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Potentiality of Using Mycorrhizae and *Pseudomonas fluorescens* In Reducing the Effect of Water Shortage on Broccoli Plants

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Abstract: Accelerating global warming and water scarcity and improving water use efficiency are considered essential factors for achieving adequate crop development and productivity. Therefore, the authors targeted the use of arbuscular mycorrhizal fungi (AMF) and *Pseudomonas fluorescens* (Ps1) for improving the growth, productivity, and water use efficiency of broccoli plants (*Brassica olercea* L. var. *italica*, cv. Belstar F_1) under various irrigation regimes i.e., 50, 75, and 100% of crop evapotranspiration (ETc). Two greenhouse experiments were carried out in clayey soil. The combined inoculation of endomycorrhizae and *Ps. fluorescens* (Ps1) improved water use efficiency and consequently vegetative growth and yield. Under the applied irrigation regime 75% ETc of broccoli inoculated with both endomycorrhizae and *Ps. fluorescens* (Ps1) showed higher head weight (616 and 647 gram) than those grown under the irrigation regime 75% ETc combined with endomycorrhizae (568 and 559 gram) during the two seasons, respectively. However, a minimum yield value of 149 and 142 grams per plant was recorded for uninoculated plants grown under a 50% irrigation regime during both seasons. In conclusion, the combined inoculation with endomycorrhizae and *Ps. fluorescens* (Ps1) under an irrigation regime of 75% ETc was the optimum combination for increasing water stress resistance and broccoli productivity under water scarcity circumstances.

1 Introduction

Broccoli (*Brassica oleracea* L. var. *italica*) belongs to the Brassicaceae family which is mainly found over the Mediterranean territory. Broccoli floret is enriched with minerals particularly Ca, K, S, P and Mg, in addition to micro-elements (Jigme et al 2015). A previous study (Zaicovski et al 2008) showed that broccoli plants subjected to water stress during the floret process were greener and more turgid additionally a decrease in crop output when compared to broccoli plants subjected to daily irrigation (Patle et al 2018).

Water has become a strategic priority risen to the top of the resource list due to population growth and climate change, which is already taking place and representing one of the most serious environmental challenges to our planet. Nowadays, plenty of studies have been conducted to investigate the optimal water demands under drought conditions to obtain the maximum crop productivity for sustainable agriculture development. Furthermore, pinpointing drought-resistant crop cultivars for each climatic element has become the main issue (Durak and Yıldırım 2017).

Mycorrhizae is the most widespread plant growth-promoting fungi that make a mutualistic relationship with plant roots and is capable of improving the nutritional status, growth, and productivity of various plants (Feddermann et al 2010). Under water stress, arbuscular mycorrhizal fungi (AMF) promote plant resistance by enlarging the surface root area into the soil and expanding their ramifying hyphae to the obtainable moisture zones for continual water absorption and translocation to plants (Smith and Read 2008). In addition, AMF increases the plant resistance to drought and improves the water-use efficiency of crops through supporting alternative physiological pathways (Abdel-Salam et al 2017).

Pseudomonas fluorescens is one of the well-known plant growth-promoting rhizobacteria (PGPR), in the soil that stimulates plant growth and development, as well as immune systemic resistance (Qessaoui et al 2019).

Both AMF and *Pseudomonas fluorescens,* are vital parts of soil microorganisms, since they have a significant impact on plant growth, especially under stress conditions through influencing numerous crucial plant physiological processes, such as seed germination ratio, root development, and branching, photosynthetic rates, and phytohormone balance in the plants. Soil remediation, like re-aggregation of soil particles, ameliorate soil pH and salinity (Cheng et al 2021, Ahanger et al 2014).

The current study was carried out to investigate the potentiality of using AMF fungi and *Pseudomonas fluorescens* on broccoli plant’s productivity and water use efficiency under different irrigation levels.

2 Materials and methods

2.1 Microorganisms treatment

Ps. fluorescens (Ps1) and a mixture of arbuscular mycorrhizal fungi (AMF) with a dominant *Glomus* sp. were obtained from Microbial Inoculants Center, Fac. Agric., Ain Shams Univ., Shubra El-Kheima, Cairo, Egypt. Each plantlet received 5 mL of AMF spore suspension (50 spores/ml).

Ps. fluorescens (Ps1) maintained on king's medium (King et al 1954) supplemented with tryptophan (1 mM/L) as auxin precursor, was investigated for indole acetic acid (IAA), cytokinins, and gibberellic acid using (HPLC) according to Tien et al (1979). Five ml cultures containing $1x10^8$ cfu/ml were added to each plant at planting and after one month.

2.2 The cultivated plant

Broccoli plant (*Brassica olercea* L. var. *italica*, cv. Belstar F_1) was transplanted on the 23rd and 25th of September 2018 and 2019, respectively. The transplants were placed in raised beds 1 m in width; each bed had two rows, and the plant spacing was 0.3 m.

2.3 Application of chemical fertilizer to broccoli seedlings

In the control treatment, the chemical fertilizers were added during the season by the fertigation system at a rate of 66 kg N, and 46 kg K_2O per feddan as ammonium nitrate and potassium sulfate, respectively. Phosphorus was added before bed raising in the form of calcium triple superphosphate at a rate of 30 kg P_2O_5 per feddan as recommended by The Egyptian Ministry of Agriculture and Land Reclamation.

