Table 1. Physical and chemical characteristics of farm soil.

Texture		Sand	Salt	Clay	pH	ds.m	Depth
		(%)	(%)	(%)		(Ec)	(cm)
Clay		25	22	53	7.3	3.8	0-30
N (ppm)	K (ppm)	P (ppm)	Cu (ppm)	Mn (ppm)	Zn (ppm)	Fe (ppm)	OC (%)
1.1	210	10	2.2	2.5	1.1	1.5	0.8

Ds.m (deciSiemens per meter); Ec: electrical conductivity.

block with 24 treatments in three replications. Irrigation is the main factor in three levels, including drought stress based on 40,70,100 mm evaporation from the evaporation pan of Class A evaporation and biofertilizer including growth-promoting bacteria (*Azospirillum*) and mycorrhiza fungus in four levels including a) no Consumption b) Seeds of growth-promoting bacteria (*Azospirillum*) c) Consumption of mycorrhiza fungus as seeds d) Concomitant use of growth-promoting bacteria *Azospirillum* with mycorrhiza fungi as seeds and ascorbic acid in two levels of foliar application, including a) No use of acid Ascorbic acid and b) Consumption of two molars ascorbic acid as a factorial was considered as a secondary factor.

In this experiment, severe and moderate drought stress treatment performed when 70 and 100 mm of evaporation from the Class A evaporation pan's surface performed, respectively. At the same time, in stress-free treatment (control), 40 mm was evaporated after evaporation. After calculating the number of seeds for each treatment for growth-promoting bacterial treatments, it poured into a polyethylene bag. It added to it, then 20 ml of gum Arabic. The bag containing the seeds and the binder was then shaken vigorously for 30 seconds to make the surface of all the seeds evenly sticky. After that, 20 g of inoculum was added to the sticky seeds. After 45 seconds of shaking and ensuring that the inoculum adhered evenly to the seeds, the inoculated seeds were spread on a clean aluminum foil and spread under the shade to dry (Heidary et al., 2017) mycorrhiza fertilizer contained a mixture of *Unneliformis Mosseae* and *Rhizophagus irregularis* with an equal population of 30 spores per gram. The mushroom inoculum was prepared from Pishtaz Varian Biotechnology Co. the substrate was placed in a layer at a depth of 2 cm, and then the seeds will be planted quickly to inoculate the inoculum. Seeds sown on planting lines at a depth of 1 cm. In this experiment, ascorbic acid at a rate of 2 M (two days after irrigation) was used as a foliar spray. To measure plant height, the number of leaves per plant and biological yield after considering the first and last line and 2 meters from the beginning and end of planting lines as a margin, an area of 2 square meters considered after planting the plants. Fresh weight was determined first, then grain weight was determined by separating the seeds, then biological yield was calculated by drying the samples. According to the mentioned margins, ten ripe leaves were selected and weighed in the field to measure the amount of relative water content (RWC). During this time, the leaves removed from the containers, and the surface was quickly recorded with a dry cloth and their

saturated weight. The leaves were then placed in paper bags and placed in an oven for 24 hours. After re-drying, they were weighed. Then according to the following formula, was determined the amount of RWC of each sample (Equation 1).

$$RWC = (FW-DW/SW-DW) \times 100 \quad (1)$$

In this formula, Fw: fresh weight, Dw: dry weight and Sw: saturated weight of the leaves.

Leaf orifice conductivity was measured using the Mk3 T-Delta Porometer (Model AP3, made in the USA) at a temperature of 25 ° C and relative humidity of 40% at 11 to noon. To measure ion leakage in terms of $\mu\text{s}/\text{cm}$, the method by Zhao et al. (2009) was evaluated. Total chlorophyll was measured by using (mg / g FW) (Munir et al., 2019). 1.5 g of plant tissue was weighed, and 20 ml of a solution containing equal proportions of methanol and deionized water was added and homogenized with a homogenizer 4 ° C. The resulting solution was centrifuged at 3000 rpm for 15 minutes to measure auxin hormone (IAA), and the supernatant was placed on a C18 chromatographic column and washed with 5 ml of deionized water. Then 3 ml of 80% methanol was passed. The extracted solution was evaporated by refrigerant at laboratory temperature, and 1 ml of 20% methanol containing 1% formic acid was added to the residue, and again 1 ml of 80% methanol was added. Also, this final solution was used to determine the number of hormones used in the next step (Hou et al., 2008). The samples were dried and ground in the shade after harvest during flowering to measure the percentage of essential oils of shoots. The essential oil was extracted by water distillation. For all samples, the harvest date and time of essential oil were performed in one day, so that 20 g of the dried plant sample was mixed in 200 ml of distilled water (1:10 PWP / BvB) then the essential oil was extracted with a Clevenger apparatus. (Sivieri et al., 2020). SAS program (Ver. 9.4) was used for statistical analysis, and the means were compared using Duncan's test at a 5% probability level.

