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Original Article

Effects of Fertilizer Treatments on Antioxidant Activities and Physiological Traits of Basil (*Ocimum basilicum* L.) under Water Limitation Conditions

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Abstract

Drought stress is a major environmental stress that restricts plant growth and production in the majority of agricultural fields of the world. The application of different fertilizers, especially biofertilizers and organic fertilizers, might play an important role in the production of medicinal plants in order to improve their resistance to deficit water stress. In order to evaluate the effects of fertilizer treatments on antioxidant enzyme activity and physiological characteristics of basils (*Ocimum basilicum* L.) under water-limited conditions, a study was arranged as a factorial layout based on a randomized complete block design with three replications. Two irrigation intervals (6, and 12 days) and six fertilizers levels [chemical fertilizers (N, P, K), vermicompost (10 t ha^{-1}) + mycorrhizal fungi (*Glomus intraradices*), vermicompost +bacterial biofertilizer [Azetobarvar1 (*Azotobacter vinelandii* as nitrogen-fixing bacteria), Phosphatebarvar2 (*Pseudomonas putida* and *Bacillus lentus* as phosphorus solubilizing bacteria), Pota barvar-2 (*Pseudomonas koreensis* and *Pseudomonas vancouverensis* as potassium releasing bacteria)], bacterial biofertilizer+mycorrhizal fungi, chemical fertilizers 50% (Basic NPK fertilizer was applied at the rate of 90–120–100 kg/ha in the form of urea, triple super phosphate, and potassium sulfate, respectively)+ bacterial biofertilizer and control] were assigned as the first and second experimental factors, respectively. The results showed that water limitation decreased the chlorophyll content and relative water content, but carotenoids and antioxidant enzyme activities (catalase, superoxide dismutase, and peroxidase) and also osmolytes (proline and sugar) contents were increased. But, the application of fertilizer sources alleviated the drought effects, so the application of fertilizers (especially chemical fertilizers 50% + bacterial biofertilizer) increased these traits at all irrigation levels. Overall, in addition to cellular mechanisms, such as osmoregulation and antioxidant defense, fertilizers sources application can improve antioxidant activities and physiological traits of basil under water-limited conditions.

Keywords: Biofertilizer, Medicinal plant, Mycorrhizae, Reactive oxygen species, Vermicompost

Introduction

Sweet basil (*Ocimum basilicum* L.) is a medicinal annual plant belonging to the Lamiaceae family. It is used in the pharmaceutical, food, cosmetic, and health industries [1]. Drought is one of the main environmental stresses throughout the world, which limits the production of crops, especially in arid and semi-arid areas. Deficit irrigation (DI) (i.e., irrigation below the optimum crop water requirements) is a strategy for water saving by which crops are subjected to a certain level of water stress either during a particular period or throughout the whole growing season. The detrimental effects of water deficit stress on growth and seed production of black cumin (*Nigella sativa* L.) are mainly due to the reduced water flow through the plants, which disturbs cellular

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metabolic pathways [3]. Plant organisms have enzymatic and non-enzymatic mechanisms that constitute a fundamental detoxification apparatus for overcoming oxidative damage induced by abiotic stress [19]. Several strategies have been developed in order to alleviate the toxic effects of severe water limitation on plant growth. Among them, the use of biofertilizers such as plant growth promoting rhizobacteria (PGPR) and Arbuscular mycorrhizal fungi (AMF), as well as organic fertilizers such as vermicompost, plays a crucial role in yield improvement, reduced application of chemical fertilizers via the improvement of soil properties in rhizosphere, enlargement of root areas, the ability to change water relations of their host plants and influence metabolism, quick activation of defense system, improvement of mineral nutrition and enhancement of drought tolerance of the host plants, while they are ecologically friendly, natural, and beneficial. Some researchers have reported improved water relations of host plants colonized with AMF and with PGPRs under moisture stress conditions [4]. Schweiger and Jakobsen [5] reported that under water stress, biofertilizers help the plants overcome the negative effects of water stress and increase significantly all growth characters. Also, Yuan *et al*. [6] showed that the combined usage of biofertilizers and chemical fertilizers influenced the physiological traits of plants. Heidari and Golpayegani [7] reported that PGPR application increased proline, chlorophyll, and water content of basils (*Ocimum basilicum* L.) under water stress conditions. Also, it has been reported that PGPR-inoculated seeds showed high antioxidant enzyme activities against water stress [2]. Ghavami *et al*. [8] indicated that sugars and proline were increased with the intensification of water deficit stress, while vermicompost and mycorrhiza consumption mitigated the severity of the increase. Also, chlorophyll *a* and *b* and catalase enzyme were enhanced by the integrated application of vermicompost and mycorrhiza as compared to the control treatment. In the present study, a field experiment was conducted to determine the effects of integrated fertilizers (N, P, K, mycorrhiza, bacterial biofertilizer, and vermicompost) on antioxidant enzyme activity and physiological responses of basils (*Ocimum basilicum*) under water-limited conditions at Miandoab climatic conditions.