2.4 Experimental design

Two greenhouse experiments were conducted over the two successive seasons of 2018/2019 at the greenhouse of the Experimental Farm of Protected Cultivation Experimental Site, Agricultural Research Center (ARC), Giza Governorate, Egypt. The experiment was designed using different combinations between three different irrigation levels (50, 75, and 100% of crop evapotranspiration (ETc)) and two types of microbial inocula (Arbuscular mycorrhizal fungi and *Pseudomonas fluorescens* (Ps1)).

The experiment was arranged in a split-plot design with three replicates. The plot area was 10 m (length) x 2 m (width). Soil physical and chemical properties were examined (**Table 1**) as reported by Page et al (1982). With a dripper system of 4 L/h broccoli plants were irrigated. The fertigation system was scheduled to work daily, and the duration of irrigation time was adopted according to treatments. For each irrigation level, a flowmeter (Metrotec, EGYPT) has been installed and between each irrigation treatment, two meters were left.

Table 1. Physicochemical analysis of soil used at the experimental site (0-30 cm depth)

Particle size distribution, %		**Soluble cations, $mmol_c$ L^{-1}**	
Sand	14.1	Ca^{2+}	14.1
Silt	9.00	Mg^{2+}	10.1
Clay	76.9	Na^{+}	10.2
Textural class	Clayey	K^{+}	4.82
$CaCO_3$, g kg^{-1}	15.0	Soluble anions, $mmol_c$ L^{-1}	
OM, g kg^{-1}	10.1	CO_3^{2-}	0.00
CEC, $cmol_c$ kg^{-1}	54.9	HCO_3^{-}	6.42
pH (1:2.5)	7.67	Cl^{-}	11.4
EC_e, dS m^{-1}	2.71	SO_4^{2-}	18.6

The climatic data concerning weather parameters, such as (temperatures, humidity, solar radiation, wind speed, and evapotranspiration (ETo)) during both successive cultivation seasons (2018 and 2019) were obtained from the weather station, that belongs to the Central Laboratory for Agricultural Climate are demonstrated in **Table 2**.

2.5 Water requirements assessment

The total amount of irrigation water was calculated according to the methods described by the FAO Penman-Monteith (Allen et al 1998). This method strongly predicted ET_o correctly in a wide range of sites and climates. Calculations of irrigation levels were done as follows:

The potential evapotranspiration (ET_o) was calculated first:

$$ET_o = \frac{0.408\Delta\,(R_n - G) + \gamma \frac{900}{T + 273} u_2 (e_s - e_a)}{\Delta + \gamma(1 + 0.34\,u_2)} \qquad (1)$$

Where:

ET_o = reference evapotranspiration (mm day^{-1}); Rn = net radiation at the crop surface (MJ m^{-2} day^{-1}); G = soil heat flux density (MJ m^{-2} day^{-1}); T = mean daily air temperature at 2 m height (°C); U_2 = wind speed at 2 m height (m s^{-1}); es = saturation vapor pressure (k Pa); ea = actual vapor pressure (k Pa); es - ea = saturation vapor pressure deficit (k Pa); Δ = slope vapor pressure curve (k Pa $^oC^{-1}$); γ = psychrometric constant (k Pa $^oC^{-1}$).

According to FAO (2012), the 2nd step was to determine crop water consumptive use (ET_c), which was calculated by multiplying the reference crop evapotranspiration, ET_o, by a crop coefficient, K_c:

$$ET_c = ET_o * K_c \quad \ldots\ldots \quad mm / day \ldots\ldots\ldots \quad (2)$$

Where:
ET_c crop evapotranspiration [mm day^{-1}].
K_c crop coefficient [dimensionless].
ET_o reference crop evapotranspiration [mm day^{-1}].

The irrigation requirements (IR) for each treatment were calculated as follows:

IR= (ET_o * K_c)* (LR)* 4.2 / Ea .(m3 / feddan/ day) (3)

Where:
LR % = Leaching requirement percentage.
Ea = the irrigation system's efficiency (assumed to be 85% of total applied water).
Leaching requirements were calculated based on Allen et al (1998).

According to FAO (1982), the water use efficiency (WUE) is the ratio of crop productivity (y) to the total amount of irrigation water used in the field throughout the growing season (IR),

WUE (kg/m^3) = Y (kg)/IR (m^3) ……………………

The irrigation water quantities for broccoli at the Dokki site throughout the two growing seasons are mentioned in **Table 3.**

Table 2. Climatic conditions of the experimental site during cultivation season 2018 / 2019

Weeks•	Temperature (^{O}C)				RH•••		SRAD••		Wind Speed		ET$_o$	
	Max		Min		(%)		(MJ/m^2/day)		(m/s)		(mm day^{-1})	
	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019	2018	2019
1	36.2	38.8	20.0	22.5	47.6	42.4	21.4	25.8	2.56	2.56	3.79	3.96
2	34.8	37.6	21.6	21.7	51.6	44.0	18.8	25.1	3.14	2.58	3.74	3.65
3	31.3	36.7	18.1	21.4	53.0	46.1	19.2	23.9	2.51	2.97	3.70	3.34
4	30.4	35.6	17.2	20.7	56.7	48.9	18.2	22.3	2.27	2.87	3.69	3.16
5	30.9	35.3	18.1	19.9	45.8	47.4	16.9	22.1	2.81	2.89	2.72	3.09
6	31.7	34.7	15.0	19.4	46.6	47.7	17.1	21.0	2.97	2.45	2.79	2.60
7	27.0	34.9	15.3	19.8	57.8	46.0	14.1	20.0	1.73	2.75	2.66	2.81
8	25.3	35.1	13.7	19.3	57.9	51.6	13.6	19.0	2.12	2.68	2.17	2.37
10	26.3	31.1	13.9	19.2	58.7	54.0	13.2	15.6	2.25	2.96	2.26	2.28
11	24.4	27.9	12.8	16.5	54.2	65.4	12.8	15.0	1.78	2.74	2.11	2.00

* The cultivation date started on the 23rd and 25th of September during the two studied seasons, respectively, and the experiment ended in the middle of December.