Results

Plant height

According to the analysis of variance (Table 2), there are significant interactions between drought stress in biological fertilizer and the effect of drought stress in

ascorbic acid. The comparing the mean results (Table 3) regarding the interaction of drought stress and biological fertilizers stated that the highest plant height was obtained from the drought stress treatment of 40 mm evaporation with an average of 74.08 cm, which is 40% more than the drought stress treatment of 100 mm evaporation And

biological fertilizers with an average of 43.75 cm. Based on the results of comparing the mean (Table 4) of the interaction effect of drought stress in ascorbic acid, the highest plant height was obtained from drought stress evaporation treatment and ascorbic acid consumption with an average of 68.12 cm, which was used evaporated

Table 2. Analysis of variance of the effect of experimental treatments on plant height, number of leaves per plant, relative leaf water content, ion leakage and total chlorophyll.

S.O. V	df	Plant height	Number of leaves per plant	Relative content	Ion leakage	Total chlorophyll
Rep	4	250.96	401.360	1.330	52.91	4.938
Drought stress	2	1041.47**	1659.791**	7058.443**	243613.701**	318.347**
Error A	8	5.556 ^{ns}	7.146 ^{ns}	1.442**	0.151 ^{ns}	0.525 ^{ns}
Bio fertilizer	3	3219.810**	4646.883**	26.488**	1060.664**	80.976**
Ascorbic acid	1	1083.068**	1338.950**	0.654*	760.013**	282.352**
Drought stress * Biological fertilizers	6	27.014**	32.210*	0.659**	13.180**	1.583**
Drought stress * Ascorbic acid	2	19.508*	71.804*	0.556*	7463.970**	3.074**
Biological fertilizers * Ascorbic acid	3	6.780 ^{ns}	3.740 ^{ns}	0.006 ^{ns}	0.024 ^{ns}	0.1107*
Drought stress * Biological fertilizers * Ascorbic acid	6	7.481 ^{ns}	14.391 ^{ns}	0.229 ^{ns}	0.283 ^{ns}	2.364**
Error B	84	6.151	14.101	0.15067	1.3569	0.3005
C.V.		13.56	16.76	10.52	14.26	7.59

S.O.V: sources of variations; df: degree of freedom; C.V: coefficient of variation. **Significant at a probability level of 1%. *Significant at a probability level of 5%. ^{ns}No significant difference.

Table 3. Comparison of the mean interaction of drought stress in biological fertilizer on plant height, number of leaves per plant, relative leaf water content and ion leakage.

Irrigation cycle	Biological fertilizers	Plant height (cm)	Number of leaves per plant	Relative content	Ion leakage ($\mu\text{s}/\text{Cm}$)
Optimal irrigation	Do not consume	51.59h	63.48f	331.49g	371.49i
	Azospirillum	62.91e	76.91d	321.13g	366.13j
	Mycorrhiza	72.01b	88.05a	308.2h	364.20k
	Mycorrhiza + Azospirillum	74.08a	90.73a	305.2h	360.20l
Medium tension	Do not consume	48.51i	60.41g	422.36d	472.36e
	Azospirillum	57.91f	71.23e	400.02e	465.02f
	Mycorrhiza	65.33d	79.39c	385.04f	461.04g
	Mycorrhiza + Azospirillum	68.01c	84.30b	382.22f	457.22h
Severe tension	Do not consume	43.75j	53.43h	540.17a	510.17a
	Azospirillum	54.16g	66.15f	485.13b	505.13b
	Mycorrhiza	58.91f	72.06e	467.74c	502.74c
	Mycorrhiza + Azospirillum	66.58cd	80.49c	462.75c	497.75d

Means with the same letters in the same column are not significantly different based on Duncan test ($\alpha=5\%$).

Table 4. Comparison of the mean interaction of drought stress in ascorbic acid on plant height, number of leaves per plant, relative leaf water content and ion leakage.

