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Original Article

Effects of Biological Fertilizers on Some Physiological Traits of Sweet Basil under Water Deficit Stress

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ABSTRACT

Today, the use of biological fertilizers in sustainable agriculture is an appropriate alternative to chemical fertilizers because the former can improve the quantitative and qualitative performance of plants, especially under stressful conditions. Therefore, this study aims in greenhouse conditions to investigate the effects of plant growth-promoting rhizobacteria on antioxidant enzyme activities and some physiological traits of sweet basil under water limitation. For this purpose, a factorial experiment was conducted based on a completely randomized block design with three replications. Three levels of water deficit stress factor involved W0 = 100% of field capacity, W1= 60% of the field capacity, and W2 = 40% of the field capacity. Also, biofertilizers factor included nine levels of F1: Pota Barvar-2, F2: Phosphate Barvar-2, F3: Azeto Barvar 1, F4 (the combination of F1 and F2), F5 (the combination of F1 and F3), F6 (the combination of F2 and F3), F7 (the combination of F1, F2, and F3); F8 (100% chemical fertilizer as a positive control) and F9 (without any fertilizer as a negative control). Results showed that water limitation increased the activity of ascorbate peroxidase (193.55%), peroxidase (416.258%), polyphenol oxidase (48.21%) enzymes, and essential oil yield (135.48%). Meanwhile, the chlorophyll index, carotenoid, and yield decreased under water deficit stress. The use of biofertilizers improved these traits under water limitation conditions and normal irrigation. Also, applying a combination of 3 biofertilizers (F7) led to an increase 29.88% in the yield compared with negative control under severe water limitation. Therefore, the use of biofertilizer can be recommended for profitable basil production under water limitation conditions.

Abbreviations: Catalase: (CAT), Peroxidase: (PROX), Ascorbate peroxidase: (ASP)

INTRODUCTION

Sweet basil *(Ocimum basilicum* L.*)* is an annual plant that belongs to the family of Lamiaceae. It is a low-growing plant that is cultivated in warm and tropical climates. Basil grows between 30 to 60 cm. its stem is square and its leaves are opposite [1], light green, silky, smooth and shiny, 3 to 7 cm long and, 1 to 3 cm wide. The flowers are large, white and, located on a terminal spike [2]. The essential oil of this plant is used in the perfumery, cosmetics and pharmaceutical industries. Basil can also be used as an anti-Alzheimer's plant. The essential oil of this plant is used in the treatment of diseases such as headache, diarrhea, cough, warts, intestinal worms and kidney failure [3]. In Iran, Khuzestan province is the largest producer of basil. The area under basil cultivation in Iran is 1139 hectares, which accounts for 23% of the total area under vegetable cultivation and 0.009% of the total area under cultivation of crops [4]. The annual production of basil essential oil as an important economic product in the world is 100 tons per year [5]. Various studies have shown that conventional agriculture with the excessive use of inputs chemical involves some serious damage to the environment. There are several solutions such as organic farming to deal with common agricultural problems. Today, organic farming is growing rapidly such that many countries, especially European ones, have included the development of biological programs in their implementation plans [6]. Biofertilizers are products that contain living cells of various types of microorganisms such as plant-growth-promoting rhizobacteria (PGPR), which play an essential role in adaptation strategies and increasing tolerance to abiotic stresses in

agricultural plants [7]. PGPR affects plant growth by several mechanisms such as the ability to produce various compounds (e.g., phytohormones, siderophores, and organic acids), nitrogen fixation, phosphate, and potassium solubilization, producing antibiotics that suppress deleterious rhizobacteria and producing biologically active substances or plant growth regulators. The last one is of the main mechanisms by which PGPR affects plant growth and development [8, 9, 10]. Because of their lower costs, biofertilizers are more economical to use compared to chemical fertilizers [11]. Inoculation with biofertilizer not only leads to increased yield of medicinal herbs but also affects the quantity and quality of its active ingredients. Different types of bio-fertilizers are used in Iran, leading to improved quantitative and qualitative production of medicinal plants such as fennel (*Foeniculum vulgare* L.) [12], borage plants (*Borago officinalis* L.) [13], and wormwood (*Artemisia absinthium* L.) [14] because of inoculation with biofertilizers.

