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Alleviating the adverse effects of water limitation on Basil (*Ocimum basillicum* L.) physiological traits and yield, by exogenous application of Biofertilizers

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Abstract

In order to investigate the effects of plant growth promoting rhizobacteria on some physiological traits of Basil (*Ocimum basillicum* L.) under water deficit stress, a factorial experiment was carried out in 2015. The first factor was soil water content [100% of field capacity; 60 % of field capacity and 40% of field capacity]; And the second factor was biofertilizer consisting of [*Pseudomonas koreensis* and *Pseudomonas vancouverensis* as potassium releasing bacteria; *Pseudomonas putida* and *Bacillus lentus* as phosphorus releasing bacteria; *Azetobacter* strains as nitrogen fixing bacteria; Combination of potassium and phosphorus releasing bacteria; Combination of potassium releasing and nitrogen fixing bacteria; Combination of nitrogen fixing bacteria and phosphorus releasing bacteria; Combination of the three kinds of bacteria; Chemical Fertilizer based on soil analysis as positive control; and No fertilizer as negative control]. Results showed that water deficit increased essential oil, proline and total soluble carbohydrates content. Whereas, chlorophyll a, chlorophyll b, carotenoid and shoot yield decreased. Application of biofertilizers improved these traits under water stress condition as well as normal irrigation. Applying combination of 3 biofertilizers led to 10.82% shoot yield increase in comparison with negative control under sever water limitation. These findings indicated that biofertilizers application can be recommended for profitable basil production under water limitation condition.

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Introduction

Basil (*Ocimum basillicum* L.) is one of the most important aromatic annual crops in the world which is used in traditional medicines and to flavor foods. In aromatic plants, growth and essential oil yield is significantly influenced by global climate change including water limitation stress (Mahajan and Tuteja, 2005). As basil is widely cultivated and used as fresh vegetable in dry and semi-dry lands of Iran, it is necessary to find an effective way to overcome drought stress in this crop.

Water deficit is at the core of serious global challenges threating production of food crops for the world's growing population, especially in arid and semiarid regions (Abedi & Pakniyat, 2010).

Drought affects morphological, physiological, biochemical and molecular processes in plants and decreases final crop yield (Cao *et al.,* 2011). Water stress directly alters the physical environment of the soil by reducing the mobility of nutrients in the soil (Schimel *et al.,* 2007). Thus, it has a strong adverse effect on nutrient availability. Under such circumstances, there is urgent need not only to improve crop productivity but also to improve soil health (Lugtenberg *et al*., 2002). However fertilization increases the availability of limited nutrients in infertile and dry soils (Dang *et al*., 2006), but acquisition of water and nutrients from soils is governed by roots and their interaction with the abiotic and biotic components of soil.

There is increasing evidence that beneficial microorganisms can induce some degree of tolerance to plants towards adverse environmental stresses like salinity (Egamberdieva, 2008), drought (Zahir *et al*., 2008), nutrient deficiency and heavy metal toxicity (Sheng, 2005). Several plant growth promoting bacteria (PGPR) strains are known to colonize plant roots and benefit plants by promoting plant growth and soil health. These beneficial microorganisms colonize the rhizosphere/endorhizosphere of plants and promote growth of the plants through various direct and indirect mechanisms such as N_2 fixation, soil mineral solubilization, production of plant-growth promoting substances (auxins, cytokinins or gibberellins), and repressing the growth of plant pathogenic microorganisms (Nia *et al*., 2012; Ramadoss *et al*., 2013), among others; thus they are able to induce abiotic stress tolerance in plants.

A better understanding of the interactions between microorganisms and abiotic stresses affecting plant health and vigor may help in programs which the objective is to improve the drought stress tolerance of crops. Microorganisms may offer a possible tool to increase plant tolerance to common abiotic stresses. Improved photosynthesis, active solute accumulation and activity of enzymatic antioxidants are claimed to be effective stress tolerance mechanisms. Therefore, the present study was taken up to investigate the favorable effect of inoculation with PGPR strains on growth, development and final yield of basil (*Ocimum basillicum* L.) under water deficit condition.