Irrigation cycle	Ascorbic acid	Plant height	Number of leaves per plant	Relative content	Ion leakage ($\mu\text{s}/\text{Cm}$)
Optimal irrigation	No use	62.17b	76.18b	87.05a	374.91e
	use	68.12a	83.41a	86.99a	356.11f
Medium tension	No use	56.70c	69.95c	69.36c	473.50c
	use	63.16b	77.72b	79.72b	454.32d
Severe tension	No use	53.83d	66.39d	61.67d	491.85b
	use	57.87c	69.68c	64.76c	516.05a

Means with the same letters in the same column are not significantly different based on danca test ($\alpha=5\%$).

in 20% more than drought stress treatment of 100 mm no ascorbic acid.

Number of leaves per plant

The analysis of variance (Table 2) regarding the number of leaves per plant showed that the interaction effects of drought stress in biological fertilizer and the interaction effect of drought stress in ascorbic acid were significant for this trait. However, other interactions were not significant. Results of mean comparison (Table 3) regarding the interaction of drought stress and biological fertilizers on the number of leaves per plant In addition to the treatments of Mycorrhiza + Azospirillum and separate application of mycorrhiza in drought stress were in a statistical group, the highest number of leaves In-plant stress treatment, 40 mm evaporation and combined application of mycorrhiza, Azospirillum and mycorrhiza fertilizers were obtained separately with averages of 90.73 and 88.05, respectively, which was 41% higher than drought stress treatment of 100 mm evaporation and no use of biological fertilizers.

Based on the results of comparing the mean (Table 4) of the interaction effect of drought stress in ascorbic acid, the highest number of leaves per plant was obtained from evaporation of drought stress treatment and ascorbic acid consumption with an average of 83.41 leaves, which is 27% more than drought stress treatment Evaporation and non-consumption of ascorbic acid.

Based on the mean comparison results (Table 4), the interaction effect of drought stress in ascorbic acid on, ion leakage of basil leaves showed that among different treatments, drought stress was 40 mm, and ascorbic acid consumption had the lowest ion leakage. The ion leakage of basil leaves in this treatment ($\mu\text{s} / \text{Cm}$) was 356.11%, the highest rate of ion leakage of basil leaves ($\mu\text{s} / \text{Cm}$) with 516.05% was related to drought stress treatment of 100 mm and no ascorbic acid, which is different from other treatments. It was meaningful. Compounds with antioxidant properties such as ascorbate can reduce drought stress damage by increasing the antioxidant capacity of the plant (Azooz et al., 2013). In this regard, Shahrajabian et al. (2019) showed in an experiment that ascorbic acid reduces electrical leakage in rice sprouts during drought stress. According to this experiment, feeding the roots with 1 mm ascorbic acid for one day reduces

ion leakage from the root cells and thus tolerates drought stress. According to the acidic hypothesis, ascorbic acid is involved in plant growth processes such as cell division and cell wall expansion (Gezaheg, 2019).

Relative leaf water content

The analysis of variance (Table 2) regarding the relative water content showed that the interactions of drought stress in biological fertilizer and the interaction of drought stress in ascorbic acid were significant for this trait, but other interactions were not significant. Based on the results of comparing the mean (Table 3) of the interaction of drought stress in biological fertilizers on the relative content of basil water showed that among different treatments, drought stress of 40 mm and application of biological fertilizers as Mycorrhiza + Azospirillum and separate application of mycorrhiza They were in a statistical group with an average of 87.76% and application of mycorrhiza fertilizer with an average of 87.57%, respectively, which had the highest relative water content, which is 35% higher than the lowest relative content of basil water, which is related to drought stress treatment of 100 mm and not use of biological fertilizers.

Ion leakage

The analysis of variance (Table 2) about ion leakage showed that the interactions of drought stress in biological fertilizer and drought stress in ascorbic acid were significant for this trait. However, other interactions were not significant. Based on the results of comparing the mean (Table 3) of the interaction of drought stress and biological fertilizers, the highest amount of ion leakage was obtained from 100 mm drought stress evaporation and non-application of biological fertilizer with an average (10s / cm) of 510.17, which is 30% higher than the treatment. Drought stress was 40 mm of evaporation and application of Mycorrhiza + Azospirillum fertilizers.

Total chlorophyll

Based on the analysis of variance, all the leading and interaction effects for this trait became significant (Table 2). Comparing the average interaction of drought stress in biological fertilizers in ascorbic acid on total chlorophyll of basil showed that among different treatments, drought

stress of 100 mm and no use of biological fertilizers and ascorbic acid had the lowest amount of this trait. Total basil chlorophyll in this treatment with an average of 2.11 (micromoles per gram of fresh weight) and the highest amount of total basil chlorophyll with an average of 12.61 (micromoles per gram of fresh weight) was related to drought stress treatment of 40 mm and application of biological fertilizers as Mycorrhiza + Azospirillum and ascorbic acid foliar application. Other results showed that the application of biological fertilizers and ascorbic acid in moisture stress leads to an increase in plant chlorophyll content (Figure 2).