One of the most important environmental factors that affect the successful growth and production of plant products is the amount of water available to the plant. Iran with an average rainfall of 250 ml per year (less than one third of the average rainfall in the world) is classified as an area with a dry climate and except for the Caspian Sea coast and small parts of the northwest, the rest of the region is part of Arid and semi-arid climates are classified [15]. If we consider the areas under drought stress as areas with an annual rainfall of less than 500 mm, it can easily be said that more than 90% of the country is under drought stress [16]. The reduction of available plant water leads to drought stress and numerous morphological, physiological and biochemical changes in plants [17]. This natural phenomenon changes the content and components of chlorophyll, inhibits the photosynthesis of plants, and damages the photosynthetic apparatus [18]. Drought-induced production of reactive oxygen species (ROS), such as superoxide (O^{2-}), hydrogen peroxide (H_2O_2), hydroxyl radicals (·OH), and singlet oxygen ($O^{\cdot}_2$), is commonly reported in the literature, which may accumulate and damage the photosynthetic apparatus [19,46]. The breakdown of the balance between the antioxidant defense and the production of reactive oxygen species is among the main mechanisms by which environmental stress inhibits the growth and photosynthetic abilities of plants (20). Plants under stress apply some defense mechanisms to protect themselves from the ROS harmful effects. ROS scavenging is one of the mechanisms against abiotic stresses [16]. ROS scavenging depends on enzymatic and non-enzymatic mechanisms. Enzymatic antioxidants include guaiacol peroxidase (POD), ascorbate peroxidase (APX), catalase (CAT), polyphenol oxidase (PPO), and glutathione reductase (GR). On the other hand, non-enzymatic includes β-carotenes, ascorbic acid (AA), α-tocopherol (α-toc), and reduced glutathione [21].

Drought stress while reducing the water content in plant tissues causes bruising and exacerbates other stresses, especially nutrient deficiency stress for the plant. The availability of different nutrients in the soil changes significantly under the influence of drought stress. Therefore, plant nutrition management in stressful conditions is one of the most critical issues in producing plant products [22]. Undoubtedly, a plant that is well-nourished and has received enough nutrients will have better drought resistance [23].

Regarding the need to manage plant nutrition and meet the nutritional needs of plants in conditions of water shortage stress and achieve sustainable agricultural goals, the purpose of this study is to investigate the effect of water stress on some quantitative and qualitative characteristics of basil and the effect of biofertilizers to compensate of dehydration stress.

MATERIALS AND METHODS

This is a factorial experiment based on a randomized complete block design experiment that was carried out in the greenhouse (12 hours of light, temperature of 18-25 oC, relative humidity of 60-80%) of Shahid Bakeri High Education Center of Miandoab, Iran. The experimental factors included three water deficit stress levels and nine biofertilizer levels. The water deficit stress levels included 100% of field capacity (W_0= control), 60% of the field capacity (W_1= moderate water limitation), and 40% of the field capacity (W_2= severe water limitation). On the other hand, the biofertilizer levels included Pota Barvar-2 (0.6 g) containing bacterial strains *Pseudomonas koreensis* and *Pseudomonas vancouverensis* as Potassium releasing bacteria (F1=K), PhosphateBarvar-2 (0.6 g) containing bacterial strains *Pseudomonas putida* and *Bacillus lentus* as phosphorus solubilizing bacteria (F2=P),

Azeto Barvar 1 (0.6 g) containing bacterial strains Azotobacter *vinelandii* as nitrogen-fixing bacteria (F3=N), the combination of F1 + F2 (F4 = KP) (0.3 g Pota Barvar-2 + 0.3 g PhosphateBarvar-2), the combination of F1 + F3 (F5=KN) (0.3 g Pota Barvar-2 + 0.3 g Azeto Barvar 1) , the combination of F2 + F3 (F6=PN) (0.3 g PhosphateBarvar-2 + 0.3 g Azeto Barvar 1) , the combination of F1+ F2 + F3 (F7=NKP) (0.2g Pota Barvar-2 + 0.2 g PhosphateBarvar-2+ 0.2 g Azeto Barvar 1)), 100% chemical fertilizer (According to the results of soil analysis table 1, 3.8 g of urea, 3.3 g of potassium sulfate and 1.1 g of superphosphate were used) as a positive control (F_8= Positive), and no fertilizer as the negative control (F_9=Negative). Biofertilizers were provided from Green Biotech Co., Iran (containing 10^8 alive and active bacteria per gram). Also, basil seeds cultivar "purple" were obtained from Esfahan Agricultural and Natural Resources Research and Education Center, Isfahan, Iran. After surface disinfection (10% sodium hypochlorite for 5 min), the seeds were inoculated with each biofertilizer, and then seeds were dried in the shade. Next, 20 seeds were sowed in each pot, and then they were thinned to 7 plants per pot. The amount of water required by the pots was estimated by daily weighing of the pots and weight difference based on the desired FC humidity and daily to the mentioned humidity. Basil was harvested 54 days after planting. The aerial part was taken from the top of the lowest stem node and their fresh weight was measured with a digital scale and then the samples were dried in the shade and their dry weight was determined. Physio-chemical properties of the soil used in this experiment are presented in Table 1.