••SRAD = Solar Radiation (MJ/m^2/day), •••RH = Relative Humidity, ET$_o$ = Evapotranspiration (mm/day).

Table 3. The average irrigation requirements for broccoli plants under different irrigation levels

Irrigation Requirements, m^3/feddan					
Season 2018			Season 2019		
100%	75%	50%	100%	75%	50%
2580	1935	1290	2648	1986	1324

2.6 Parameters measured

2.6.1 *Ps. fluorescens* (Ps1) population densities in the rhizosphere of broccoli plants

Total viable counts (cfu/g) of *Ps. fluorescens* (Ps1) were determined in the rhizosphere using the standard plate count on King's agar medium (King et al 1954) where three plants were randomly selected from each experimental plot at zero time, after 30, 60, and 90 days after sowing in each season.

2.6.2 Arbuscular mycorrhizal fungi (AMF) root colonization

Three plants were randomly selected from each experimental plot two months from infection to estimate mycorrhizal root infection ratio by visual observation of fungal colonization according to Phillips and Hayman (1970).

2.6.3 Plant growth parameters

Three plants were randomly selected from each experimental plot to estimate the growth parameters and yield of broccoli. The number of leaves per plant, fresh weight (g/plant), head weight (g), head length (cm), and head width (cm) were determined.

2.6.4 Biochemical analysis of the leaves

The selected broccoli leave samples were oven-dried and digested in H_2SO_4/H_2O_2 mixture as described by Page et al (1982); the total nitrogen was determined using the micro-Kjeldahl method, as described by the FAO (1982). According to Watanabe and Olsen (1965), phosphorus concentration was evaluated using a Spectrophotometer (SPAD-502Plus). Potassium content was measured with a Flame photometer (flame photometer 410) as reported by Chapman and Pratt (1961).

2.6.5 Biochemical parameters in broccoli heads

Total soluble solids (TSS) were examined using the refractometer (ATC 2E) (AOAC 1990). Additionally, vitamin C determinations were performed according to the protocol of Pearson (1970). Protein contents were calculated using data obtained from total nitrogen contents using the micro-Kjeldahl method (AOAC 1990).

2.7 Statistical analysis

Data were presented as a mean of at least three independent replicates and statistically analyzed using the SAS program (SAS 1976). The difference among means was considered significant at $P \leq 0.05$ referring to the LSD value according to Waller and Duncan (1969).

3 Results and Discussion

3.1 Growth regulators produced by *Ps. fluorescens* (Ps1)

Analysis of King's medium, *Ps. fluorescens* (Ps1) supernatant revealed the capability to produce zeatin (ZE), gibberellins (GAs), and indole-3-acetic acid (IAA), with concentrations of 159.0, 45.1, and 15.0 µg/ml, respectively these results are compatible with those revealed by {Mona, 2017 #19}.

3.2 Endomycorrhizal colonization as influenced by different treatments

Results recorded in **Table 4** show that using *Ps. fluorescens* (Ps1) as a helper had a stimulatory effect on plant root infection with endomycorrhizae **Fig 1**, while the uninoculated plants showed no endomycorrhizal colonization at any of the irrigation levels. These results are compatible with Pivato et al (2009) as they reported that *Pseudomonas fluorescens* C7R12 promoted both the growth and root colonization by *Glomus mosseae* BEG12, indicating that it acted as endomycorrhizal helper bacteria. Barea et al (1998) reported that two different strains of *Pseudomonas fluorescens* stimulated spores germinating and mycelial development of *Glomus mosseae* in the soil as well as plant root colonization.

Table 4. Percentage of arbuscular mycorrhizal fungi (AMF) root colonization in association with *Ps. fluorescens* (Ps1) under different irrigation levels (ETc)

Treatments		AMF Root colonization %
50% ETc	Control	0
	AMF	90
	AMF and *Ps. fluorescens* (Ps1)	96
	Ps. fluorescens (Ps1)	0
75% ETc	Control	0
	AMF	81
	AMF and *Ps. fluorescens* (Ps1)	87
	Ps. fluorescens (Ps1)	0
100% ETc	Control	0
	AMF	70
	AMF and *Ps. fluorescens* (Ps1)	78
	Ps. fluorescens (Ps1)	0

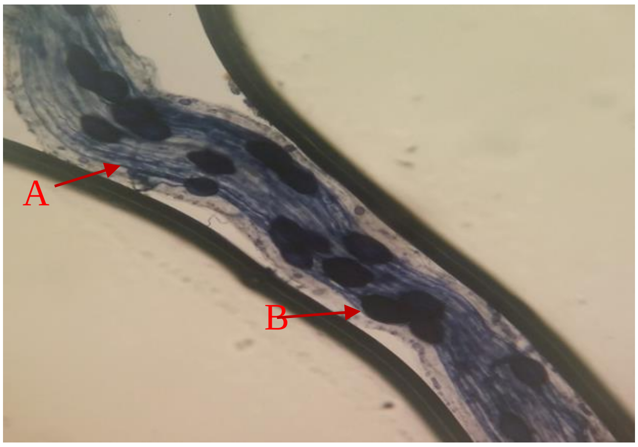

Fig 1. AMF fungal structures in Broccoli roots A) arbuscules B) vesicles

Intriguingly, the highest observed mycorrhizal colonization ratio was combined with inoculation with *Ps. fluorescens* (Ps1) under 50% ETc followed by plants inoculated with endomycorrhizal spores only under the same ETc level being 96 and 90 %, respectively.

