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Evaluation the Effect of Bio-Fertilization on Some Wheat (*Triticum Aestivum*) Growth Parameters under Drought Conditions

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Abstract

Field experiment was conducted during 2018- 2019 in loam soil at the research field of the Department of Biology, College of Science, Baghdad University, Baghdad, Iraq, to study the effect of bio-fertilizers and two levels of chemical fertilization (50% and 100%) in some agronomic traits of wheat *Triticum aestivum* L. cultivar IPA 99 by the genus *Azotobacter chroococum* and AMF *Glomus mosseae* singly or in combination under drought condition. The experimental design was a Completely Randomized Block Design (CRBD)with three replications. The results revealed that the application of bio-fertilizers reduced the negative impacts of water deficit. However, (Azoto+AMF) were significantly increased the means of plant height, flag leaf area, flag leaf chlorophyll content and the fresh weight of total vegetative (86.81 cm, 65.45 cm², 49.55 SPAD, 37.93 g) respectively compared to the control treatment at 20% water deficit and 50% fertilization. Besides, there was no antagonism between *A. chroococum* and *Glomus mosseae*, which can recommend to use them as bio-fertilizer.

Keywords: bio-fertilizers, *Azotobacter* sp., *Glomus mosseae*, Wheat, drought conditions.

تقييم تأثير الاسمدة الحيوية في بعض صفات النمو لنبات *Triticum aestivum* L. تحت ظروف الجفاف

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الخلاصة

نفذت التجربة الحقلية خلال الفترة 2018 - 2019 في حقول ذات تربة مزيجية تابعة لوحدية ابحاث قسم علوم الحياة في كلية العلوم - جامعة بغداد - بغداد / العراق. وذلك لدراسة تأثير الاسمدة الحيوية ومستويان من الاسمدة الكيميائية (50% و 100%) في بعض صفات نمو نبات الحنطة صنف أباء 99 باستخدام الجنس *Azotobacter chroococum* وفطريات المايكورايزا *Glomus mosseae* بصورة مفردة أو متحدة تحت ظروف الجفاف. استخدم تصميم القطاعات الكاملة العشوائية بترتيب الألواح المنشقة (CRBD) مع ثلاث مكررات. أذ بينت النتائج ان إضافة الاسمدة الحيوية قد اختزلت التأثير السلبي لنقص الماء. علاوة على ذلك، معاملة التسميد الحيوي Azoto+AMF قد زادت معنوياً من معدل ارتفاع النبات، مساحة ورقة العلم، محتوى الكلوروفيل في ورقة العلم والوزن الطري للمجموع الخضري بنسبة (86.81 سم، 65.45 سم²، 49.55

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SPAD, 37.93 (على التوالي, بالمقارنة مع معاملة السيطرة تحت ظروف الري 20 % وتسميد 50 % . بالإضافة لذلك عدم وجود تضاد بين *Glomus mosseae* و *A. chroococcum* مما يسمح باستخدامها معاً ضمن توليفة الاسمدة الحيوية.

Introduction

Wheat (*Triticum aestivum* L.) is cerealia that belongs to Poaceae (Gramineae) family, which is organized from subtropical area and it contains 13% protein, 11% gluten and 69 % carbohydrate [1]. It has high nutrient content and diverse products made of wheat as staple for a third of population in the world [2]. Due rapidly increasing population and changing dietary manner, the demand for wheat by 2050 is expected to increase by 31% over the 683 million tons consumed in 2008 [3]. Abiotic stresses, such as drought, soil salinity, and extreme temperatures, adversely affect the output and quality of economically important crops throughout the world. Drought stress is one of the central limitations to crop productivity so high- yielding crops even in environmentally stressful conditions are fundamental [4]. Drought affects morphological, biological, physiological and molecular processes in plants resulting in growth inhibition [5]. One of pains to solve such drought stress on wheat is through mycorrhizal inoculation. Mycorrhiza is a mutualistic symbiosis between fungus and the plant's roots. The mycorrhizal fungus could increase the plant's growth by increasing the nutrient adsorption, particularly P_2O_4 , increasing plant's resistance to drought, controlling root infection by pathogens, stimulating activities of some advantageous organisms to improve structure, production the growth stimulants compounds, and soil aggregation as well as mineral nutrient allocation [6]. As a result, mycorrhizal plants are often more competitive and are able to tolerate environmental stresses contrast to non- mycorrhizal plants. Nitrogen -fixing bacteria (*Azotobacter*) supplied additional nitrogen in an eco-friendly mode. *Azotobacter* has been found to synthesize plant growth promoting substances like gibberellins, cytokinins, auxin and some antibiotics metabolites [7]. It can impact plant growth indirectly by increasing the population of beneficial microorganisms in the rhizosphere, these microorganisms serve as a applicable alternative to nitrogenous fertilizers and involve comparatively lower cost. Diverse workers have reported significant increase in yield in diverse crops through the use of *Azotobacter* [8].