Materials and methods

This experiment was conducted in the greenhouse of Shahid Bakeri University, Miandoab, Iran, during 2015. This study was carried out as a factorial experiment based on a randomized block design.

The first factor was soil water content [100% of field capacity (W_1 as well watered); 60 % of Fc (W_2as mild water stress); 40% of Fc (W_3 as severe water stress)]; and the second factor was Biofertilizer, consisting of 1) *Pseudomonas koreensis* and *Pseudomonas vancouverensis* as potassium releasing bacteria (K); 2) *Pseudomonas putida* and *Bacillus lentus* as phosphorus solubilizing bacteria (P); 3) *Azetobacter* strains as nitrogen fixing bacteria (N); 4) combination of K and P (KP); 5) combination of K and N (KN); 6) combination of N and P (NP); 7) combination of N, K and P (NKP); 8)chemical fertilizer based on soil analysis as positive control (C_1); 9) No fertilizer as negative control (C_0)].

Biofertilizers were provided from Green Biotech Co., Iran, (containing 10^8alive and active bacteria per gram), and basil seeds cultivar "purple" were obtained from Esfahan Agricultural and Natural Resources Research and Education Center, Isfahan, Iran. Seeds were first surface sterilized with 17% sodium hypochlorite for five minutes and then were inoculated with bacteria strains. Then 20 seeds were sowed in each pot, and then they were thinned to 7 plants per pot each pot was containing 6 Kg soil. Physio-chemical properties of the soil used in this experiment was presented in table1.

Measurements

Photosynthetic pigment content

Chlorophyll content was measured in 0.1g fresh leaf tissue, which was gradually extracted with 5ml of 80% acetone in the dark. The mixture was filtered and the absorbance at 645, 663 and 470nm was recorded by a spectrophotometer. Chlorophyll and carotenoids content were obtained based on the following equations (Arnon, 1949):

Chlorophyll *a* (Chl a) = (19.3 × A663 - 0.86 × A645) V/100 W

Chlorophyll *b*(Chl b) = (19.3 × A645 - 3.6 × A663) V/100 W

Total Chlorophyll = Chlorophyll *a*+ Chlorophyll *b*

Carotenoid = (1000 A470 - 1.82 Ca - 85.02 Cb) /198

Essential oil content

Quantitative determination of the essential oil from basil subjected to the different treatments was achieved by placing the air-dried shoot in a 2l flask with distilled water (1:5 w/v) and using a Clevenger apparatus, as described by Charles and Simon (1990). The average essential oil content of aerial parts is reported as percent of plant dry matter.

Proline Content

In order to measure proline, 0.5 g of plant fresh tissue was crushed in 10 ml sulpho-acetic acid solution to obtain a homogeneous mixture. Then, the solution was smoothed using whit-man and 2 ml dimenhydrinate reagent and 2 ml glacial acetic acid were added. The extract was mixed and stirred on bain-marie at 100 °C for one hour and then 4 ml toluene added and the extract was vortexes to form two separate phases. The supernatant was read at 520 nm by a spectrophotometer (Bates *et al*, 1973).

Soluble Carbohydrates were determined based on phenol sulfuric acid method (Dubois *et al.,* 1956).

Statistical analysis

Data were subjected to statistical analysis of variance and mean comparisons using SPSS software. The main and interaction effects were tested using the Duncan test.

Results and discussion

Photosynthetic pigment content

The results indicated that water limitation and bio fertilizers had a significant effect on photosynthetic pigments. The highest values for chl a (1.19 and 1.213mg g^{-1} FW), chl b (1.42 and 1.29 mg g^{-1} FW), total chlorophyll (2.614 and 2.51 mg g^{-1}FW) and carotenoid (0.35 and 0.36 mg g^{-1} FW) were observed in well watered as W_1 and application of NPK releasing bio fertilizers as NKP respectively (Table 2), while the lowest of values were obtained at severe water limitation as W_3 and application of no fertilizers as C_0 (Table 2). 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 (Sultana *et al.* 1999). However, chlorophyll and carotenoid content decreased significantly as water stress was increased from normal to sever water deficit stress, significant increase was found in interaction between water stress and inoculation with bacterial species ($p<0.01$). Means comparison of interaction effect of drought stress levels × PGPR streatments on chl a, chl b, Total Chlorophyll and Carotenoid content showed that the values of these pigments increased due to PGPR inoculation with all strains, but the highest values was obtained under the inoculation with bio fertilizers containing combination of bacteria strains releasing all three minerals (NPK) (Table3).