3.3 Proliferation of *Ps. fluorescens* (Ps1)

Data illustrated by **Fig 2** reveal the average total viable count of *Ps. fluorescens* (Ps1) in the rhizosphere of broccoli plants as influenced by different water irrigation regimes and the combined inoculation with AMF in the two successive seasons of 2019/2020.

Generally, at zero time the initial population density of *Ps. fluorescens* (Ps1) in the rhizosphere of broccoli plants in all treatments ranged approximately from 8.44 to 8.78 x10^7 cfu/g dry rhizosphere soil. The population of *Ps. fluorescens* (Ps1) revealed a gradual increase in density followed by a slight decrease in all irrigation levels.

The densities of *Ps. fluorescens* (Ps1) as influenced by inoculation with AMF under 50% ETc are plotted in **Fig 2-a**. The *Ps. fluorescens* (Ps1) density after 60 days of cultivation reached, 17.0 x 10^7, 15.0 x 10^7, 11.2 x 10^7 and 12.6 x 10^7 cfu/g dry rhizosphere soil, in broccoli plants inoculated with AMF and *Ps. fluorescens* (Ps1), *Ps. fluorescens* (Ps1), AMF only and the uninoculated control in respective order.

Fig 2-b show that *Ps. fluorescens* (Ps1) density after 60 days of cultivation broccoli plants subjected to 75% ETc reached 4.2 x 10^7, 12.5 x 10^7, 9.9 x 10^7, and 7.5 x 10^7 cfu/g dry rhizosphere soil, in broccoli plants inoculated with AMF and *Ps. fluorescens* (Ps1), *Ps. fluorescens* (Ps1), AMF only and the control treatments in respective order.

Data in **Fig 2-c** show that *Ps. fluorescens* (Ps1) density after 60 days of cultivation, reached 12.2 x10^7, 9.1 x 10^7, 7.9 x 10^7 and 6.4 x 10^7 cfu/g dry rhizosphere, in broccoli plants inoculated with AMF and *Ps. fluorescens* (Ps1), *Ps. fluorescens* (Ps1), AMF only and the control in respective order.

Our results are compatible with Manaf and Zayed (2015), Edwards et al (1998)who indicated that inoculation of plants with endomycorrhizae and *Ps. fluorescens* had a synergistic effect on the population of both microorganisms in addition to increasing mycorrhizal root colonization percentage.