Material and Methods

In order to evaluate the effects of fertilizer sources on antioxidant enzyme activity and physiological characteristics of basils under water-limited conditions, a study was arranged as a factorial layout based on a randomized complete block design with three replications in the Research Farm of the Shahid Bakeri Higher Education Center of Miandoab, University of Urmia, Iran, during the growing season of 2017. Some meteorological data of the experimental site in the growing seasons and the soil physicochemical characteristics are given in Tables 1 and 2, respectively.

The first factor was assigned to two irrigation intervals of 6 and 12 days, and the second experimental factor was assigned to six fertilization levels including chemical fertilizers (N, P, K), vermicompost (10 t ha^{-1})+mycorrhizal fungi (*Glomus intraradices*), vermicompost+bacterial biofertilizer [Azetobarvar1 (*Azotobacter vinelandii* as nitrogen-fixing bacteria), Phosphatebarvar2 (*Pseudomonas putida* and *Bacillus lentus* as phosphorus solubilizing bacteria), Pota barvar-2 (P*seudomonas koreensis* and *Pseudomonas vancouverensis* as potassium releasing bacteria)], bacterial biofertilizer+ mycorrhizal fungi, chemical fertilizers 50%+ bacterial biofertilizer, and control.

Basic NPK fertilizer was applied at the rate of 90–120–100 kg/ha in the form of urea, triples super phosphate, and potassium sulfate, respectively. All phosphorus and potassium were applied at the time of seedbed preparation. But, half of these amounts were applied in the chemical fertilizer 50%+ bacterial biofertilizer treatment. Nitrogen fertilizer was applied half at sowing and half at 6–8 leaf stage. Before seed sowing, 30 g inoculation (approximately 1200 spores) of *Glomus intraradices* was applied into each planting hole. The vermicompost was applied at the rate of 10 t ha^{-1} into the top 15 cm of soil in experimental plots. All plots consisted of eight crop rows 4 m in length with an on-row plant spacing of 0.4 m. Plots and blocks were separated by a buffer space of 1.5 m and 3 m, respectively. The seeds were sown on May 12, 2017. Before the seed plantation, the related biofertilizers (108 CFU) + 100 cm^3 water and sugar cube (20%) were blended completely to cover the whole seeds after which the seeds were shadow-dried. Bacterial biofertilizer was provided from Zist Fanavar Sabz Company.

All plots were irrigated immediately after sowing, but subsequent irrigations were carried out according to the treatments. Weeds were controlled by hand during plant growth and development as required. Basil aerial parts were harvested at 50% flowering stage for determined antioxidant activities and physiological traits.

Antioxidant enzymes assay

Anti-oxidative enzymes were extracted according to Syros *et al*. [9]'s method. Peroxidase activity was calculated by Ngo and Lenhoff [10]'s method. Superoxide dismutase activity was calculated by Minami and Yoshikawa [11]'s method. Catalase activity was determined by the decrease in absorbance of H_2O_2 [12].

Measurement of chlorophylls, sugar and proline content

The chlorophylls and carotenoids (carotene and xanthophyll) content of leaves were measured with Lichtenthaler and Welbum [13]'s method. Sugar content was measured with Desingh and Kangaraj [14]'s method. Also, proline content was measured with Bates [15]'s method.

Statistical analysis

Analysis of variance and means comparison were performed using the SAS software package. Duncan's multiple range test ($p \leq 0.05$) was performed to determine the significance of the results.

Results

In this study, irrigation intervals and fertilizer sources had significant effects on all studied traits of basils except for peroxidase enzymes. Also, analysis of variance showed a significant interaction between irrigation intervals and fertilizer sources on the carotenoid, soluble sugars content, proline and SOD enzymes (Table 3).

Chlorophylls and carotenoids

Chlorophyll (Chl) *a* and *b* contents decreased under water-limited conditions (Fig. 1a, b). But, carotenoid content was increased from the 12-day irrigation interval. The highest Chl *a* and *b* contents were obtained from the 6-day irrigation interval, whereas the lowest values were observed in irrigation after 12 days. There was a decrease of about 24.26 and 43.75% in the content of Chl *a* and *b* in irrigation after 12 days. Nevertheless, fertilizer sources mitigated the adverse effect of water deficit stress on Chl *a* and *b* contents. The highest Chl *a* and *b* contents were obtained by the application of chemical fertilizers 50% + bacterial biofertilizer treatment (Fig. 1c, d), but the lowest values were observed in control (no fertilizer application). When fertilizer sources were applied, carotenoid content was increased with the increase in irrigation interval to 12 days. With the application of chemical fertilizers 50%+bacterial biofertilizer, carotenoids were increased by 65.47% as compared to control (no fertilizer application in the 6-day irrigation interval) (Table 4).