Measurements

Enzymatic Antioxidants Activities

About 0.1 g of fresh leaf tissue was homogenized in 0.9 ml of 0.1 M sodium phosphate buffer (pH 7.2) at 4°C. The homogenate was centrifuged at 20,000 rpm for 15 min. The supernatant served as an enzyme source.

Polyphenol oxidase activity was carried out according to Mayer et al. [24]. The reaction mixture consisted of 1250 µl of 50 µM sodium phosphate buffer (pH 7.2) and 6 µl of the enzyme extract. The reaction was initiated by adding 700 µl of 0.1M catechol and the activity was expressed as a change in absorbance at 495 nm at 30-s intervals for 3 min. The enzyme activity was expressed as a change in absorbance min^{-1} g fresh weight of leaves.

Peroxidase was assayed according to Chance and Maely method [12]. The reaction mixture consisted of 20 µL enzyme source, 1920 µL sodium phosphate buffer (pH 7.2), 20 µL guaiacol and 10 µL H_2O_2 solution. After mixing the reaction absorbance in optical density at 470 nm, it was continuously recorded every 5 s (for 1 min). Ascorbate peroxidase was assayed following the instructions of Nakano and Asada [22]. The reaction mixture consisted of 20 µL enzyme source, 1650 µL sodium phosphate buffer (pH 7.2), 200 µL EDTA, and 10 µL H_2O_2 solution. After mixing the reaction absorbance in optical density at 290 nm, it was continuously recorded every 5 sec (for 1 min).

Chlorophyll content (SPAD)

Leaf chlorophyll content was measured using a hand-held chlorophyll content meter (CCM-200, Opti-Science, USA) (26).

Carotenoids

The total carotenoids were assessed based on the method proposed by Arnon et al. [25]. In this process, we used an extraction with 80% acetone and read the solution absorbance by a spectrophotometer at 645, 663, and 470 nm wavelengths using the following formula: Total carotenoids = [1000 A470 − 1.9 Ca − 63.14 Cb]/214

Essential Oil Percentage

Quantitative determination of the essential oil from basil subjected to the different treatments was achieved by placing the air-dried herbage in a 1 L flask with distilled water (1:15 w/v) and using a Clevenger apparatus, as described by Charles and Simon [26]. The average essential oil content of aerial parts is reported as the dry matter percentage of the plant.

Total Phenolic Content

Total phenolics in extracts were determined with the Folin-Ciocalteau reagent using the method by Gao et al. [27]. For this purpose, 0.1 g of each sample was grounded in 2 ml methanol and centrifuged. Next, to 50 µl of the extract, 450 µl distilled water and 2.5 ml of 10% Folin-Ciocalteau reagent were added and put in a dark place for 6 min. Afterward, 2 ml of Na_2CO_3 (7.5%, w/v) was added and incubated at room temperature for 1.5 h in the dark. The absorbance of all samples was measured at 760 nm. Gallic acid was used as standard and results are

expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dw).

Statistical analysis

Data analysis was performed using the analysis of variance (ANOVA) in SPSS software (v. 24.0). Also, Duncan's Multiple Range Test was used to measure the significant differences between treatments ($P < 0.05$).

RESULTS AND DISCUSSION

Growth Trait

Fresh and dry weights of aerial parts are the main yield component in vegetative plants like basil. The yield was significantly affected by bio-fertilizers, water limitation, and their interaction (Table 2). On the other hand, it decreased by the increase in water limitation. The results showed that using biofertilizers, especially NPK biofertilizers, improved plant tolerance, and increased fresh and dry weights (Table 3). The highest fresh and dry weight 43.71 and 11.35 g/Plant, respectively was obtained in normal irrigation (W_1) and when using biofertilizer as F_7 (Figs. 1 and 2). The lowest yield (9.67 g/Plant) was obtained in severe water limitation (W_3) and without using biofertilizer application of K-releasing biofertilizers. Rezaei et al. [28] reported that Severe water stress decreased plant biomass, essential oil content, yield, and chlorophyll a content by relative to normal irrigation. Water stress affects all metabolic processes by affecting enzyme activity [29]. However, at low concentrations, they influence plant growth and performance. In this regard, important bioactive molecules that stimulate plant growth, fix nitrogen enhance water and mineral uptake by plants [30], balance the nutrition of plants, and increase plant yield. Noorieh *et al*. [9] proposed co-inoculation with biofertilizer as an efficient procedure to increase plant growth.