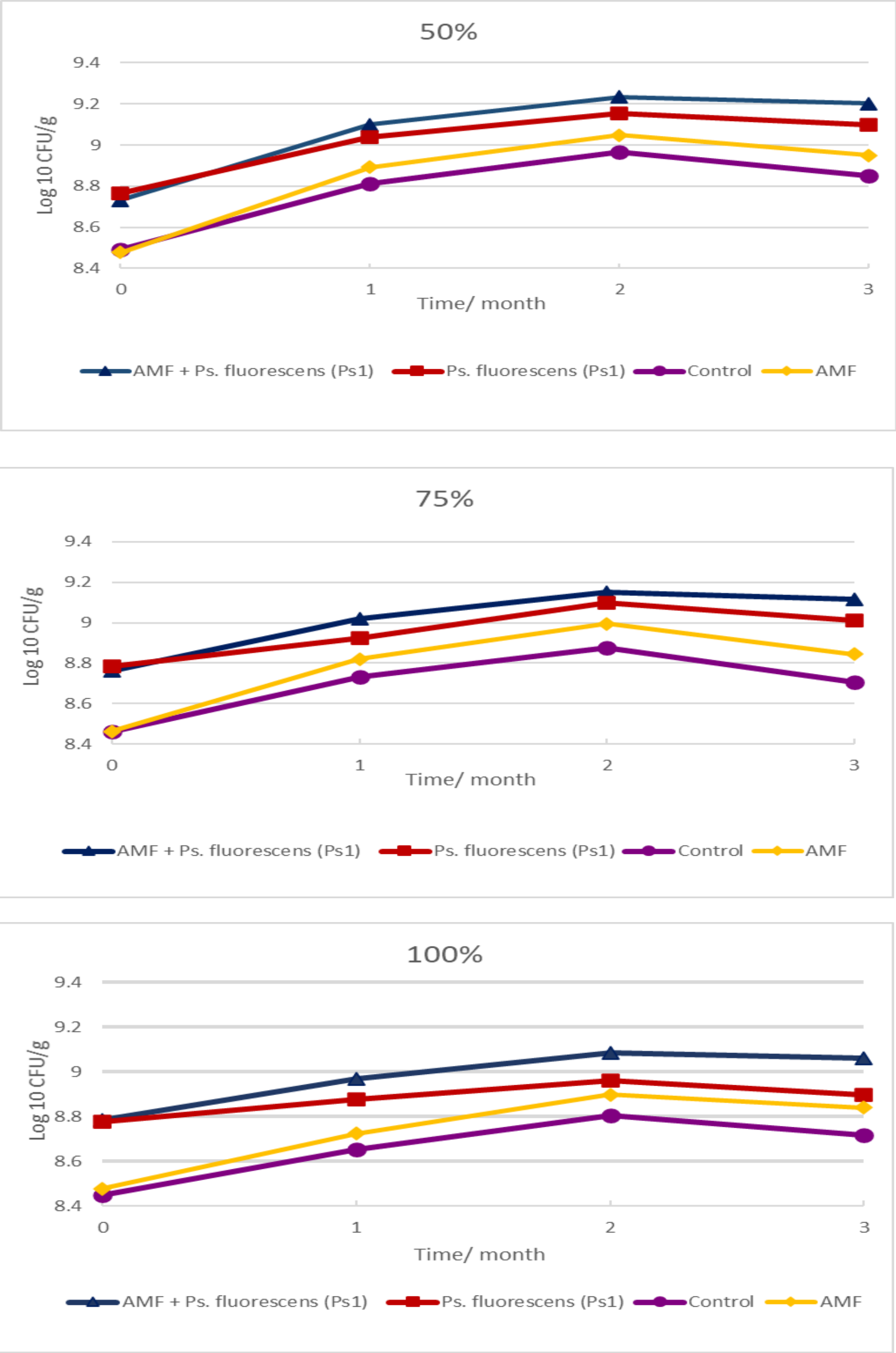

Fig 2. *Ps. fluorescens* (Ps1) counts in the rhizosphere of broccoli plants as affected by (A) %50 (B) 75% and (c) 100% of water requirements "irrigation levels" and the combined inoculation with AMF fungi.

3.4 Vegetative growth and yield of plants

Data displayed in **Table 5** reveal the influence of different ETc treatments and different microbial inocula on the growth parameters of broccoli plants during two successive seasons (2018-2019). Generally, by estimating the effect of ETc it is clear that increasing the ETc reveals a significant increase in the number of leaves and total plant fresh weight in the two successive seasons. The highest significant increases in the number of leaves and total plant fresh weight were recorded with plants subjected to 100% ETc in the two seasons being 24.1 & 23.3 and 985 & 1028 g/plant respectively. While broccoli plants revealed a decrease in total chlorophyll by increasing the ETc, the highest significant total chlorophyll was recorded with plants subjected to 50% ETc being 63.1 and 60.3 µg Chl./cm respectively. The effect of different combinations between *Ps. fluorescens* (Ps1) and AMF on broccoli plants revealed that using both *Ps. fluorescens* (Ps1) and AMF on broccoli plants recorded the highest significant growth parameters in no. of leaves (24.3 & 23.8), total plant fresh weight (1208 and 1249 g/plant) and total chlorophyll 62.1 and 67.3 µg Chl./cm) in both seasons respectively.

The different combinations between ETc and biofertilizers (*Ps. fluorescens* (Ps1) and AMF) revealed the highest significant No. of leaves and total plants fresh weight with plants subjected to 100 ETc and inoculated with (*Ps. fluorescens* (Ps1) and AMF) in the two seasons being 26.8 & 26.4 and 1365&1501 g/plant respectively, while the highest chlorophyll content was recorded with plants subjected to 50% ETc and inoculated with *Ps. fluorescens* (Ps1) and AMF being 68.6 and 68.2 µg Chl/cm respectively.

It is clear in the current study that lower irrigation levels reduced the recorded plant growth parameters, which could be attributed to its effect on cell division in the meristems of the shoot and root as well as arresting cell growth. The reduction in photosynthetic rate induced by water scarcity is directly linked to the disturbance of cell division or expansion (Benabdellah et al 2011). In case of water stress, tissues to tissues water balance is disturbed, and the loss of turgor has negatively impacted cell development, therefore the cells keep small. Furthermore, the synthesis of the cell wall is affected by the reduction in cell development, which subsequently causes shrinking leaves and decreasing photosynthesis (Abdrabbo et al 2015, Asghari 2008). As a result of decreasing photosynthesis, the protein and chlorophyll are negatively impacted (Chen et al 2013).

Table 5. Vegetative growth parameters of broccoli plants as influenced by different irrigation levels and microbial inoculants throughout the two studied seasons of 2018 and 2019

	1st season					**2nd season**				
	Microbial inocula					**Microbial inocula**				
Irrigation level of ETc	**•AMF**	**••Ps1**	**AMF+Ps1**	**Control**	**Mean(B)**	**•AMF**	**••Ps1**	**AMF+Ps1**	**Control**	**Mean(B)**
	No. of leaves					**No. of leaves**				
50%	21.2 i	21.7 g	23.1 d	21.5 g	21.9 B	20.6 h	21.9 e	21.5 f	19.1 i	20.8 C
75%	22.2 e	21.4 h	23.0 d	21.9 f	22.1 B	21.9 e	20.7 g	23.4 b	20.7 g	21.7 B
100%	25.0 b	23.2 c	26.8 a	21.4 h	24.1 A	22.7 c	22.0 e	26.4 a	22.2 d	23.3 A
Mean (A)	22.8 B	22.1 C	24.3 A	21.6 D		21.7 B	21.5 B	23.8 A	20.7 C	
	Total plant fresh weight (g/plant)					**Total plant fresh weight (g/plant)**				
50%	738 g	539 i	998 e	473 j	687 B	753 ef	539 i	1048 d	449 j	697 C
75%	1090 d	770 f	1260 b	646 h	941 A	1068 d	737 g	1197 b	569 h	893 B
100%	1161 c	737 g	1365 a	675 h	985 A	1150 c	770 f	1501 a	689 g	1028 A
Mean (A)	996 B	682 C	1208 A	598 C		990 B	682 C	1249 A	569 D	
	Total chlorophyll µg Chl./cm (SPAD)					**Total chlorophyll µg Chl./cm (SPAD)**				
50%	67.5 b	67.2 b	68.6 a	49.1 h	63.1 A	64.1 c	60.2 d	68.2 a	48.5 h	60.3 A
75%	61.1 d	55.6 e	62.5 c	46.5 i	56.5 B	57.0 f	58.0 e	67.9 a	46.7 i	57.4 B
100%	54.3 f	53.2 g	55.1 ef	40.9 j	50.9 C	58.1 e	56.0 g	65.9 b	45.8 j	56.4 B
Mean (A)	61.0 A	58.6 B	62.1 A	45.5 C		59.7 B	58.1 B	67.3 A	47.0 C	

•AMF Arbuscular mycorrhizal fungi, •• Ps1 *Pseudomonas fluorescens* (Ps1)

Data displayed in **Table 6** present the influence of different ETc treatments and different combinations between AMF and *Ps. fluorescens* (Ps1) on the yield and quality parameters of broccoli plants during two successive seasons (2018, 2019).