For this reason, the central goal was to study the effect of bacteria *Azotobacter chroococum* and *Glomus mosseae* on shortage of water irrigation lacking on some wheat (*Triticum aestivum* L.) growth parameters under field conditions.

Material and methods

Soil collection: Four soil samples were collected from Al Ramadi; samples were picked from (10 – 15) cm below the surface from Rhizosphere of roots of two crops (wheat and barley), which collected in March 2018 in pored polythene bags and stored at room temperature to use for arbuscular mycorrhiza fungi isolation.

Isolation and identification of AM fungi from root -soil mixtures

The spores of AM fungi were isolated by using the wet sieving and decanting method describes by [9]. The procedure used was as follows:

- The root-soil mixture was vigorously mixed with a glass rod for 30 sec.
- Leave the mixture 10 sec to settle heavier particles and organic material, the remaining soil-root-hyphae-spores suspension is slowly poured through a set of three sieves. The sieves used are those with pores of diameters of 85, 65, and 50 respectively.
- The extract was washed away from the sieves to petri dishes of 10cm diameter.
- Using a dissecting microscope, spores, aggregates, and sporocarps are picked by means of pipette or forceps.
- The fresh spores were used for identification based on morphology of spores, spore –bearing structures, sporocarps morphology [10].

Isolation of *Azotobacter chroococcum* from soi

- Grad dilutions preparation of soil solution (10^{-3} , 10^{-5}) for each sample .
- One ml from each dilution was placed in 250 ml flask containing 50 ml of N- free Jensen's broth and incubated at 30° C for 2-5days .

- The flasks were examined for a film of surface growth formation, and prepare a wet mount preferably of the surface film and observe with compound microscope .
- Plates of N – free Jensen's agar were streaked and incubated at 30 ° C for 1-2 days .
- The plates were examined for colonies presence, the colonies wet mounted and gram stain examined .
- The pure colonies were examined and used as inoculums for a slant of N - free Jensen's agar medium .
- All the isolates of *Azotobacter* sp. were subjected to biochemical characterizations : Gram stain reaction , Growth on N- free medium containing 1 % (sucrose , mannitol , and rhamnose) as a sole carbon sources [11].

Field experiment: Experiment was conducted on (2018-2019) at the research field of the Department of Biology, College of Science, Baghdad University, Baghdad, Iraq. The chemical and physical characteristics of field soil were measured in the laboratory of soil Department, college of agriculture, Baghdad University (Table-1). Field plots (48 plots) (1×2 m) were prepared in the field equipped with rainfall transparent shed to avoid rainfall during winter season. The plots were separated from each other by a plastic sheet inserted vertically in the soil to 35 cm depth in order to prevent the possible horizontal movement of irrigated water and inoculant grains of wheat cultivar (IPA 99) were sown manually in their respective plots in rows of two meter each with a distance of 20 cm between rows (3 rows per plot) and at seed rate of 10g per row (100 kg/ha). The plot was treated with bio-fertilizer consisted of *Glomus mosseae* , *Azotobacter chroococcum* and their combination. Chemical fertilizers used were urea and super phosphate (P_2O_5) at 100 kg ha⁻¹. All phosphorus fertilizer was added at the beginning during seed bed preparation, while urea was divided into two equal amounts(25 kg for acre) The first amount was added during the land preparation prior to planting, the second was added 40 days after sowing (during the early tillering stage).The seeds of wheat *Triticum aestivum* L. cultivar (IPA 99) were sowed (on 28 November 2018). Water stress was applied by irrigated the plots to the soil filed capacity then withheld next irrigation until the soil moisture the respective plots reached 50, and 20 % of soil field capacity. All weeds were hand weeded during the course of study. Soil moisture of the plots was recorded by weight basis method [12].