Soluble sugar content

Our results showed that irrigation intervals enhanced sugar content in leaves of basil under fertilizer treatments (Table 4). Fertilization significantly increased sugar content at the severe water deficit stress levels compared to normal irrigation. The maximum values were obtained for vermicompost+mycorrhizal fungi. Under severe water deficit stress versus normal irrigation, the application of vermicompost+mycorrhizal fungi increased soluble sugars content by 71.56%.

Proline Content

Proline content was increased in the leaves of basils treated with fertilizers × irrigation intervals (Table 4). The highest proline content was recorded for the combined use of bacterial biofertilizer + mycorrhizal fungi, which showed approximately 67.62% under severe water deficit stress, respectively.

Table 1 Average rainfall, temperature and relative humidity of the experimental site during 2017 growing seasons.

Parameter	April	May	June	July	August	September
Monthly average temperature (°C)	13.1	18.5	22.5	26.3	25.7	21.8
Monthly average rainfall (mm)	1.2	0.4	0.04	0	0	0
Relative humidity (%)	51	41	36	38	40	46

Table 2 Physical and chemical properties of the soil and vermicompost.

	Texture	pH	EC (dS.m^{-1})	Organic matter (%)	Total N (%)	Available phosphorus (mg kg^{-1})	Available potassium (mg.kg^{-1})
Soil	Silty clay	7.79	0.92	0.82	0.09	11.24	189
Vermicompost	-	8.45	3.91	7.75	3.02	28900	31000

Table 3 Analysis of variance (mean of squares) for effects of irrigation intervals and fertilizer sources on studied traits of basil

S.O.V	DF	Chlorophyll a	Chlorophyll b	Carotenoid	Soluble sugars content	Proline	Catalase	Peroxidase	Superoxide dismutase	Relative water content
Replication	2	ns	ns	ns	*	ns	ns ns	ns	ns	ns
Irrigation (I)	1	**	**	*	**	**	*	ns	**	**
Fertilizer source (F)	5	**	**	**	**	**	**	ns	**	*
F×I	5	ns	ns	*	**	*	ns	ns	*	ns
CV (%)		7.47	9.46	11.38	7.54	13.25	6.71	17.67	13.60	4.26

*, ** and ns: are significant at 5 and 1% probability levels and non-significant, respectively.

Table 4 Mean comparisons for interaction effects of irrigation intervals and fertilizers sources on carotenoid content, soluble sugars content, proline and SOD (Unit/ g FW. Min) in leaves of basil. Means within each column followed by at least one letter in common are not significantly different based on Duncan's multiple range test P≤0.05.

Irrigation intervals (day)	Fertilizer treatments	Carotenoid (mg/g FW)	Soluble sugars content (mg/g DW)	Proline (mg/g DW)	Superoxide dismutase (Unit/ g FW. Min)
6 days	Control	0.733 e	1.457 f	2.033 c	0.693 e
	Vermicompost+ Mycorrhizal fungi	1.43 bc	2.497 de	3.01 bc	1.403 d
	Chemical fertilizers 50% + Bacterial biofertilizer	1.216 cd	2.203 de	2.57 bc	1.19 d
	Chemical fertilizers (N, P, K)	0.943 de	2.107 e	2.267 c	1.263 d
	Vermicompost+ Bacterial biofertilizer	0.863 de	2.577 cd	2.98 bc	1.210 d
	Bacterial biofertilizer+ Mycorrhizal fungi	1.39 bc	2.240 de	2.993 bc	1.367 d
12 days	Control	1.41 bc	2.933 c	3.4 b	1.41 d
	Vermicompost+ Mycorrhizal fungi	1.853 a	5.107 a	6.273 a	2.95ab
	Chemical fertilizers 50% + Bacterial biofertilizer	2.066 a	4.217 b	5.717 a	2.353 c
	Chemical fertilizers (N, P, K)	1.71 ab	4.197 b	5.570 a	2.303 c
	Vermicompost+ Bacterial biofertilizer	1.98 a	4.253 b	5.750 a	2.72 bc
	Bacterial biofertilizer+ Mycorrhizal fungi	2.123 a	4.54 b	6.277 a	3.167 a