Generally, to estimate the effect of ETc it is clear that, 75% ETc revealed the highest significant increase in head weight, head length, and head width in the two successive seasons being 453 and 457 g/plant, 16.0 and 12.4 cm and 26.7 and 25.8 cm, while no significant differences were recorded in the head length of broccoli plants subjected to 75% and 100% ETc.

The different combinations between AMF and *Ps. fluorescens* (Ps1) in **Table 6** revealed that using both of *Ps. fluorescens* (Ps1) and AMF on broccoli plants recorded the highest significant yield and quality parameters in the two seasons being 465 and 476 g/plant for head weight, 18.8 and 15 cm for head length and 32.2 and 30.5 cm for head width respectively.

The different combination between ETc and biofertilizers (*Ps. fluorescens* (Ps1) and AMF) revealed the highest significant head weight and head width within plants subjected to 75% ETc and inoculated with (*Ps. fluorescens* (Ps1) and AMF) in the two successive seasons being 616 and 647 g/plant and 36.8 and 34.9 cm respectively. The highest significant head length in the first season was recorded within broccoli plants subjected to 75% ETc and inoculated by (*Ps. fluorescens* (Ps1) and AMF) being 19.5 cm while in the second season was recorded within plants subjected to 100% ETc and inoculated by (*Ps. fluorescens* (Ps1) and AMF) being 15.9 cm. The growth and biochemical constituents of plants could be elucidated by the capability of *Pseudomonas* spp. to stimulate some defense mechanisms in the plant toward biotic and abiotic stresses. For instance, they produce some biochemical signals such as indole acetic acid and gibberellins, leading to stimulation and boosting the length, expanding surface area, and the number of root tips. Accordingly, nutrient uptake is improved, and plant fresh, dry weights and yield increase under stress conditions (Hussain et al 2016, Neetu et al 2012).

Table 6. Yield parameters of broccoli plants as influenced by different treatments throughout the two successive seasons of 2018 and 2019

Irrigation level of ETc	**1st season**					**2nd season**				
	Microbial inocula					**Microbial inocula**				
	•AMF	**••Ps1**	**AMF+Ps1**	**Control**	**Mean (B)**	**•AMF**	**•Ps1**	**AMF+Ps1**	**Control**	**Mean (B)**
	Head weight (g/plant)					**Head weight (g/plant)**				
50%	353 g	227 h	370 f	149 j	275 C	258 h	256 h	332 g	142 k	247 C
75%	568 b	421 c	616 a	206 i	453 A	559 b	413 e	647 a	210 i	457 A
100%	392 e	362 f	408 d	203 i	341 B	489 c	360 f	449 d	178 j	369 B
Mean (A)	438 B	337 C	465 A	186 D		435 B	343 C	476 A	177 D	
	Head length (cm)					**Head length (cm)**				
50%	16.5 e	10.8 i	18.7 b	8.60 k	13.7 C	12.8 e	8.93 j	14.2 cd	7.50 k	10.9 B
75%	17.8 cd	15.0 f	19.5 a	11.7 h	16,0 A	14.3 c	10.9 h	15.0 b	9.34 i	12.4 A
100%	17.3 d	14.1 g	18.3 bc	9.83 j	14.9 B	14.0 d	11.3 g	15.9 a	11.9 h	13.3 A
Mean (A)	17.2 A	13.3 B	18.8 A	10.0 C		13.7 B	10.4 C	15.0 A	9.60 C	
	Head width (cm)					**Head width (cm)**				
50%	23.6 e	20.4 g	27.8 d	11.2 j	20.7 C	21.2 f	19.5 g	24.6 e	12.7 i	19.5 C
75%	32.0 b	21.9 f	36.8 a	16.1 h	26.7 A	29.9 c	24.3 e	34.9 a	14.2 h	25.8 A
100%	29.4 c	21.9 f	32.1 b	14.2 i	24.4 B	25.6 d	21.2 f	32.0 b	14.5 h	23.3 B
Mean (A)	28.3 B	21.4 D	32.2 A	13.8 C		25.5 B	21.6 C	30.5 A	13.8 D	

•AMF Arbuscular mycorrhizal fungi, ••Ps1 *Pseudomonas fluorescens* (Ps1)

3.5 NPK contents in leaves

Data in **Table 7** shows the percentages of N, P, and K in broccoli plant leaves as influenced by different treatments throughout the 2018 and 2019 growing seasons.

Results show that the highest significant N, P, K contents in leaves were observed in 50% of ETc being 4.95, 0.63, and 2.55 in the first season and 5.00, 0.62, 2.45 in the second season, respectively. Regarding the use of different biofertilizers, the combined inoculation with AMF and *Ps. fluorescens* (Ps1) showed the highest significant content of N, P, and K in plant leaves being 4.88, 0.60, and 2.61 in the first season and 4.79, 0.58 and 2.57 in the second season respectively.