Stomatal guidance

The analysis of variance (Table 5) about stomatal conductance showed that the interactions of drought stress in biological fertilizer and the interaction of drought stress in ascorbic acid were significant for this

trait. However, other interactions were not significant. The interaction effect of drought stress in biological fertilizers on stomatal conductance of basil showed that among different treatments, drought stress of 100 mm and non-application of biological fertilizers had the lowest amount of stomatal conductance. The stomatal conductance of basil in this treatment (Cm / s) was 4.43, and the highest amount of stomatal conductance of basil with 10.38 (Cm / s) was related to the drought stress treatment of 40 mm and the use of biological fertilizers in the form of Mycorrhiza + Azospirillum. Other results of this treatment indicated that, in general, the use of biological fertilizers in both stress and irrigation conditions increases stomatal conductance (Table 6).

With increasing stress, the osmotic potential of the environment around the root also increases, which increases the secretion of abscisic acid from the root. This closes the pores and reduces the pore conductance (Hernandez-Santana et al., 2016). On the other hand, it

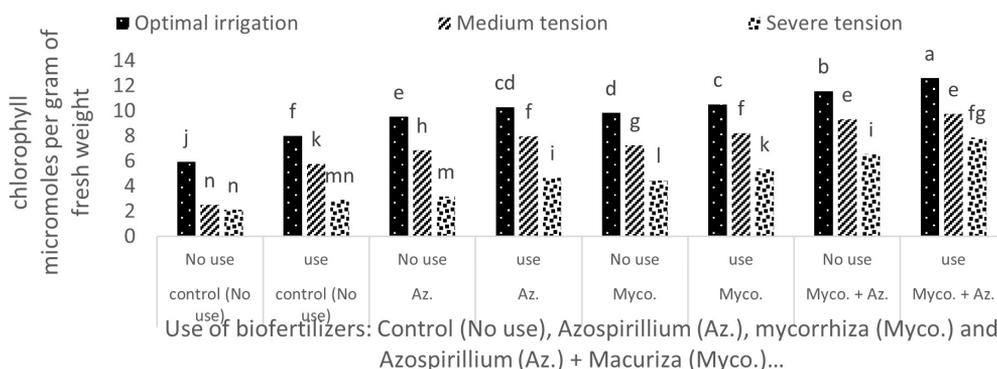


Figure 2. Interaction of drought stress in biological fertilizers in ascorbic acid on total chlorophyll content.

Table 5. Analysis of variance of the effect of experimental treatments on stomatal conductance, leaf essential oil percentage, proline content, auxin and biological yield.

S.O. V	df	Stomata function	Essential percentage	Proline	Auxin	Biological yield
Rep	4	1.920**	43.608**	2.535**	857.123**	57001.85 ^{ns}
Drought stress	2	118.080**	2577.836**	260.123**	39468.336**	18937789.39**
Error A	8	0.063 ^{ns}	16.572*	0.090 ^{ns}	19.371 ^{ns}	70658.2 ^{ns}
Bio fertilizer	3	47.188**	1410.807**	55.664**	16633.066**	8275585.47
Ascorbic acid	1	13.844**	1898.925**	26.01**	5198.41**	188942.01*
Drought stress * Biological fertilizers	6	0.291*	38.137**	15.492**	272.191**	442624.21**
Drought stress * Ascorbic acid	2	1.201**	31.230*	1.801**	180.829**	458648.12**
Biological fertilizers * Ascorbic acid	3	0.082 ^{ns}	1.311 ^{ns}	0.071 ^{ns}	53.219 ^{ns}	162730.35 ^{ns}
Drought stress * Biological fertilizers * Ascorbic acid	6	0.054 ^{ns}	0.615 ^{ns}	0.036 ^{ns}	25.087 ^{ns}	148942.01 ^{ns}
Error B	84	0.107	7.459	0.059	21.227	73300.06
C.V.		13.31	11.2	9.94	10.1	13.43

**Significant at a probability level of 1%. *Significant at a probability level of 5%. ^{ns}No significant difference.

was stated that Arbuscular mycorrhiza increased stomatal conductance under stress (Augé et al., 2015). Thus, seed pretreatment with bacteria caused root expansion and better access to water resources, reducing abscisic acid and increasing stomatal conductance (Zhu et al., 2016). Decreased stomatal conductance indicates a change in the osmotic position of the root, which rapidly affects water relations in the shoots (Eroğlu et al., 2020).