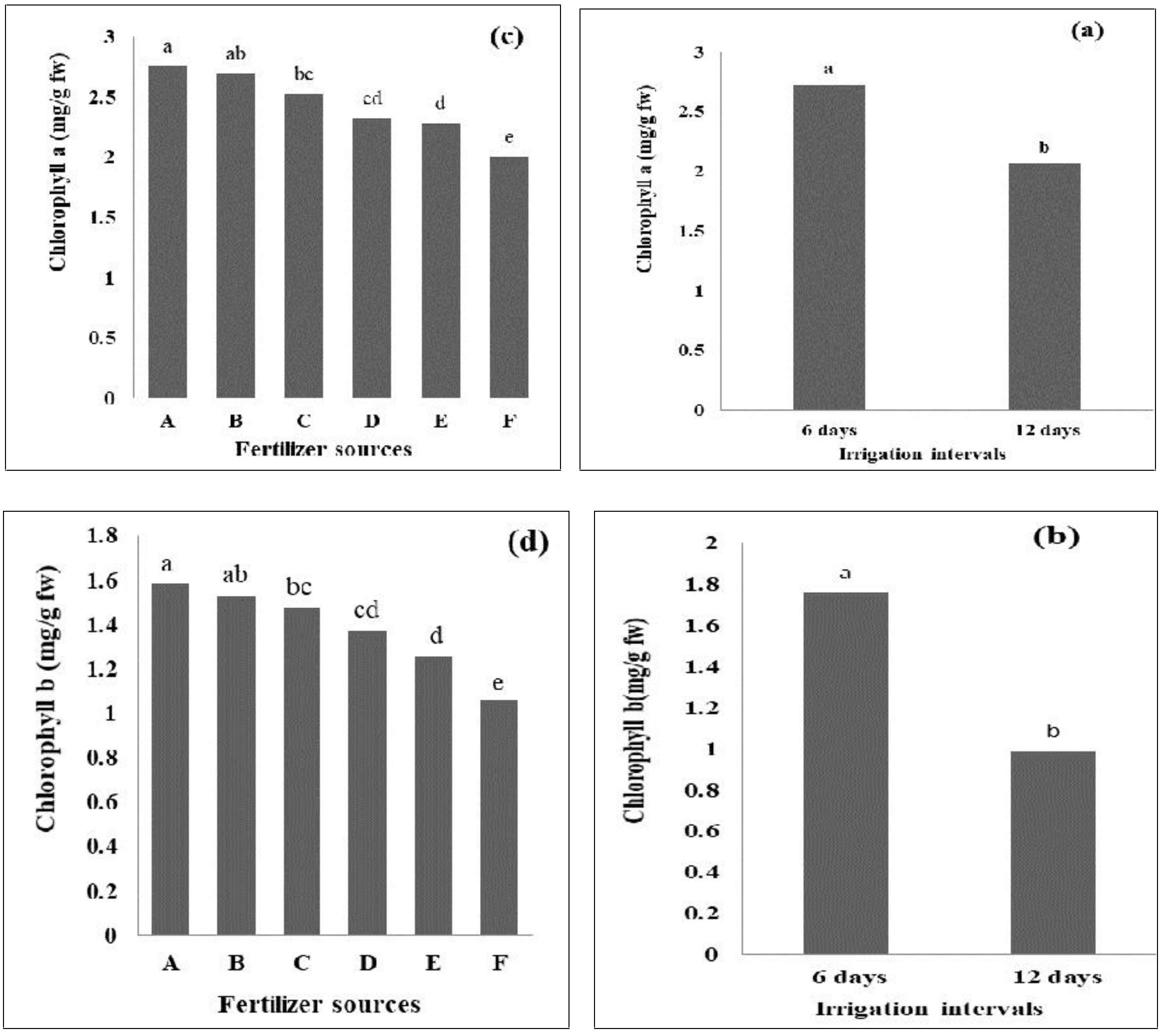

Fig. 1 Mean comparisons for the effects of irrigation intervals and fertilizers sources on chlorophyll a (a, c) and chlorophyll b (b, d) in basil. Means with different letters in each figure are not significantly different based on Duncan's multiple range test P≤0.05. A-chemical fertilizers 50%+bacterial biofertilizer; B- vermicompost+ mycorrhizal fungi; C-bacterial biofertilizer+ mycorrhizal fungi; D-vermicompost+ bacterial biofertilizer; E- chemical fertilizers (N, P, K); F-control.

Relative Water Content (RWC)

RWC content was decreased under irrigation intervals (Fig. 2a), but it was increased significantly by the application of fertilization sources. Also, the highest RWC of 78.67% was observed with combined application of chemical fertilizers 50%+bacterial biofertilizer, and the lowest RWC of 66.33% was obtained from control (no fertilizer application) (Fig. 2b)

Antioxidant Enzymes

Catalase (CAT) activity

CAT activity was significantly increased with severe water limitation (Fig. 3a). Severe water limitation (12-day irrigation interval) increased CAT activity by as much as 32.94% as compared to plants irrigated at 6-day intervals. The maximum values were obtained when chemical fertilizers 50%+bacterial biofertilizer was applied (Fig. 3b) so that the application of NPK+biofertilizers increased CAT activity by 31.32%.