After flowering stage, the biological parameters (plant height (cm), flag leaf area (cm²) , flag leaf chlorophyll content (SPAD) and fresh weight of total vegetative (g)) were taken by randomly and for ten replicates of each plot.

The experiment was conducted in split plot design with three replications for each treatment. The data were analyzed using analysis of variance (ANOVA). The Least significant difference (LSD) at $P < 0.05$ probability level was calculated.

Results

Isolation and identification of AM fungi from root – soil mixtures

Results of isolation and identification of AM fungi from rhizosphere of root of two plant species (wheat and barley) showed that the most common AM fungi were *Glomus mosseae*. the common species found in Iraq soils (Figure-1).



Figure 1- The single spore isolated of *Glomus mosseae* (40X)

Physical and chemical properties of soil**Table 1-** Some physical and chemical properties of experiment soil

Sand (g/kg soil)	Silt (g/kg soil)	Clay (g/kg soil)	Texture soil	Field capacity	pH	EC ds/ m	Available nutrients mg. kg ⁻¹		
							N	P	K
320	430	250	Loam	31	7.4	1.1	14.58	24.36	375.16

The agronomic traits result**a-The height of the plant**

Results in Table-2 revealed that plant height significantly affected by water deficit at different fertilization treatments. The greatest mean of the plant high was 95.56 cm for the 50% water deficit, while the lowest was 84.47cm for the 20% water deficit. Besides, the highest mean of higher plant was 91.22 cm at 100% fertilization, but the lowest was 88.80 cm at 50% fertilization. Hence, the interaction between the fertilization and water deficit were significantly affected, the highest mean was 97.29 cm at 100% fertilization and 50% water deficit, while the lowest was 83.79 cm at 50% fertilization and 20% water deficit.

Also, the interaction between the fertilization and the treatments were significantly affected, the highest value was 93.75 cm of Azoto +AMF at 100% fertilization , while the lowest was 84.03 cm of control at 50% fertilization. Moreover, the interaction between water deficit and treatments were significantly affected, the highest value was 98.83 cm of Azoto +AMF at 50% water deficit, but the lowest was 80.39 cm of control at 20% water deficit.

Furthermore, the triple interaction between fertilization, water deficit, and treatments were significantly affected. the result in Table-2 showed that the highest value was 100.22 cm of Azoto +AMF at 100% fertilization and 50% water deficit, while the lowest was 79.26 cm of control at 50% fertilization and 20% water deficit.

Table 2- Effect of bio-fertilizer, Water deficit and Treatments in the plant height (cm)

F: Fertilizer %	H: Water %	T: Treatments				F x H	Mean FH
		AMF	Azoto	Azoto + AMF	Control		
50	20	84.31	84.79	86.81	79.26	83.79	88.81
	50	93.64	95.22	97.64	88.81	93.83	
100	20	86.82	85.03	87.28	81.52	85.16	91.23
	50	98.47	98.26	100.22	92.23	97.29	
LSD	--	LSD FHT = 4.888 *				LSD FH= 3.20 *	LSD F= 1.72 *
F x T						Mean of F	
F: 50		88.97	90.01	92.22	84.03	88.80	LSD FT = 7.76 *
F: 100		92.65	91.64	93.75	86.87	91.22	
H x T						Mean of H	
H: 20		85.56	84.91	87.04	80.39	84.47	90.01
H: 50		96.06	96.74	98.93	90.52	95.56	
LSD		LSD HT = 3.55 *				LSD H= 1.72 *	
Mean of T		90.81	90.82	92.99	85.45	LSD T= 2.44 *	

* (P<0.05).

* = significant

b-The flag leaf area

The interaction between the fertilization and the treatments were significantly affected. Table- 3 showed that the highest value was 71.65 cm² of Azoto +AMF at 100% fertilization, while the lowest was 63.21cm² of control at 50% fertilization. Although the highest mean of flag leaf area was 69.76 cm² at 100% fertilization, the lowest was 97.91cm² at 50% fertilization.

As well as, the interaction between the fertilization and water deficit were significantly affected, the highest mean was 74.36 cm² at 100% fertilization and 50% water deficit, while the lowest was 63.61cm² at 50% fertilization and 20% water deficit. Also, the highest mean between the treatments was 70.90 cm² of Azoto +AMF, but the lowest was 64.80 cm² of control.