According to the Duncan test, a comparison of the mean interaction effect of drought stress in ascorbic acid on basil stomatal conductance showed that among different treatments, drought stress of 40 mm and ascorbic acid consumption had the highest stomatal conductance. The stomatal conductance of basil in this treatment (Cm / s) was 9.49 and the lowest basil with 5.75 (Cm / s) was related to 100 mm drought stress treatment and no ascorbic acid consumption. These results show the positive effect of an

ascorbic acid application under water stress conditions on improving the stomatal conduction performance of basil (Table 7).

Leaf essential oil

The results of the analysis of variance (Table 5) regarding leaf essential oil yield showed that the interaction effects of drought stress in biological fertilizer and the interaction effect of drought stress in ascorbic acid were significant for this trait. But other interactions were not significant. The results of comparing the mean (Table 6) of the interaction of drought stress in ascorbic acid on the percentage of basil essential oil showed that among different treatments, drought stress of 100 mm and application of biological fertilizers in combination with Mycorrhiza + Azospirillum had the highest percentage of essential oil.

Table 6. Comparison of the mean effect of drought stress in biological fertilizer on stomatal conductance, leaf essential oil percentage, proline content, auxin and biological yield.

Irrigation cycle	Biological fertilizers	stomata function	Essential percentage	Proline	Auxin	Biological yield
Optimal irrigation	Do not consume	7.49e	46.05h	2.58j	91.47f	4982.71c
	Azospirillum	8.95c	58.01g	2.69j	119.79c	6289.21b
	Mycorrhiza	9.78b	60.93f	2.9i	135.41b	8068.31a
	Mycorrhiza + Azospirillum	10.38a	65.15de	2.35k	154.79a	7982.11a
Medium tension	Do not consume	6.25g	57.96g	6.76c	70.35h	3528.11e
	Azospirillum	7.40e	64.18e	5.37e	89.42f	4604.71d
	Mycorrhiza	8.21d	66.56d	3.90g	104.12e	6425.11b
	Mycorrhiza + Azospirillum	8.45d	70.91c	3.35h	113.57d	6624.71b
Severe tension	Do not consume	4.43i	65.38de	8.08a	44.65j	3048.51f
	Azospirillum	5.83h	71.61b	7.92b	63.65i	3947.31e
	Mycorrhiza	6.56f	73.81b	6.22d	74.11g	5401.51c
	Mycorrhiza + Azospirillum	7.23e	77.98a	5.06f	89.93f	5154.32c

Means with the same letters in the same column are not significantly different based on danca test ($\alpha=5\%$).

Table 7. Comparison of mean drought stress traits in ascorbic acid on stomatal conductance traits, leaf essential oil percentage, proline content, auxin and biological yield.

Irrigation cycle	Ascorbic acid	Stomata function	Essential percentage	Proline	Auxin	Biological yield
Optimal irrigation	No use	8.81b	54.35d	2.96e	117.92b	6559.01b
	use	9.49a	60.72c	2.38f	132.81a	7002.21a
Medium tension	No use	7.25d	61.75c	5.18c	87.58d	4172.81e
	use	7.90c	68.05b	4.51d	101.15c	5918.41c
Severe tension	No use	5.75f	67.63b	7.97a	64.28f	4331.81e
	use	6.28e	76.75a	6.67b	71.88e	4844.01d

Means with the same letters in the same column are not significantly different based on danca test ($\alpha=5\%$).

Proline content

The analysis of variance (Table 5) concerning proline content showed that the interactions of drought stress in biological fertilizer and drought stress in ascorbic acid were significant for this trait. Regarding the interaction of drought stress and biological fertilizers, the highest proline content was obtained from drought stress treatment of 100 mm evaporation and application of Mycorrhiza + Azospirillum fertilizer with an average of 80.08 (mg / g), which evaporated 70% more than drought stress treatment of 40 mm. No use of biological fertilizers. However, the most significant effect of biological fertilizers on proline content was obtained in the treatment of severe stress, so that in the treatment of severe moisture stress and with the use of biological fertilizer, the proline content of basil was 37% lower than the treatment of severe stress and no use of biological fertilizers. there was observed medium moisture stress treatment (Table 6).

Auxin

The analysis of variance (Table 5) regarding the content of auxin showed that the interaction effects of drought stress in biological fertilizer and the interaction effect of drought stress in ascorbic acid were significant for this trait. However, other interactions were not significant. The results of comparing the mean (Table 6) of the interaction of drought stress in ascorbic acid on basil auxin showed that among different treatments, drought stress of 40 mm and application of biological fertilizers in the form of Mycorrhiza + Azospirillum had the highest auxin.