Superoxide Dismutase Activity (SOD)

SOD was significantly influenced by the interaction between fertilizer sources and irrigation intervals (Table 4). The increase in water deficit stress severity significantly increased SOD under the application of different fertilizer sources. For instance, the application of fertilizer sources, especially bacterial biofertilizer+mycorrhizal fungi and vermicompost+mycorrhizal fungi treatments under severe water deficit stress (12-day irrigation interval), increased SOD by 78.06% and 76.50% as compared to control (no fertilizer application and 6-day irrigation interval), respectively.

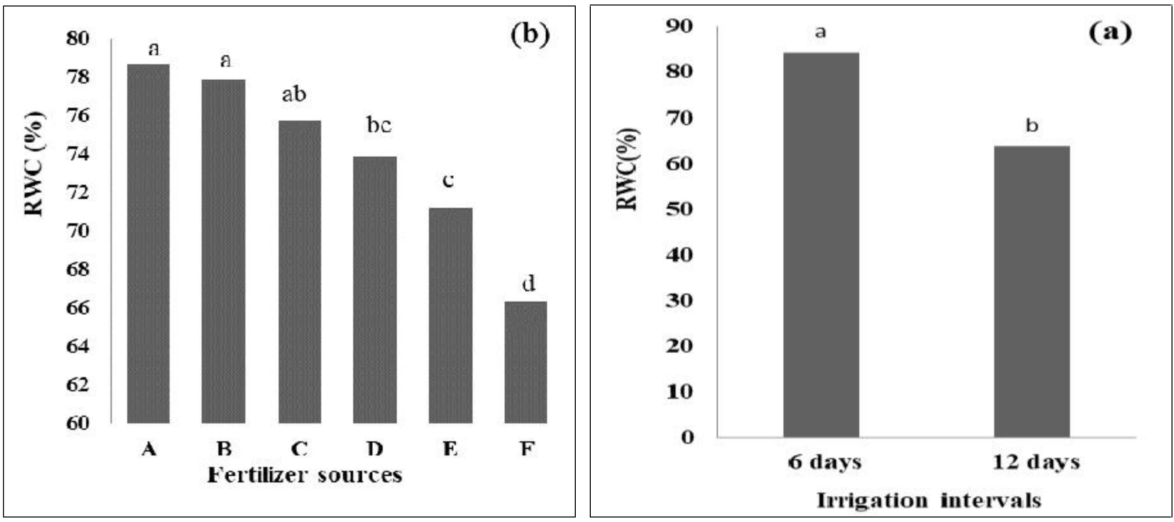

Fig. 2 Mean comparisons for the effects of fertilizer treatments (a) and irrigation intervals (b) on relative water content (RWC) in leaves of basil. Means with different letters in each shape are not significantly different based on Duncan's multiple range test P≤0.05. A-chemical fertilizers 50%+bacterial biofertilizer; B-vermicompost+ mycorrhizal fungi; C-bacterial biofertilizer+ mycorrhizal fungi; D-vermicompost+ bacterial biofertilizer; E-chemical fertilizers (N, P, K); F-control.