Our results in this case are in accordance with findings reported by Jaleel *et al.* (2007) in *Catharantus roseus* and Ghorbanpour *et al.* (2013) in *Hyoscyamus niger*, and Arshad *et al.* (2008) in *Pisum sativum* who reported alleviative effects of PGPRs on photosynthetic pigments content in plants under drought stress. As fertilizing with 100% chemical fertilizer containing all three minerals of nitrogen, phosphorus and potassium, yielded less photosynthetic pigments in comparison with biofertilizers alone or in combination, we may presume that the main mechanism of photosynthesis enhancement is related to direct effect of the tested PGPR on basil plants physiological status rather than to nitrogen fixation or P and K releasing ability alone. The favorable effects of the combination of N+P+K releasing biofertilizers may be explained based on the beneficial effects of them on the improvement of soil physical and biological properties resulting in more nutrient availability and improved physiological processes in plants such as photosynthetic activity. Kleiner *et al.* (1992) claimed that good soil fertility increase the ability of plants to maintain relatively high levels of growth, stomatal conductance and photosynthesis under drought conditions.

Proline and Soluble Carbohydrates

Proline and carbohydrate content were significantly affected by both factors and their interactions (Table 1). Maximum values for proline (13.69 and 11.15 mg g^{-1} FW) and soluble solids (3.31 and 2.9 mg g $^{-1}$) were obtained under W$_3$ and NPK treatments respectively (Table 2). Prolineand soluble carbohydrate contents increased significantly due to severe water limitation and application of bio fertilizers. Thus, all bio-fertilizer treatments individually or combined proved significant increases in the mean values of proline and sugar content compared with control treatment (Figs. 1 & 2).

Table 1. physical and chemical properties of the experimental soil.

Property	EC (dsm^{-1})	pH	K	P	N (%)	Organic carbon (%)	Sand (%)	Silt (%)	Clay (%)
			(mgKg^{-1})						
Amount	1.8	7.65	183	11.8	0.08	0.8	44	44	12

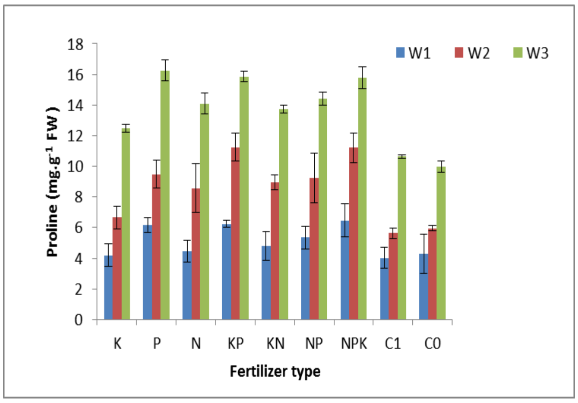

Fig 1. The effect of biofertilizer and soil water content on leaf Proline content of basil.

W$_1$, W$_2$ and W$_3$ indicative for well watered (100% of field capacity), mild water tress (60% of FC) and sever water stress (40% of field capacity); K, P, N, KP, NK, NP, NKP, C$_1$, and C$_0$ indicative for seed inoculation with releasing PGPR, chemical fertilizer based on soil analysis as positive control and No fertilizer as negative control respectively.

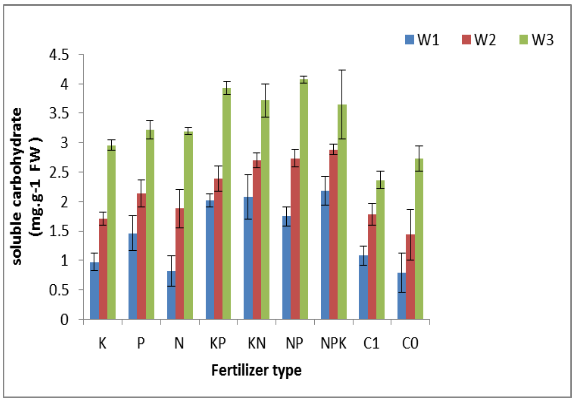

Fig. 2. The effect of bio fertilizer × water limitation on soluble carbohydrates content of basil.