In addition to, the interaction between water deficit and treatments were significantly affected, the highest value was 75.48 cm² of Azoto +AMF at 50% water deficit, but the lowest was 60.74 cm² of control at 20% water deficit. Although the triple interaction between fertilization, water deficit, and treatments were significantly affected, the highest value was 76.11 cm² of Azoto +AMF at 100% fertilization and 50% water deficit, while the lowest was 59.91 cm² of control at 50% fertilization and 20% water deficit.

Table 3- Effect of Bio-fertilizer, Water deficit and Treatments in the flag leaf area (cm²)

F: Fertilizer %	H: Water %	T: Treatments				F x H	Mean FH
		AMF	Azoto	Azoto + AMF	Control		
50	20	64.82	64.28	65.45	59.91	63.61	67.92
	50	73.30	74.23	74.86	66.51	72.22	
100	20	66.15	65.78	67.20	61.58	65.17	69.77
	50	75.15	74.98	76.11	71.21	74.36	
LSD	--	LSD FHT = 5.190 *				LSD FH= 3.038 *	LSD F= 1.83 *
F x T						Mean of F	
F: 50		69.06	69.25	70.15	63.21	67.9 1	LSD FT = 6.58 *
F: 100		70.65	70.38	71.65	66.39	69.7 6	
H x T						Mean of H	
H: 20		65.48	65.03	66.86	60.74	64.39	68.84
H: 50		74.22	74.61	75.48	68.86	73.29	
LSD		LSD HT = 3.53 *				LSD H= 1.83 *	
Mean of T		69.85	69.82	70.90	64.80	LSD T= 2.59 *	
* (P<0.05).							

* = significant

c-The flag leaf chlorophyll content (SPAD)

Results in Table-4 show that means of flag leaf chlorophyll content (SPAD) significantly affected by water deficit stress at different fertilization. The highest mean of flag leaf chlorophyll content was 51.89 SPAD for the 50% water deficit, while the lowest was 46.07 SPAD for the 20% water deficit. In addition, the highest mean of flag leaf chlorophyll content was 49.78 SPAD at 100% fertilization, but the lowest was 48.17 SPAD at 50% fertilization. Otherwise, the highest mean between the treatments was 52.45 SPAD of Azoto +AMF, while the lowest was 44.5 SPAD of control.

Although the interaction between the fertilization and water deficit were significantly affected, the highest mean was 53.17 SPAD at 100% fertilization and 50% water deficit, while the lowest was 45.74 SPAD at 50% fertilization and 20% water deficit. As well as, the interaction between the fertilization and the treatments were significantly affected, the highest value was 52.81 SPAD of Azoto +AMF at 100% fertilization but the lowest was 42.40 SPAD of control at 50% fertilization.

Also, the interaction between water deficit and treatments were significantly affected, the highest value was 55.25 SPAD of Azoto +AMF at 50% water deficit, while the lowest was 40.75 SPAD of control at 20% water deficit.

Moreover, the triple interaction between fertilization, water deficit, and treatments were significantly affected, the highest value was 55.86 SPAD of Azoto +AMF at 100% fertilization and 50% water deficit, while the lowest was 40.06 SPAD of control at 50% fertilization and 20% water deficit.

Table 4- Effect of Bio-fertilizer, Water deficit and Treatments in The flag leaf chlorophyll content(SPAD)

F: Fertilizer %	H: Water %	T: Treatments				F x H	Mean of FH
		AMF	Azoto	Azoto + AMF	Control		
50	20	46.90	46.44	49.55	40.06	45.74	48.18
	50	51.11	51.99	54.63	44.73	50.61	
100	20	47.53	46.86	49.76	41.43	46.40	49.78
	50	53.36	53.06	55.86	50.40	53.17	
LSD	--	LSD FHT = 3.703 *				LSD FH= 3.089 *	LSD F= 1.30 *
F x T						Mean of F	
F: 50		49.01	49.21	52.09	42.40	48.17	LSD FT = 4.47 *
F: 100		50.45	49.96	52.81	45.91	49.78	
H x T						Mean of H	
H: 20		47.21	46.65	49.66	40.75	46.07	48.98
H: 50		52.24	52.52	55.25	47.56	51.89	
LSD		LSD HT = 2.75 *				LSD H= 1.30 *	
Mean of T		49.73	49.59	52.45	44.5	LSD T= 1.85 *	
* (P<0.05).							