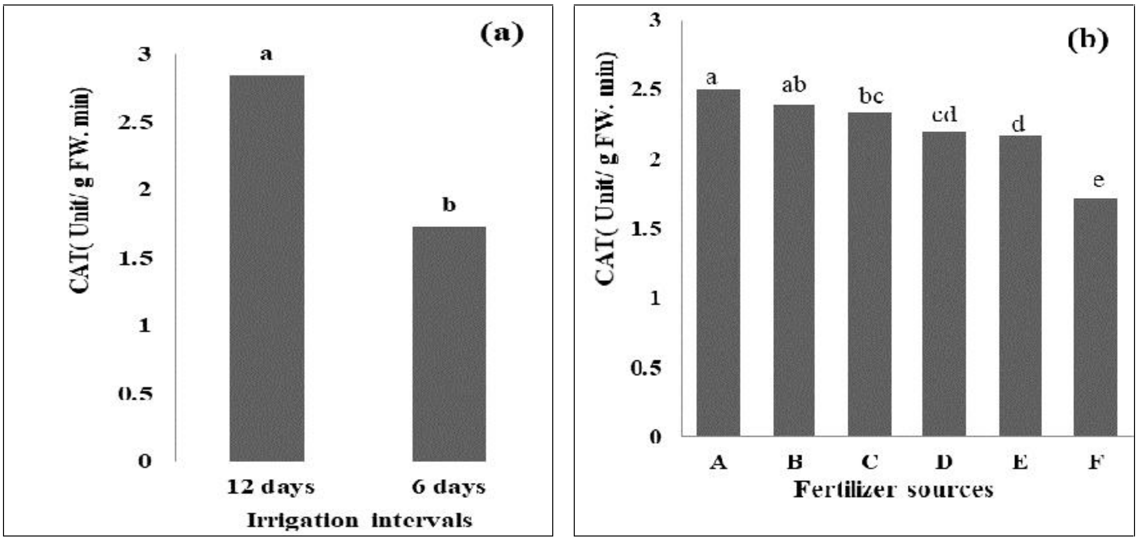

Fig. 3 Mean comparisons for the effects of irrigation intervals (a) and fertilizers sources (b) on catalase activity in leaves of basil. Means with different letters in each figure are not significantly different based on Duncan's multiple range test P≤0.05. Different letters indicate significant difference (*P*<0.05) between fertilizers at control and drought treatments. A-chemical fertilizers 50%+bacterial biofertilizer; B-bacterial biofertilizer+ mycorrhizal fungi; C-vermicompost+ mycorrhizal fungi; ; D- vermicompost+ bacterial biofertilizer; E- chemical fertilizers (N, P, K); F-control.

Discussion

Plants usually contain more than 70% by weight of water, and it is essential to maintain satisfactory water content for plant growth and metabolism [19]. Some plants are, to some degree, adapted to water stress. Fertilizer sources are a particularly effective biocontrol agent against abiotic stresses, especially water stress, in plants, including basil. In the present study, we tested whether the physiological and biochemical induced by application fertilizer sources especially biofertilizer and organic fertilizer are able to prime basil plants for enhanced tolerance to a water stress of mild intensity, a condition that recurrently occurs during the growing season.

Water stress influences enzyme activity and therefore influences all metabolic processes [16]. Chlorophyll concentration is one of the key factors in determining the intensity of photosynthesis and dry matter production. Under water stress conditions, a significant decrease was seen in total Chl, Chl *a* and Chl *b* concentrations in plants [17]. Degradation by reactive oxygen species (ROS), Beta carotene destruction and Zea xanthin formation were previously reported as the main reasons for the decrease in chlorophyll under water deficit stress [18]. Mineral elements including zinc, nitrogen and magnesium are essential constituents of chlorophyll molecules. Fertilizer sources increased the chlorophyll content in leaves by improving the nutrient availability. These conditions could effectively improve the mobilization and uptake of nitrogen, potassium, phosphorus and micronutrients. Rezaei-Chiyaneh *et al*. [3] indicated that the amount of Chl *a* and *b* and their ratio were significantly decreased under water deficit stress in black cumin (*Nigella sativa* L.). In drought stress, nitrogen recovery is reduced, and chloroplasts require nitrogen to produce chlorophyll which results in reduced rate of chlorophyll production and as a result, leaves become more susceptible to photoinhibition [18]. Consistent with them, we found that Chl content was decreased under drought stress, but Chl content was increased with the application NPK 50%+ biofertilizer treatment. Also, these results are in agreement with Ashraf and Harris [19] who reported that Chl concentration was decreased and carotenoid concentration was increased with the increasement in drought stress.

Low chlorophyll content under stress was reported as a result of lower chlorophyll synthesis that destroys the PSII reaction center, inhibits carbonic anhydrase and nitrate reductase activities, causes an imbalance in the ion flux inside plants, affects membrane stability index, and reduces RWC [20]. On the other hand, the reduction of chlorophyll and other pigments finally results in the loss of photosynthesis efficiency.

Plants protect cells and subcellular systems against reactive oxygen radicals through both enzymatic and non-enzymatic antioxidant systems such as carotenoids [19]. Our results are consistent with them. Enzymatic activity and carotenoid content were increased under drought conditions. That increase was higher in fertilizer treatments than in control. The change in the activity of antioxidant enzymes is a defense mechanism of plants under oxidative stress induced by environmental stresses [21]. In the present study, the interaction between drought and fertilizer showed that the highest activity of CAT was obtained from the use of NPK50%+ biofertilizer. Also, mycorrhizae + biofertilizer treatment showed the highest SOD activity under drought stress.

Proline and soluble sugars content contribute to osmotic adjustment during stress and protect the structure of macromolecules and membranes during extreme dehydration [22]. The beneficial effect of higher osmolyte concentration is reflected in the maintenance of higher RWC and stabilization of essential enzyme proteins such as catalase, POD, and SOD resulting in higher activity under stress [23]. Proline reduces cytoplasmic pH and maintains the proper ratio of $NADP^+$/NADPH in metabolism and increases different enzymatic activities [24]. The highest content of proline and soluble sugars was observed in vermicompost treatment, but the minimum of these osmolytes was observed in control plants. An increase has been reported in soluble sugars and proline accumulation and CAT and POX activity in black cumin under water deficit stress [3].