W$_1$, W$_2$ and W$_3$ indicative for well watered (100% of field capacity), mild water tress (60% of Fc) and sever water stress (40% of field capacity); K, P, N, KP, NK, NP, NKP, C$_1$, and C$_0$ indicative for seed inoculation with releasing PGPR, chemical fertilizer based on soil analysis as positive control and No fertilizer as negative control respectively.

Accumulation of proline and other solutes, such as soluble solids is a major adaptation mechanism for plants to overcome salinity and water deficit stress through osmotic adjustment (Ramanjulu and Sudhakar, 2000) as well as by stabilizing many functional units like complex II of the electron transport system, proteins and enzymes (Ashraf and Foolad 2007). Qudsaia *et al.* (2013) reported that inoculating maize with *Azospirillum lipoferum* increased growth, soluble sugars, and proline during drought stress.

Essential oil content

Essential oil content extracted from the leaves was affected by drought stress levels, PGPRs and their interaction (Table 2). These secondary metabolites increased significantly in response to water stress and use of PGPR, the highest Essential oil content was observed in drought and combined use of biofertillizers (Fig 3). PGPR and osmotic stresses are classified as biotic and abioticelicitors for production of secondary metabolites in medicinal plants (Jaleel *et al.* 2009), because in this condition, more metabolites are produced to prevent oxidization in the cells (Aliabadi Farahani *et al.,* 2009). Khalid (2006) reported a significance increase in essential oil percentage and the main constituents of essential oil under the influence of water stress in two species of *Ocimum basilicum* L. (sweet basil) and *Ocimum americanum* L. (American basil). However drought stress reduces plant biomass, which is a key determinant of essential oil yield per plant.

Table 2. Effects of bio fertilizers on Chlorophyll and careened content, proline, essential oil yield, soluble carbohydrates and dry weight of basil under water limitation.

	Dry Weight (g)	Chl a (mg g^{-1} FW)	Chl b (mg g^{-1} FW)	Chl T (mg g^{-1} FW)	Caretenoid (mg g^{-1} FW)	Essential oil yield (ml 100g DW^{-1})	Proline (mg. g^{-1}FW)	soluble carbohydrate µmolmg protein-1 min-1)
water limitation								
W_1 = well watered	11.44 a	1.192 a	1.422 a	2.614 a	0.347 a	0.905 c	5.109 c	1.462 c
W_2=mild water tress	10.29 b	0.964 b	1.069 b	2.034 b	0.311 b	1.006 b	8.545 b	2.183 b
W_3=severe water stress	9.28 c	0.834 c	0.856 c	1.69 c	0.288 c	1.184 a	13.692 a	3.313 a
Bio fertilizers								
K	9.67 d	0.92 bc	1.025 bc	1.945 cd	0.298 c	0.822 e	7.769 c	1.879 cd
P	10.51 bc	1.05 ab	1.118 abc	2.169 bc	0.342 ab	0.937 d	10.631 a	2.27 b
N	9.97 cd	1.047 ab	1.066 bc	2.113 bc	0.293 c	1.0669 c	9.033 b	1.963 c
KP	10.48 bc	1.123 ab	1.21 ab	2.331 ab	0.354 a	1.192 b	11.111 a	2.777 a
KN	10.97 ab	0.969 b	1.055 bc	2.024 c	0.302 c	1.121 bc	9.168 b	2.833 a
NP	10.54 bc	1.057 ab	1.262 a	2.319 ab	0.32 bc	1.309 a	9.663 b	2.852 a
NPK	11.35 a	1.123 a	1.294 a	2.507 a	0.364 a	1.347 a	11.15a	2.903 a
C_1	9.88 cd	0.892 bc	1.007 c	1.9 cd	0.304 c	0.803 e	6.768 d	1.741 cd
C_0	9.67 d	0.699 c	1.006 c	1.706 d	0.261 d	0.686 f	6.744 d	1.653 d
W	**	**	**	**	**	**	**	**
F	**	**	**	**	**	**	**	**
W*F	**.	**	*	**	**	**	**	**
C.V.	13.35	32.35	28.12	25.98	16.75	41.78	44.05	44.35

The same letters in each column show non- significant difference at $P \leq 0.05$ by Duncan test.

ns and *, ** show no significant and significant differences at 0.05, 0.01 probability level, respectively.