Antioxidant Enzymes Activities

ANOVA results indicated the significant effects of drought stress levels, PGPRs, and their interaction with POX, PPO, and APX activities in basil leaves (Table2). The results revealed an increase in enzyme activities in leaves of basil plants under drought stress (Table 3). The results also showed that the highest activity of POX (10.54 and 7.9 OD µmol/mg protein/min), APX (21.37 and 16.42 OD µmol/mg protein/min), and PPO (0.0166 and 0.0158 OD µmol mg/protein/min) occurred in W_3 and F_7 = NPK, respectively (Table 3). In supporting our finding, some previous studies have reported increased POD and PPO activity under drought stress conditions in various plants such as sunflower [23], poplar [31], and brassica species [32]. Environmental stresses result in the formation of reactive oxygen species in plants, causing destructive oxidative processes such as lipid peroxidation, chlorophyll bleaching, protein oxidation, and damage to nucleic acids, and finally reducing plant vigor and yield. The activity rate of antioxidative enzymes determines the amount of damage that will occur in the plant [32]. A rapid increase in antioxidative enzyme activity in this study might indicate that POX, APX, and PPO are major enzymes detoxifying hydrogen peroxide in plants under water deficit stress. Application of biofertilizers under water limitation significantly increased POX, PPO, and APX enzyme activities (Figs. 3, 4, and 5). Stefan *et al*. [33] and Noorieh *et al*. [32] suggested that biofertilizers significantly improve PPO, SOD, and POD activities, alleviating the oxidative damage induced by drought and salinity. Kleiner et al. [34] claimed that good soil fertility increases the ability of plants to maintain relatively high levels of growth, stomatal conductance, and photosynthesis under drought conditions, but fertilizing with 100% chemical fertilizer did not improve better antioxidant activity compared with biofertilizers. Therefore, one may assume that PGPR strains can prevent oxidative stress by increasing antioxidant enzyme activities with intense photosynthesis.

Total Phenolic Content and Essential Oil Percentage

According to Table 2, the values of essential oil percentage and total phenolic content extracted from the leaves of basil plants were affected by drought stress levels, PGPRs, and their interaction. These two secondary metabolites increase significantly in response to water stress and the use of PGPRs. Such an increase was most pronounced with increasing the severity of drought and combined use of biofertilizers (Figs. 6 and 7). PGPR and osmotic stresses are classified as biotic and abiotic elicitors for generating secondary metabolites in medicinal plants [12]. The reason is that in this condition, more metabolites are produced to prevent oxidization in the cells [35]. The increase in essential oil content under drought stress may be related to decreased leaf area and increased number

of essential oil glands [36]. Besides, the stimulation of essential oil production under water stress may be due to a higher terpene production [36]. Khalid [37] reported a significant increase in essential oil percentage and the main constituents of essential oil under the influence of water stress in two species of *O. basilicum* L. (sweet basil) and *O. americanum* L. (American basil). However, drought stress reduces plant biomass (which is a key determinant of essential oil percentage per plant) and the application of biofertilizer elevates biosynthesis of secondary metabolites content.

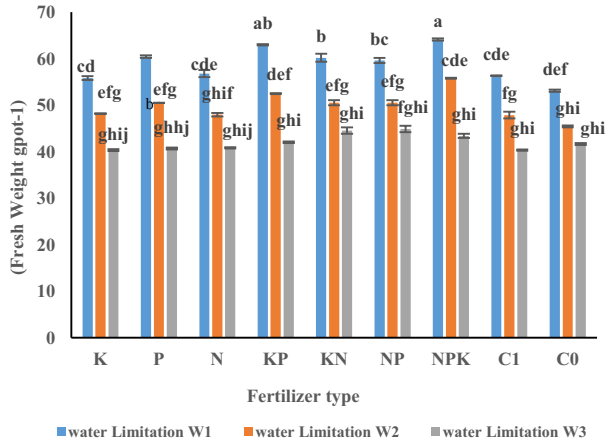

Fig. 1 Effects of biofertilizer × water limitation on Fresh Weight Yield of basil

W1, W2, and W3 denote normal irrigation (100% of field capacity), moderate water stress (60% of field capacity), and severe water stress (40% of field capacity). F1, F2, F3, F4, F5, F6, F7, F8, and F9 show seed inoculation with K-, P-, N-, KP-, NK-, NP-, and NKP-releasing PGPR, 100% chemical fertilizer, and without inoculation, respectively.