Concerning irrigation and inoculation interaction, the treatment of 50% ETc inoculated with AMF and *Ps. fluorescens* (Ps1) gave the highest significant nutrient content (N, P, and K) in plant leaves. Different researchers recorded that increasing the irrigation water level causes an increase in the leachability of the nutrients from the soil profile (Hashem et al 2014). Regarding the interaction among AMF and *Ps. fluorescens* (Ps1) it is clear that *Ps. fluorescens* (Ps1) enhances mutualistic interactions between plants and AMF, as well as the growth performance of mycorrhizal plants by causing better root structure that allows the extra radical hyphae to expand beyond plant rhizosphere depletion zones toward available moisture areas, which permits more effective absorption of water and low-mobile nutrients under water stress (Qurashi and Sabri 2012, Giri et al 2004).

Table 8, shows the effect of different irrigation levels on biochemical constituents of broccoli heads. The highest significant constituents were (6.22 and 6.50%) for TSS, (78.4 and 82.1 mg/100 g FW) for vitamin C, and (2.82 and 2.60 g/100 g FW) for protein were obtained when broccoli was supplied with irrigation water equal to 50% ETc.

The combined inoculation of broccoli plants by AMF and *Ps. fluorescens* (Ps1) significantly increased the TSS, vitamin C, and protein, during the two studied seasons being 7.06 and 6.845% for TSS, 77.0 and 87.3 mg/100 g FW for vitamin C and 3.09 and 3.03 g/100 g FW for protein.

With respect to the interaction between different irrigation levels and microbial inoculants, data revealed that the highest significant head chemical compounds were obtained by combined inoculation of AMF and *Ps. fluorescens* (Ps1) under 50% ETc during the two studied seasons being 7.47 and 7.56 % for TSS, 85.2 and 98.3 mg/100 g FW for vitamin C and 3.26 and 3.19 g/100 g FW for protein. While the lowest chemical compounds of broccoli plants were obtained by 100% ETc without inoculation. These results are compatible with (Emmanuel and Babalola 2020, Erdogan and Bagdatli 2017, Zhang et al 2011) who reported that mycorrhiza can delay drought effect

Table 7. Content of N, P, and K in leaves of broccoli plants as influenced by different treatments throughout the two seasons of 2018 and 2019

Irrigation level of ETc	**1st season**					**2nd season**				
	Microbial inocula					**Microbial inocula**				
	•AMF	**••Ps1**	**AMF+Ps1**	**Control**	**Mean(B)**	**•AMF**	**••Ps1**	**AMF+Ps1**	**Control**	**Mean(B)**
	N (%)					**N (%)**				
50%	5.39 b	4.41 c	5.94 a	4.06 ef	4.95 A	5.10 b	4.85 c	5.64 a	4.40 e	5.00 A
75%	4.13 de	3.99 f	4.51 c	3.78 g	4.10 B	4.07 fg	3.99 g	4.60 d	3.47 j	4.03 B
100%	3.85 g	3.64 h	4.21 d	3.64 h	3.83 C	3.82 h	3.68 i	4.12 f	3.43 j	3.76 C
Mean (A)	4.46 B	4.01 C	4.88 A	3.83 D		4.33 B	4.17 B	4.79 A	3.77 C	
	P (%)					**P (%)**				
50%	0.65 b	0.63 b	0.71 a	0.56 b	0.63 A	0.68 b	0.56 c	0.72 a	0.51 d	0.62 A
75%	0.49 de	0.47 ef	0.58 c	0.47 ef	0.50 B	0.52 d	0.45 ef	0.55 c	0.38 g	0.47 B
100%	0.43 g	0.45 fg	0.51 d	0.39 h	0.44 B	0.44 f	0.46 ef	0.48 e	0.33 h	0.43 B
Mean (A)	0.52 B	0.51 B	0.60 A	0.47 C		0.54 B	0.49 C	0.58 A	0.41 D	
	K (%)					**K (%)**				
50%	2.62 c	2.55 d	2.85 a	2.21 g	2.55 A	2.54 c	2.20 e	2.90 a	2.14 f	2.45 A
75%	2.48 e	2.22 fg	2.72 b	1.95 g	2.34 B	2.34 d	2.20 e	2.66 b	1.98 h	2.30 B
100%	2.08 h	1.80 i	2.27 f	1.81 i	1.99 C	1.98 h	2.08 g	2.15 e	1.80 i	1.99 C
Mean (A)	2.39 B	2.19 C	2.61 A	1.99 D		2.29 B	2.16 C	2.57 A	1.97 D	

•AMF -Arbuscular mycorrhizal fungi, ••Ps1 *Pseudomonas fluorescens* (Ps1)

Table 8. TSS (%), vitamin C, and protein contents in broccoli fruits as influenced by different treatments throughout the two seasons of 2018 and 2019