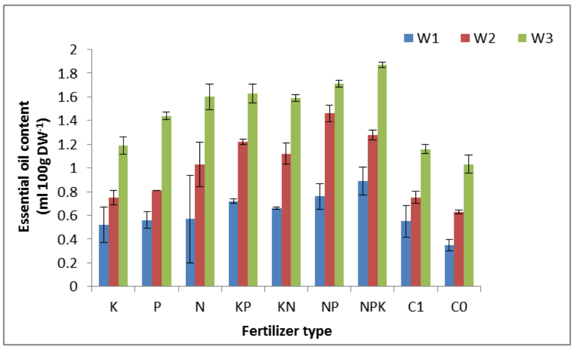

Fig. 3. The effect of bio fertilizer × water limitation on Essential Oil content of basil.

W_1, W_2 and W_3 indicative for well watered (100% of field capacity), mild water tress (60% of Fc) and sever water stress (40% of field capacity);
K, P, N, KP, NK, NP, NKP, C_1, and C_0 indicative for seed inoculation with releasing PGPR, chemical fertilizer based on soil analysis as positive control and No fertilizer as negative control respectively.

Yield

Dry weight of aerial parts is the main yield component in vegetative plants like basil. Shoot yield was significantly affected by bio fertilizers, water limitation and their interaction. Shoot yield was decreased by the increase of water limitation, but application of biofertilizers, especially NPK biofertilizer improved plant tolerance and increased dry weight (Fig. 4). The highest Shoot yield (11.35 g/Plant) was obtained in well waterd and application of triple biofertilizer (NKP) (Fig. 5). The lowest yield (9.67 g/Plant) was obtained in severe water limitation (W_3) and without application of biofertilizer and application of K releasing biofertilizers. Based on these results, we can conclude that ability of biofertilizersto 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 (Cakmakci *et al.* 2007) among other mechanisms, result in balanced nutrition of plants and increase plant yield. Noorieh *et al.* (2013) have proposed co-inoculation with bio fertilizer as an efficient procedure to increase plant growth.

Table 3. Effect of PGPR on chlorophyll a, b, total chlorophyll and carotenoid content of basil under water limitation.

Bio Fertilizers	Chlorophyll a (mg g^{-1} FW)			Chlorophyll b (mg g^{-1} FW)		
	water limitation levels			water limitation levels		
	W_1	W_2	W_3	W_1	W_2	W_3
K	1.219±0.294 a-d	0.724±0.4278 e-h	0.816± 0.524 d-h	1.34±0.079 bc	0.802±0.604 hk	0.935±0.049 d-k
P	1.245±0.092 a-d	1.096±0.066 a-e	0.81±0.141 d-h	1.33±0.058 bc	1.09±0.067 c-k	0.929±0.069 d-k
N	1.282±0.039 abc	1.041±0.067 a-f	0.817±0.046 d-h	1.341±0.094 bc	1.069±0.146 c-k	0.788±0.18 k
KP	1.476±0.385 a	1.035±0.01 a-f	0.859±0.0958 c-h	1.591±0.165 ab	1.191±0.06 c-f	0.841±0.123 ghk
KN	1.099±0.25 a-e	0.994±179 b-f	0.815±0.218 d-h	1.26±0.057 cd	1.05±0.1 c-k	0.853±0.2 f-k
NP	1.153±0.066 a-e	1.073±0.03 a-f	0.944±0.065 b-f	1.8±0.311 a	1.143±0.2 c-h	0.847±0.136 ghk
NPK	1.445±0.146 a	1.281±.079 abc	0.914±0.101 b-f	1.782±0.306 a	1.214±0.05 cde	0.89±0.086 e-k
C_1	1.346±0.03 ab	0.442±0.581 h	0.89±0.127 c-g	1.192±0.06 c-f	1.054±0.025 c-k	0.77±0.06 k
C_0	0.467±0.626 gh	0.991±0.08 b-f	0.641±0.086 fgh	1.158±0.048 ab	1.01±0.033 c-k	0.849±0.021 f-k