Biological yield

The analysis of variance (Table 5) concerning biological yield showed that the interactions of drought stress in biological fertilizer and drought stress in ascorbic acid were significant for this trait. However, other interactions were not significant. Based on the results of comparing the mean (Table 6) of the interaction effect of drought stress in biological fertilizers on the biological yield of basil showed that among the different treatments, drought stress of 40 mm and application of biological fertilizers as a separate application of mycorrhiza with an average of 3307.1 kg/ha The use of Mycorrhiza + Azospirillum with an average of 3183.91 kg/ha was obtained. Also, these two treatments were in the same statistical group. The lowest biological yield of basil with 974.8 kg/ha was related to drought stress treatment of 100 mm and no biological fertilizers.

Discussion

The coexistence of arbuscular mycorrhizae can improve the ability of plants to avoid drought (Shabani et al., 2022). These results have been reported by other researchers (Covacevich et al., 2006). Increased basil plant height was reported due to inoculation with biological fertilizers (Azotobacter, Azospirillum and Pseudomonas) (Tahami et al., 2017). Other reports in this regard indicate that the basil plant under the treatment

of Azospirillum growth-promoting bacteria recorded maximum root weight, plant height and essential oil yield (Ordoookhani et al., 2011; Agami et al., 2016). According to the findings of Zare et al. (2021), Arbuscular mycorrhiza (AM) had a significant effect on the plant height of basil. Other results of this treatment indicated that ascorbic acid at all levels of irrigation increased the height of the basil plant. In this regard, it was stated that if the basil plant is treated with ascorbic acid, its resistance to UV rays will increase, and some It increases its biochemical traits (Sakalauskaite et al., 2012). The positive effect of ascorbic acid use by other researchers has also been reported (Masi et al., 2006; El-Hak et al., 2012; Yazdanpanah et al., 2011). It was also stated that ascorbic acid spraying increased plant height, 1000-seed weight, grain yield, total chlorophyll, the relative water content of leaves and decreased proline and activity of superoxide dismutase and catalase enzymes in basil (Nassar et al., 2019). It seems that due to the positive role of biological fertilizers in the production and regulation of growth-stimulating hormones, the use of these biological fertilizers increases root surface and depth and increases water and nutrient uptake, which improves growth and increases photosynthesis (Kour et al., 2020).

Hasanpour et al. (2012) have reported decreased area and number of leaves due to drought stress. The results of other studies showed that increasing the concentration of ascorbic acid gradually all growth parameters (plant height, number of branches, and number of leaves, stem diameter, root length, and fresh and dry weights of all plant organs) well as the percentage of total carbohydrates. Affected nitrogen, phosphorus and potassium (Mazher et al., 2011). It was reported in an experiment that ascorbic acid increases growth by increasing the leaf area of the flag (Dolatabadian and Jouneghani, 2009). Similar results have been reported regarding the positive effect of mycorrhiza inoculation on the relative water content of corn leaves under drought stress conditions (Zhu et al., 2012).

The researchers (Masi et al., 2006) said that basil almost increases the activities of glycolate oxidase, hydrogen peroxide (H₂O₂) and proline under drought stress, but the relative water content decreases under drought stress. Another report states that the use of fertilizer for growth-promoting bacteria under the influence of drought stress has increased the relative water content of basil (Keshavarz et al., 2020). Based on the mean comparison results (Table 4), the interaction effect of drought stress in ascorbic acid on the relative water content of basil showed that among different treatments, drought stress of 40 mm and ascorbic acid consumption had the highest relative water content. The relative content of basil leaf water in this treatment was 86.99%. However, these results were in the same statistical group with 40 mm evaporation and no ascorbic acid consumption. The lowest relative content of basil water, with 61.67%, was related to drought stress treatment of 100. Mm and no ascorbic acid consumption was significantly different from other treatments. Other studies have shown that the use of ascorbic acid results in the highest water use efficiency of the plant (Farjam et al., 2015; Ergin et al., 2014). Ascorbic acid treatment simultaneously with salinity stress increased the relative water content of flat flax leaves of salinity stress

(El-Hariri et al., 2010). These results indicate that drought stress increases ion leakage and decreases the relative water content of leaves under drought stress conditions. In this regard Taha et al. (2020), stated that drought stress reduces the relative amount of leaf water and increases electrolyte leakage from the plant cell membrane. On the other hand, several experiments have shown that biological fertilizers increase plant resistance under environmental stresses such as drought and salinity (Darabi et al., 2020; Ghavami et al., 2017; Begum et al., 2019).