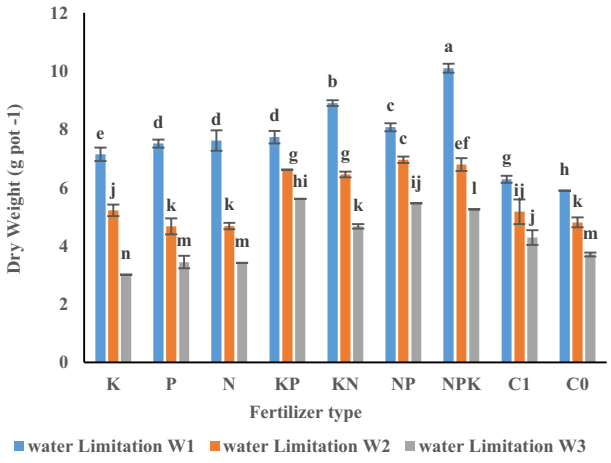

Fig. 2 Effects of biofertilizer × water limitation on Dry Weight Yield of basil

W1, W2, and W3 denote the normal irrigation (100% of field capacity), moderate water stress (60% of field capacity), and severe water stress (40% of field capacity);
F1, F2, F3, F4, F5, F6, F7, F8, and F9 show seed inoculation with K-, P-, N-, KP-, NK-, NP-, and NKP-releasing PGPR, 100% chemical fertilizer, and without inoculation, respectively.

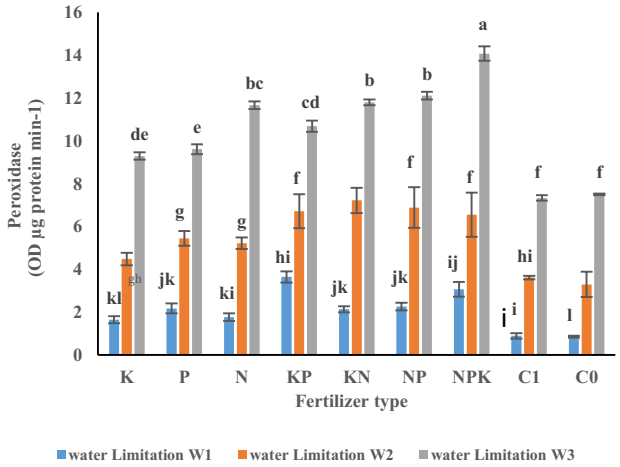

Fig. 3 Effects of biofertilizer × water limitation on POX enzyme activity of basil

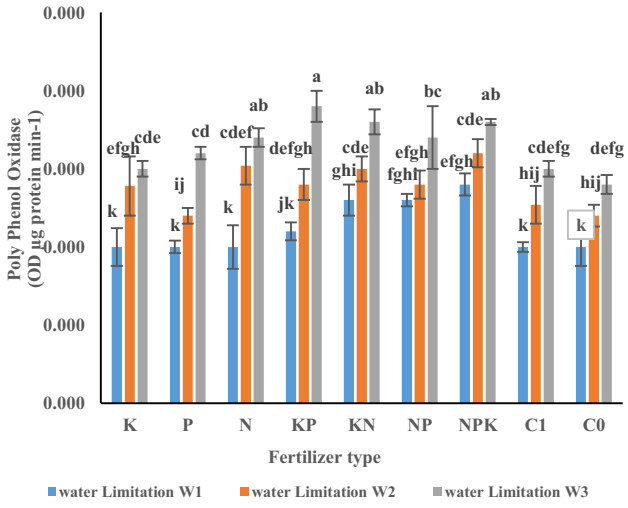

Fig. 4 Effects of biofertilizer × water limitation on PPO enzyme activity of basil