Bio Fertilizers	Total Chlorophyll (mg g^{-1} FW)			Caroteniod (mg g^{-1} FW)		
	water limitation levels			water limitation levels		
	W_1	W_2	W_3	W_1	W_2	W_3
K	2.559±0.369 b-e	1.523±0.416 j	1.751±0.052 g-j	0.343±0.029 b-e	0.305±0.019 d-h	0.245±0.033 jk
P	2.58±0.148 b-e	2.186±0.123 d-h	1.74±0.191 g-j	0.387±0.023 ab	0.350±0.006 bcd	0.289±0.004 e-j
N	2.623±0.071 bcd	2.11±0.084 d-i	1.605±.018 ij	0.327±0.011 c-f	0.302±0.007 d-i	0.249±0.024 ijk
KP	3.067±0.521 ab	2.227±0.07 d-g	1.7±0.218 g-j	0.420±0.059 a	0.357±0.038 bcd	0.285±0.043 f-j
KN	2..359±0.279 def	2.044±0.239 e-j	1.668±0.365 hij	0.308±0.04 d-h	0.267±0.025 g-k	0.330±0.036 c-f
NP	2.949±0.362 abc	2.216±0.193 d-g	1.791±0.198 g-j	0.321±0.023 c-g	0.282±0.04 f-j	0.357±0.028 bcd
NPK	3.227±0.316 a	2.495±0.032 c-f	1.8±0.176 g-j	0.394±0.032 ab	0.372±0.021 abc	0.328±0.01 c-f
C_1	2.537±0.0485 c-f	1.496±0.562 j	1.666±0.154 hij	0.318±0.046 c-g	0.311±0.013 d-h	0.284±0.026 f-j
C_0	1.626±0.659 ij	2±0.062 f-j	1.49±0.068 j	0.305±0.039 d-h	0.255±0.002 h-k	0.224±0.014 k

W_1, W_2 and W_3 indicative for well watered (100% of field capacity), mild water tress (60% of Fc) and sever water stress (40% of Fc); K, P, N, KP, NK, NP, NKP, C_1, and C_0 indicative for seed inoculation with releasing PGPR, chemical fertilizer based on soil analysis as positive control and No fertilizer as negative control respectively.

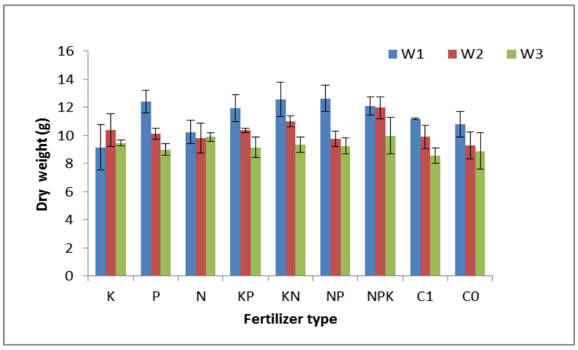

Fig. 4. The effect of bio fertilizer × water limitation on Dry Weight Yield of basil.

W_1, W_2 and W_3 indicative for well watered (100% of field capacity), mild water tress (60% of Fc) and sever water stress (40% of field capacity);
K, P, N, KP, NK, NP, NKP, C_1, and C_0 indicative for seed inoculation with releasing PGPR, chemical fertilizer based on soil analysis as positive control and No fertilizer as negative control respectively.

Conclusions

The results showed that water limitation reduced shoot yield and chlorophyll content of basil plants, but soluble carbohydrates, essential oil and proline contents increased. Also PGPR strains alone or in combination increased yield, chlorophyll content, proline and soluble carbohydrates under water limitation conditions. It seems that plants apply defensive mechanisms such as accumulation of salutes like soluble sugars and proline to alleviate the effects of stress and application of biofertilizers can be recommended for profitable basil production under water deficit stress condition.

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