The decrease in pigments is due to the increase in the production of oxygen free radicals, which cause peroxidation and consequently the decomposition of chlorophylls (Damalas, 2019; Tolay, 2021). Ascorbic acid, known as one of the best non-enzymatic compounds with antioxidant properties, can reduce the damaging effects of stress on photosynthetic pigments by purifying oxygen species, thereby increasing chlorophyll content (Ardebili et al., 2015). Other reports also suggest that the use of growth-promoting bacteria (PGPR) is involved in the uptake of iron and manganese elements, which can improve the leaf area (chlorophyll a, chlorophyll b and total chlorophyll) in basil in bacterial inoculation conditions (Etesami and Adl, 2020; Kenneth et al., 2019; Lobo et al., 2019). Similar results have been reported that the use of ascorbic acid in green leaves reduces water stress due to pore closure, nutrient uptake, total chlorophyll, protein synthesis, photosynthesis and plant growth (Xu et al., 2006). The application of ascorbic acid modulates the destructive effect of drought stress by increasing stomatal conductance. This may be due to the antioxidant role of ascorbic acid by neutralizing harmful oxidants (Malik and Ashraf, 2012). The percentage of basil essential oil was 0.43% in this treatment. The lowest percentage of basil essential oil with 0.25% was related to 40 mm drought stress treatment and no biological fertilizers. Although secondary metabolites in medicinal and aromatic plants are commonly affected by their genotypes, their biosynthesis is strongly influenced by environmental factors. This means that environmental and environmental factors affect the growth parameter, yield of essential oil and its components (Aziz et al., 2008).

In another study, it was stated that mycorrhizal fungus increased the essential oil and biomass of basil (Zolfaghari et al., 2013). Alternatively, according to Copetta et al. (2006), mycorrhiza can have different effects on a plant and increase the essential oil yield and quantitative and qualitative characteristics of basil. Based on the results of comparing the mean (Table 7) regarding the interaction of drought stress in ascorbic acid, it was stated that severe drought stress of 100 mm evaporation and consumption of ascorbic acid had the highest leaf essential oil. Basil leaf essential oil was 0.42% in this treatment, and the lowest basil leaf essential oil with 0.3% was related to 40 mm drought stress treatment and no ascorbic acid consumption. According to Khorasaninejad et al. (2011), Drought stress has reduced the total growth parameters and yield of essential oil and its percentage. The highest growth parameters and percentage of essential oil and essential oil yield were observed in severe stress. In another study, it was stated that the use of ascorbic acid significantly improves

the amount of essential oil, which can be attributed to the positive relationship between photosynthesis and the production of secondary metabolites, so that exogenous treatment of plants with ascorbic acid can maintain photosynthesis. Moreover, photosynthetic pigments improve plant growth under stress conditions (Narimani et al., 2020; Hamada and Al-Hakimi, 2009). The amino acid proline is an osmotic regulating compound whose concentration increases under environmental stresses such as salinity and drought (Ding et al., 2021). Accumulation of proline and other osmolites for plant cell turbidity preservation is part of drought stress resistance mechanisms (Ibrahim et al., 2020). Proline has been reported to be higher in mycorrhiza inoculated seedlings compared to inoculated seedlings. Inoculated seedlings had leaf water potential (Ψ), photosynthetic rate (P_n), stomatal conductance, relative water content (RWC) and lower leaf temperature compared to non-inoculated seedlings (Wu and Xia, 2006). Regarding the interaction effect of drought stress in ascorbic acid, the lowest proline content was obtained from drought stress treatment of 100 mm evaporation and consumption of ascorbic acid with an average of 79.7 (mg / g), which is 70% more than drought stress treatment of 40 mm evaporation and no acid consumption. It was ascorbic. (Table 7). Due to ascorbic acid's antioxidant role in stresses and because ascorbic acid is a cofactor of the enzyme proline hydroxylase, the conversion of proline to hydroxyproline reduces the amount of free proline (Alqurainy, 2007).