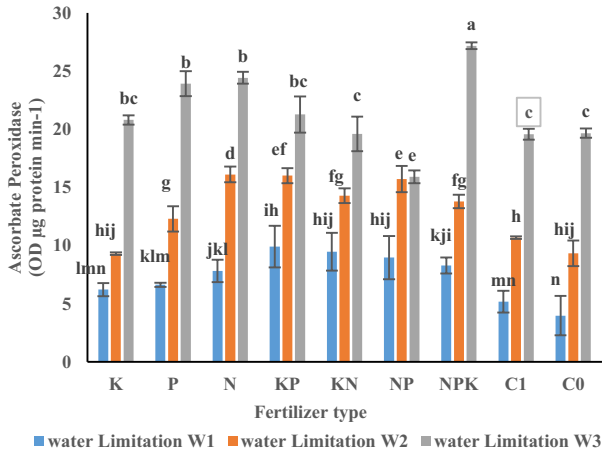

Fig. 5 Effects of biofertilizer × water limitation on APX enzyme activity of basil

W1, W2, and W3 denote the normal irrigation (100% of field capacity), moderate water stress (60% of field capacity), and severe water stress (40% of field capacity);
F1, F2, F3, F4, F5, F6, F7, F8, and F9 show seed inoculation with K-, P-, N-, KP-, NK-, NP-, and NKP-releasing PGPR, 100% chemical fertilizer, and without inoculation, respectively.

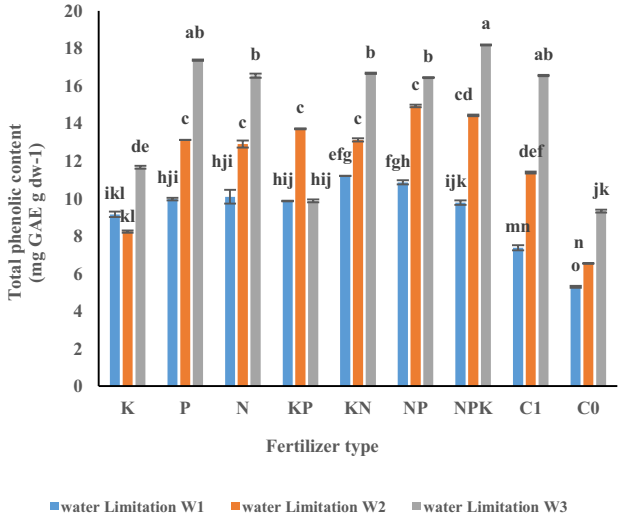

Fig. 6 Effects of biofertilizer × water limitation on Total Phenolic Content of basil

W1, W2, and W3 denote the normal irrigation (100% of field capacity), moderate water stress (60% of field capacity), and severe water stress (40% of field capacity);
F1, F2, F3, F4, F5, F6, F7, F8, and F9 show seed inoculation with K-, P-, N-, KP-, NK-, NP-, and NKP-releasing PGPR, 100% chemical fertilizer, and without inoculation, respectively.

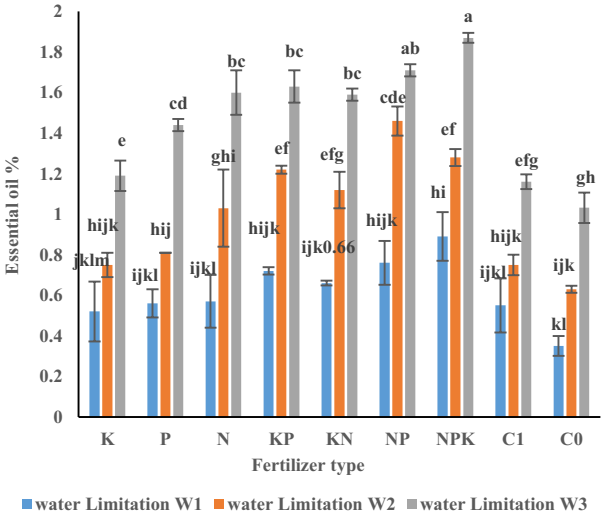

Fig. 7 Effects of biofertilizer × water limitation on Essential Oil % of basil

W1, W2, and W3 denote the normal irrigation (100% of field capacity), moderate water stress (60% of field capacity), and severe water stress (40% of field capacity);
F1, F2, F3, F4, F5, F6, F7, F8, and F9 show seed inoculation with K-, P-, N-, KP-, NK-, NP-, and NKP-releasing PGPR, 100% chemical fertilizer, and without inoculation, respectively.

Photosynthetic Pigment Content (SPAD)

Leaves chlorophyll content was affected by water restriction and biological fertilizers (Table 2). The obtained data revealed that the concentration of photosynthetic pigment in terms of chlorophyll index (40.35 and 36.51) was higher in plants grown under normal irrigation as W_1 and application of NPK biofertilizers as F_7 (Table 3). Meanwhile, the lowest values were obtained at severe water limitation as W_3 and application of no fertilizers as F9 (Table 3).