Based on these results, we can conclude that ability of biofertilizers to produce various phytohormones which at low concentrations influence plant growth and performance, and important bioactive molecules that stimulate plant growth, fix nitrogen, and enhance water and mineral uptake by plants among other mechanisms, result in balanced nutrition of plants and increase antioxidant activities and physiological traits[2].

Photosynthetic pigments and proline are both synthesized from the same substrate [25]. Thus, an increase in the synthesis of proline leads to the decrease in chlorophyll content in water stress conditions. The reduction of chlorophyll and other pigments finally impairs the efficiency of photosynthesis. A decrease in this ratio results from the photosynthetic electron transport impairment [26]. There was a negative significant correlation ($P<0.05$) between chlorophyll and proline content in our plants.

It was found that plants that accumulated higher proline content during drought were more tolerant to drought [27]. Therefore, fertilizer treatments caused basil plants to tolerate drought conditions better than others. The same response was, also, observed in Ghavami *et al*. [8] who revealed that water deficit stress increased proline content of basils, while the higher amounts of vermicompost fertilizer consumption alleviated the damages of water deficit stress rising from its higher water retention capacity.

Relative water content (RWC) is used as the most meaningful index to contrast the differences in dehydration tolerance [28]. Kazeminasab *et al*. [29] reported that the relative water content of basils was positively affected by biofertilizer application under water stress. Therefore, drought decreased RWC by 10.86% in comparison with normal irrigation. Also, the consumption of 5 t/ha vermicompost increased it by 9.17% as compared to the control treatment.

High RWC is a resistant mechanism to stress those results from more osmotic regulation or less elasticity of tissue cell wall [30]. It was, also, found that higher RWC indicates a better plant water status. Higher RWC in biofertilizer-treated plants may be beneficial for moving water through the plants to the evaporating surfaces and maintaining stomata open in leaves [31]. In the present study, RWC was decreased under drought stress, but plants that were treated with fertilizers outperformed control plants in retaining their water. There was a positive significant correlation ($P \leq 0.05$) between chlorophyll and RWC content in our plants.

Conclusion

The results of the study showed that the application of biofertilizer and organic fertilizers had significant improvements in some physiological characteristics of basils. Because of more nutrient uptake and symbiosis with mycorrhizal fungi, water, and mineral uptake was increased, and thereby the negative effects of drought stress were alleviated. Due to global attention to sustainable agriculture, environment, and human health, organic fertilizers and biofertilizers can be as alternative fertilizer sources. Furthermore, the results suggest that soluble sugars, proline, and antioxidant enzymes can possibly play a role in the tolerance of basils to water deficit.

References

1. Penuelas J, Munne-Bosch S. Isoprenoids: an evolutionary pool for photoprotection. Trends Plant Sci. 2005;10:166-169.
2. Khalilzadeh R, Seyed Sharifi R, Jalilian J. Antioxidant status and physiological responses of wheat (*Triticum aestivum* L.) to cycocel application and bio fertilizers under water limitation condition. J Plant Interact. 2016;1:130-137.
3. Rezaei-Chiyaneh E, Seyyedi SM, Ebrahimian E, Siavash Moghaddama S, Damalasd CA. Exogenous application of gamma-aminobutyric acid (GABA) alleviates the effect of water deficit stress in black cumin (*Nigella sativa* L.). Ind Crops Prod. 2018;112:741-748.
4. Kaushal M, Wani SP. Plant-growth-promoting rhizobacteria: drought stress alleviators to ameliorate crop production in drylands. Ann Microbiol. 2016;66:35-42.
5. Schweiger PF, Jakobsen I. Direct measurement of arbuscular mycorrhizal phosphorus uptake into field-grown winter wheat. Agron J. 1999;91:998-1002.
6. Yuan J, Ruan Y, Wang B, Zhang J, Waseem R, Huang Q, Shen Q. Plant growth promoting rhizobacteria strain Bacillus amyloliquefaciena NJN- 6- enriched bio-organic fertilizer suppressed Fusarium wilt and promoted the growth of banana plants. J Agric Food Chem. 2013;61:3774-378.
7. Heidari M, Mousavinik SM, Golpayegani A. Plant growth promoting rhizobacteria (PGPR) effect on physiological parameters and mineral uptake in basil (*Ociumum basilicm* L.) under water stress. J Agric Biol Sci. 2012;6:6-11
8. Ghavami A, Abdossi V, Rafiee M, Khalighi A. The effect of mycorrhiza and vermicompost bio-fertilizers on some physiological characteristics of sweet basil plant (*Ocimum basilicum* L.) under the stress condition caused by water deficit. Ukr J Ecol. 2017;7:325-329.
9. Syros T, Yupsanis T, Economou A. Factors affecting the determination of peroxidase activity of Ebenus cretica L. cuttings- A preliminary survey. Propag. Ornam. Plants. 2001;1:50-53.
10. Ngo T, Lenhoff M. A sensitive and versatile chromogenic assay for peroxidase and peroxidase-coupled reactions. Anal Biochem. 1980;105:389–397.
11. Minami M, Yoshikawa H. A. Simplified assay method of superoxide dismutase activity for clinical use. Clin Chem Acta. 1979;92:337-342.
12. Aebi H. Catalase in vitro Methods Enzymol. 1984;105:121-126.
13. Lichtenthaler HK; Welbum AR. Determination of total carotenoids and chlorophylls a and b leaf extracts in different solvents. Biochem Soc Trans. 1983;11:591-592.
14. Desingh R, Kangaraj G. Influence of salinity stress on photosynthesis and antioxidative system in two cotton varieties. J Plant Physiol Pathol. 2007;33:221-234.
15. Bates L. Rapid determination of free proline for water stress studies. Plant Soil. 1973;39:205-207.
16. Esfandiari E, Shakiba MR, Mahboob SA, Alyari H, Shahabivand S. The effect of water stress on the antioxidant content, protective enzyme activities, proline content and lipid peroxidation in wheat seedling. Pak J Biol Sci. 2008;11:1916-1922.