Irrigation level of ETc	1st season					2nd season				
	Microbial inocula					Microbial inocula				
	•AMF	Ps1••	AMF+Ps1	Control	Mean(B)	•AMF	••Ps1	AMF+Ps1	Control	Mean(B)
	TSS (%)					TSS (%)				
50%	6.53 b	5.70 d	7.47 a	5.18 ef	6.22 A	6.46 c	5.99 e	7.56 a	5.99 e	6.50 A
75%	6.01 c	5.23 e	7.55 a	4.85 g	5.91 B	6.31 d	6.19 d	6.74 b	5.45 f	6.17 B
100%	5.15 f	4.71 g	6.16 c	4.21 h	5.06 C	4.98 g	4.67 h	6.21 d	5.08 g	5.23 C
Mean (A)	5.89 B	5.21 BC	7.06 A	4.75 C		5.91 B	5.62 C	6.84 A	5.51 C	
	Vitamin C (mg/100 g FW)					Vitamin C (mg/100 g FW)				
50%	83.0 b	75.8 c	85.2 a	69.4 e	78.4 A	87.7 b	78.8 d	98.3 a	63.4 h	82.1 A
75%	70.7 de	63.2 f	74.4 c	54.0 h	65.6 B	81.1 c	76.4 e	87.5 b	72.8 f	79.5 B
100%	63.4 f	57.6 g	71.4 d	45.6 i	59.5 C	69.3 g	60.0 i	75.9 e	57.6 j	65.7 C
Mean (A)	72.4 B	65.5 C	77.0 A	56.3 D		79.4 B	71.7 C	87.3 A	64.6 D	
	Protein (g/100 g FW)					Protein (g/100 g FW)				
50%	3.24 a	2.43 d	3.26 a	2.35 e	2.82 A	2.88 b	2.45 c	3.19 a	1.86 e	2.60 A
75%	3.06 b	2.30 e	3.11 b	1.85 g	2.58 B	3.07 ab	2.26 d	2.95 b	1.53 f	2.45 B
100%	2.83 c	2.12 f	2.90 c	1.82 g	2.42 C	2.38 d	1.95 e	2.95 b	1.36 f	2.16 C
Mean (A)	3.04 A	2.28 B	3.09 A	2.01 C		2.78 B	2.22 C	3.03 A	1.58 D	

•AMF Arbuscular mycorrhizal fungi, ••Ps1 *Pseudomonas fluorescens* (Ps1)

according to the colonization ratio, which may vary according to irrigation levels, plant species, and some ecological parameters, especially under the low irrigation level (Polcyn et al 2019).

It is clear from the previous data that nutrient contents in the broccoli heads were lower under high irrigation levels compared to lower irrigation levels, which resulted in low yield quality. These results could be attributed to increasing irrigation levels, increased humidity in plants, and decreasing solidity, which decreased the quality of broccoli (Hashem and Abd-Elrahman 2016).

3.6 Water use efficiency (WUE)

Data in **Table 9** show the calculated WUE of broccoli plants under different irrigation levels and different combinations between AMF and *Ps. fluorescens* (Ps1) during the two studied seasons of 2018 and 2019.

The treatment of 75% ETc showed the highest significant values of WUE (2.34, and 2.30 kg/m^3) in the two seasons respectively.

The effect of microbial inoculant treatments on water use efficiency was significantly noticeable during both seasons. The combined inoculation of AMF and *Ps. fluorescens* (Ps1) gave the highest significant WUE being 2.54 and 2.49 kg/ m^3 in the two seasons respectively followed by those inoculated by AMF. The lowest broccoli Water use efficiency was obtained with control groups during both seasons.

The combined inoculation of AMF and *Ps. fluorescens* (Ps1) combined with 75% ETc gave the highest significant values compared to the other treatments being 3.18 and 3.26 kg/ m^3 followed by 50% ETc under the same treatment being 2.87 and 2.51 kg/ m^3 in the two seasons respectively. These results agreed with those reported by Langeroodi et al (2020), and Rouphael et al (2015) who found that the addition of different combinations of biofertilizers especially the AMF improved the efficiency of water use and protected the plants from stress.

Increasing water use efficiency can be attributed to the combined inoculation of AMF and *Ps. fluorescens* (Ps1) as both of them improve the rate of delivering macro and micronutrients to the plants and improve crop resistance to water stress (Abdrabbo et al 2015). Supporting this notion, Jiao et al (2011) stated that inoculation of plants with AMF enhanced water use efficiency in vegetable plants by improving water absorption, leading to more efficient water usage.

Table 9. Water use efficiency (WUE) of broccoli plants under different irrigation water levels and microbial inoculant types in the two seasons of 2018 and 2019

Irrigation level of ETc	**1st season**					**2nd season**				
	Microbial inocula					**Microbial inocula**				
	•AMF	**••Ps1**	**AMF+Ps1**	**Control**	**Mean (B)**	**•AMF**	**••Ps1**	**AMF+Ps1**	**Control**	**Mean (B)**
	WUE (kg/ m^3)•••					**WUE** (kg/ m^3)				
50%	2.74 c	1.76 e	2.87 bc	1.16 h	2.13 A	1.95 cd	1.93 def	2.51 b	1.07 g	1.87 B
75%	2.94 b	2.18 d	3.18 a	1.06 h	2.34 A	2.81 b	2.08 c	3.26 a	1.06 g	2.30 A
100%	1.52 efg	1.40 g	1.58 ef	0.79 i	1.32 B	1.85 ef	1.36 g	1.70 f	0.67 h	1.39 C
Mean (A)	2.40 A	1.78 B	2.54 A	1.00 C		2.20 B	1.79 C	2.49 A	0.93 D	

•AMF Arbuscular mycorrhizal fungi, ••Ps1 *Pseudomonas fluorescens* (Ps1), •••WUE Water use efficiency

4 Conclusion

This study can assume that using vesicular arbuscular mycorrhizal fungi (AMF) and *Ps. fluorescens* (Ps1) in combination with 75% of ETc of irrigation level for broccoli plants was a promising combination to increase the productivity under water stress management. Additionally, using arbuscular mycorrhizal fungi (AMF) and *Ps. fluorescens* (Ps1) improved the leaves and fruit's nutrient content especially the quality characteristics when combined with a low level of irrigation water which saved macronutrients, increased WUE of the studied plants, and ameliorate the adaptability to stress.

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