Table 1 Physical and chemical properties of the soil studied

Property	EC (ds/m)	pH (ds/m)	K (mg/kg)	P (mg/kg)	N (%)	Organic carbon (%)	Sand (%)	Silt (%)	Clay (%)	Texture
Amount	1.8	7.6	183	11.8	0.08	0.8	44	44	12	Sandy loam

Table 2 Analysis of variance for experimented factors effect on evaluated traits in Sweet Basil

Mean of square										
S.O.V	df	Fresh Weight (g)	Dry Weight (g)	POX(OD µmol mg/protein/min)	PPO (OD µmol mg/protein/min)	APX (OD µmol mg/protein/min)	Chlorophyll content (SPAD)	Caretenoids (mg/g FW)	Essential Oil%	Phenol (mg GAE/g FW)
Replication	2	0.001 ^{ns}	0.003^{ns}	0.431 ^{ns}	3.848E-7 ^{ns}	0.99 ^{ns}	6.06 ^{ns}	0.002 ^{ns}	0.03 ^{ns}	0.63 ^{ns}
Water limitation	2	2133.5 **	71.310 **	491.14 **	0.000 **	1337.09 **	626.81 **	0.024 **	4.85 **	282.1**
Fertilizer	8	71.17 **	7.200**	18.162 **	1.693E-5 **	35.163 **	11.19 **	0.010 **	0.491**	56.8 **
Water limitation × Fertilizer	16	4.05 **	0.86^{0**}	2.603 **	2.555E-6 **	16.755 **	5.24 **	0.003 **	0.033 **	2.82 **
Error	52	0.510	0.03	0.366	9.653	0.938	3.746	0.001	0.012	0.65
CV		18.2	13.35	29.11	20.13	30.9	14.1	16.12	14.9	25.46

^{ns}, * and ** non- significant and significant at 5 and 1% probability level, respectively.

Table 3 Effects of biofertilizers on the activity of POX, PPO, and APX enzymes, fresh weight, chlorophyll content **(**SPAD), carotenoid content, phenolic content, and essential oil percentage of basil under water limitation conditions

	Fresh Weight (g)	Dry Weight (g)	POX(OD µmol/mg protein/ min	PPO (OD µmol/mg protein/min)	APX (OD µmol/mg protein/ min)	chlorophyll content (SPAD)	Caretenoids (mg/g FW)	Essential Oil%	Phenol (mg GAE g/FW)
Water limitation									
W_1 = Normal irrigation	43.71 a	7.54 a	2.042 c	0.0112 c	7.382 c	40.35 a	26.92 a	0.62 c	9.066 c
W_2 = Moderate water limitation	35.05 b	5.05 b	5.49 b	0.0139 b	13.056 b	32.64 b	19.81b	1.00 b	12.043 b
W_3 = Severe water limitation	25.93 c	2.76 c	10.542 a	0.0166 a	21.67 a	31.49 c	13.68 c	1.46a	15.524 a
Biofertilizers									
F_1= K	33.12 f	5.28 b	5.35 d	0.0131 d	12.1 c	33.71 bc	18.90 cde	0.80 e	9.02 d
F_2= P	33.35 f	5.09 c	5.74 cd	0.0126 d	14.28 b	35.26 ab	18.15 de	0.94d	13.483 ab
F_3= N	33.2 f	5.15 bc	6.21 c	0.0142 c	16.12 a	34.53bc	19.47 cd	1.06 c	13.18 b
F_4= KP	37.71 b	5.74 a	7.01 b	0.0147 bc	15.731 a	35.27 ab	22.25 a	1.2 b	13.475 ab
F_5= KN	35.43d	5.70 a	7.05 b	0.0155 ab	14.45 b	34.93 ab	20.60 b	1.10 bc	13.669 ab
F_6= NP	34.94 e	5.05 c	7.08 b	0.0147 bc	13.53 b	35.97 a	17.67 e	1.30 a	14.081 a
F_7=NPK	39.61 a	5.65 a	7.9 a	0.0158 a	16.42 a	36.51 a	19.71c	1.35 a	14.127 a
F_8= 100% chemical	36.51 c	4.05 e	3.94 e	0.0124 d	11.81 cd	34.42ab	18.39 cde	0.80 e	11.776 c
F_9= no fertilizer	30.22 g	4.35 d	3.88 e	0.0122 d	10.99 d	32.85 c	15.75 f	0.7 f	7.057 e

The same letters in each column show non-significant difference at P≤0.05 by Duncan test. APX: Ascorbate Peroxidase; POX: Peroxidase; PPO: Polyphenol Oxidas