17. Ranjbar Fordoei A, Dehghani Bidgholi R. Impact of salinity stress on photochemical efficiency of photosystem II, chlorophyll content and nutrient elements of nitere bush (*Nitraria schoberi* L.) plants. J Range Sci. 2016;6:3-9.
18. Paknejad F, Majidi heravan E, Noor Mohammadi Q, Siyadat A, Vazan S. Effects of drought stress on chlorophyll fluorescence parameters, chlorophyll content and grain yield of wheat cultivars. J Biol Sci. 2007;7:841-847.
19. Ashraf M, Harris PJC. Photosynthesis under stressful environments: an overview. Photosynthetica. 2013;51:163-190.
20. Talaat NB, Shawky BT. 24-Epibrassinolide ameliorates the saline stress and improves the productivity of wheat (*Triticum aestivum* L.). Environ Exp Bot. 2012;82:80-88.
21. Gao S, Ouyang C, Wang S, Xu Y, Tang L, Chen F. Effects of salt stress on growth, antioxidant enzyme and phenylalanine ammonia lyase activities in *Jatropha curcas* L. seedlings. Plant Soil Environ. 2008;54:374-381.
22. Farhoudi R, Modhej A, Afrous A. Effect of salt stress on physiological and morphological parameters of rapeseed cultivars. J Sci Res Dev. 2015;2:111-117.
23. Ashraf M, Foolad MR. Roles of glycine betaine and proline in improving plant abiotic stress resistance. Environ Exp Bot. 2007;59:206-216.
24. Szabados L, Savouré A. Proline: a multifunctional amino acid. Trends Plant Sci. 2010;15:89-97.
25. Aspinall D, Paleg LG. Proline accumulation: physiological aspects, In: Paleg LG, Aspinall D, eds. The Physiology and biochemistry of drought resistance in plants. Australia: Academic Press, Sydney. 1981;205-241.
26. Pereira WE, De Siqueira DL, Martínez CA, Puiatti M. Gas exchange and chlorophyll fluorescence in four citrus rootstocks under aluminum stress. J Plant Physiol. 2000;157:513-520.
27. Barnabas B, Jager K, Feher A. The effect of drought and heat stress on reproductive processes in cereals. Plant Cell Environ. 2008;31:11–38
28. Sinclair TR, Ludlow MM. Influence of soil water supply on the plant water balance of four tropical grain legumes. Aust. J Plant Physiol. 1986;13:329-341.
29. Kazeminasab A, Yarnia M, Lebaschy MH, Mirshekari B, Rejali F. The Effect of Vermicompost and PGPR on Physiological Traits of Lemon Balm (*Melissa officinalis* L.) Plant under Drought Stress. J Med Plant & By-product. 2016;2:135-144.
30. Ritchie SW, Nguyen HT, Holaday AS. Leaf water content and gas exchanges parameters of two wheat genotypes differing in drought resistance. Crop Sci. 1990;30:105-111.
31. Nelsen CE, Safir GR. The water relations of well-watered, mycorrhizal and non-mycorrhizal onion plants. J Am Soc Hortic Sci. 1982;107:271-274.