The most important antioxidant compounds include glutathione, tocopherol, phallonoids and ascorbate, directly involved in the clearance of reactive oxygen species. Also, antioxidant enzymes such as catalase, superoxide dismutase, peroxidase, polyphenol oxidase and ascorbate peroxidase are involved in the clearance of reactive oxygen species in the cell ascorbic (Dolatabadian et al., 2008). Basil auxin in this treatment was 154.79 (micromoles per gram of fresh weight). The lowest basil auxin with 44.65 (micromoles per gram of fresh weight) was related to drought stress treatment of 100 mm and no biological fertilizers. Similar to these results, it was stated that inoculation of different plant species with PGPR bacteria increased root growth or increased the formation of secondary roots through the secretion of auxin hormone by these bacteria, followed by increased effective root level and finally, crops increase water uptake and Nutrients under drought stress conditions (Mahmoud et al., 2020; Raheem et al., 2018; Jochum et al., 2019). The results of comparing the mean interaction of drought stress in ascorbic acid (Table 7) on basil auxin showed that among different treatments, drought stress of 40 mm and ascorbic acid consumption had the highest auxin. Basil auxin in this treatment was 132.81 (micromoles per gram of fresh weight), and the lowest basil auxin with 64.28 (micromoles per gram of fresh weight) was related to drought stress treatment of 100 mm and no ascorbic acid. Ascorbic acid also regulates plant hormonal conditions and increases auxin levels under non-stress conditions. Accordingly, it has been suggested that ascorbic acid in wheat plants increases the amount of auxin and ABA and prevents the reduction of cytokinin in drought stress (Shamsipur et al.,

2012). The positive effect of ascorbic acid on plant growth can be attributed to its role as an important cofactor in the biosynthesis of some plant hormones involved in cell growth and cell division, including gibberellin, auxin and pigment stability of the photosynthetic apparatus (Khan et al., 2011). Other studies showed that drought stress has a significant effect on dry basil weight and basil yield decreases compared to the control (Sirousmehr et al., 2014). In this regard, according to biomass measurements, mycorrhizal plants inoculated with mycorrhizal fungi compared to non-mycorrhizal plants inoculated with mycorrhiza fungi were more stable under drought stress (Jayne and Quigley, 2014; Rezaei et al., 2017). The results showed the effect of biological fertilizers on the number of main branches, inflorescence per flowering plant, dried flower yield, and essential oil yield of German chamomile (*Matricaria chamomilla* L.) was positive and significant (Fallahi et al., 2009). Another study showed that the combined application of biological and chemical fertilizers improves plant dry yield and essential oil yield in conditions of plant water supply. In conditions of drought stress, the application of biofertilizers works better than other fertilizers (Zand et al., 2017). The results of comparing the mean (Table 7) of the interaction effect of drought stress in ascorbic acid on the biological yield of basil showed that among different treatments, drought stress 40 mm and ascorbic acid consumption had the highest biological yield. The biological yield of basil in this treatment 2813.51 Kg/ha, and the lowest biological yield of basil with 140.41 kg/ha was related to drought stress treatment of 100 mm and no ascorbic acid consumption, which was significantly different from other drought stress treatments. In this regard, the results of various studies showed that ascorbic acid spray (100 ppm) in wheat reduced the adverse effects of drought stress (Noreen et al., 2009; Ameer Khan and Ammadmr, 2006). Ascorbic acid has been reported to increase cell division, increase the dry and fresh weight of leaves in plants, and reduce radial oxygen damage by antioxidants, which is the product of drought stress (Silva et al., 2019). Ascorbic acid increases stem length, root length, fresh and dry weight of terrestrial and aerial parts (Olle et al., 2012). The application of ascorbic acid increases resistance to drought stress (Hussein and Khursheed, 2014).

Conclusion

Drought stress due to increasing irrigation distance significantly reduced all yield components and ultimately biological yield, and overall application of biofertilizers and ascorbic acid reduced the adverse effects of drought stress, although biofertilizers showed greater efficacy than ascorbic acid. Regarding the use of biological fertilizers, the use of mycorrhiza and the combined use of mycorrhiza and Azospirillum bacteria showed a greater effect was more on improving the characteristics of basil than the separate Application of Azospirillum bacteria (Witness). Ascorbic acid as an antioxidant can have a favourable effect on the quantitative and qualitative characteristics of basil under environmental stress, so that the percentage of plant

essential oil under the influence of severe drought stress and consumption of ascorbic acid with an average of 0.42% was obtained, which was 12% higher than the ascorbic acid non-consumption treatment. Therefore, according to the results of this experiment, it can be said that the use of mycorrhizal fungus and Azospirillum bacteria simultaneously and ascorbic acid on basil can play the role of replacing plant enzymes and consequently reduce the damage of biomarkers. Degradation and increase of growth-promoting hormones in the plant ultimately improve the quantitative and qualitative characteristics of basil in different moisture conditions.

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