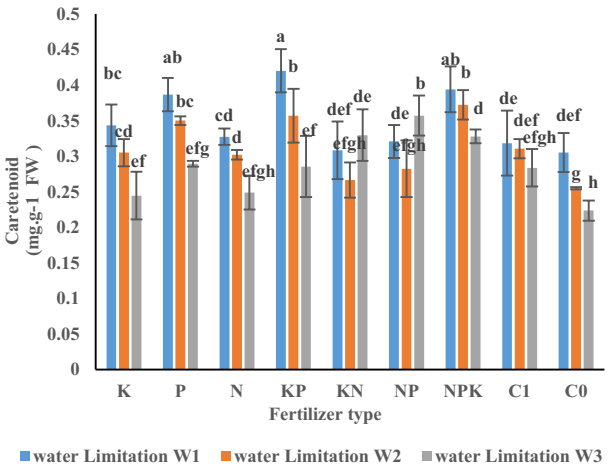

Fig. 8 Effects of biofertilizer × water limitation on Carotenoids Content of basil

W1, W2, and W3 denote the normal irrigation (100% of field capacity), moderate water stress (60% of field capacity), and severe water stress (40% of field capacity);
F1, F2, F3, F4, F5, F6, F7, F8, and F9 show seed inoculation with K-, P-, N-, KP-, NK-, NP-, and NKP-releasing PGPR, 100% chemical fertilizer, and without inoculation, respectively.

In studies by Farouk et al. [38] water deficit stress significantly decreased leaf photosynthetic pigment content. Degradation by reactive oxygen species (ROS), beta carotene destruction, and zeaxanthin formation was previously reported as the main reason for the decrease in chlorophyll under water deficit stress [28]. Drought stress significantly decreased chlorophyll content [6,39,40]. In comparison, fertilizers increased the photosynthetic pigment content by improving nutrient availability. These conditions can effectively improve the mobilization and uptake of trace elements, nitrogen, potassium, and phosphorus. Our results are consistent with the results reported by Jaleel *et al*. [41] in Catharanthus roseus, Arshad *et al*. [42] in Pisum sativum, and Ghorbanpour *et al*. [43] in *Hyoscyamus niger*. In this respect, Batool *et al*. [44] reported positive effects of PGPRs on photosynthetic pigment content in potatoes under drought stress. They showed that carotenoid content increased significantly as water stress was increased from well-watered to severe water deficit stress. In another study, Abdalla *et al*. [45] reported the same results in wheat and showed a significant increase in the interaction between water stress and inoculation with bacterial species ($P< 0.01$). Chemical fertilizer, as a positive control treatment containing three minerals of nitrogen, phosphorus, and potassium, produced less photosynthetic pigments than bio-fertilizers alone or in combination. We may presume that the main mechanism of photosynthesis enhancement is related to the direct effect of the tested PGPR on basil plant physiological status rather than to nitrogen fixation. The favorable effects of the combination of N+P+K-releasing biofertilizers may be explained based on their beneficial effects on the improvement of soil physical and biological properties. As a result, they lead to more release of available nutrient elements available to the plant roots and have physiological processes such as photosynthesis activity.

CONCLUSIONS

The results showed that water limitation reduced the yield and chlorophyll content of basil plants and increased the activity of POX, APX, and PPO enzymes, essential oil, and total phenolic compounds. Also, PGPR strains increased yield, chlorophyll content, and the activity of antioxidant enzymes under water limitation conditions. It seems that plants apply defensive mechanisms such as the synthesis of antioxidant enzymes, and phenolic compounds to alleviate the effects of stress The results showed that water limitation reduced the yield and chlorophyll content of basil plants and increased the activity of POX, APX, and PPO enzymes, essential oil, and total phenolic compounds. Also, PGPR strains alone or in combination increased yield, chlorophyll content, and the activity of antioxidant enzymes under water limitation conditions. It seems that plants apply defensive mechanisms such as the synthesis of antioxidant enzymes, and phenolic compounds to alleviate the effects of stress. Due to the fact that with the exception of the Caspian Sea coast and small parts of the northwest, other parts of Iran such as Fars, Yazd, Kerman, Semnan and, Khorasan provinces, which are classified as arid and semi-arid regions, then biofertilizers can be used to increase the drought resistance of basil to profitable production. This experiment was performed in greenhouse conditions, which is recommended to be performed in field conditions.

Conflict of Interest

No potential conflict of interest was reported by the authors.

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