*= significant

d- The fresh weight of total vegetative

The interaction between the fertilization and water deficit was significantly affected, the highest mean was 41.00 g at 100% fertilization and 50% water deficit, while the lowest was 35.51 g at 50% fertilization and 20% water deficit. As well as, the highest mean of the fresh weight of total vegetative was 40.61 g at 50% water deficit, but the lowest was 36.11 g at 20% water deficit.

Otherwise, there was no significant effect between fertilization, the highest mean of the fresh weight of total vegetative was 38.86 g at 100% fertilization, but the lowest was 37.86 g at 50% fertilization. Hence, the highest mean between the treatments was 40.85 g of Azoto +AMF, while the lowest was 34.91g of control.

Furthermore, the interaction between the fertilization and the treatments were significantly affected, the highest value was 41.10 g of Azoto +AMF at 100% fertilization, while the lowest was 34.08 g of control at 50% fertilization. Besides, the interaction between water deficit and treatments were significantly affected, the highest value was 43.43 g of Azoto +AMF at 50% water deficit, but the lowest was 31.63 g of control at 20% water deficit.

Nevertheless, the triple interaction between fertilization, water deficit, and treatments were significantly affected, the highest value was 43.60 g of Azoto +AMF at 100% fertilization and 50% water deficit, while the lowest was 31.16 g of control at 50% fertilization and 20% water deficit.

Table 5- Effect of Bio-fertilizer, Water deficit and Treatments in biomass of fresh weight of total vegetative (g)

F: Fertilizer %	H: Water %	T: Treatments				F x H	Mean of FH
		AMF	Azoto	Azoto + AMF	Control		
50	20	36.01	36.93	37.93	31.16	35.51	37.86
	50	40.23	40.40	43.26	37.00	40.22	
100	20	38.36	37.83	38.60	32.10	36.72	38.86
	50	40.56	40.43	43.60	39.40	41.00	
LSD	--	LSD FHT = 4.263 *				LSD FH= 2.67 *	LSD F= 1.51 NS
F x T						Mean of F	
F: 50		38.12	38.66	40.60	34.08	37.86	LSD FT =4.057 *
F: 100		39.46	39.13	41.10	35.75	38.86	
H x T						Mean of H	
H: 20		37.19	37.38	38.26	31.63	36.11	38.36
H: 50		40.39	40.41	43.43	38.20	40.61	
LSD		LSD HT = 2.802 *				LSD H= 1.51 *	
Mean of T		38.79	38.90	40.85	34.91	LSD T= 2.13 *	
* (P<0.05).							

*= significant

NS = No Significant

Discussion

Screening for drought tolerance is a useful tool to select the most drought tolerant genotypes. This can be done under laboratory, green- house in pots and under field condition; however, water deficit is considered as a major environmental factor affecting many aspects of physiology and biochemistry [5].

Results of the present work have shown differential response of the test cultivar of wheat to water deficit stress, the reduction in all measured traits by water deficit stress applied under field conditions coincided with the findings of Bayoumi *et al.*[13], when they mention that drought caused reduction in days to 50% heading, plant height, leaf area, chlorophyll content and the fresh weight of total vegetative and in this study showed Tables-(2,3,4 and 5) reduced by 79.26, 59.91 ,40.06 and 31.16 of control, respectively. The present result revealed that all mention traits were decreased under 20% of field capacity, which is also reported by several investigators.

Bio-fertilizers can help in solving the problem of feeding an increasing global population at a time when agriculture is facing various environmental stresses. It is important to realize the useful aspects of bio-fertilizers and implement its application to modern agricultural practices. One of the goals of the study is to gain an understanding of survival mechanisms which may be used for improving

drought tolerant cultivars for areas where proper irrigation sources are scarce or drought conditions are common.

So the results of this study showed that the treatment of bio-fertilizers (Azoto+AMF) significantly increased the mean of plant height, flag leaf area, flag leaf chlorophyll content and the fresh weight of total vegetative (86.81 cm, 65.45 cm², 49.55 SPAD, 37.93 g) respectively. In this study all growth characters were greatly increased by all treatments in comparison to the control with significant differences in most cases. The values correspondingly increased by adding chemical fertilizers more when its combination with bio-fertilizers. The results revealed that the highest value for agronomic traits was the treatment of Azoto+AMF of 100% fertilizer (recommended dose). This increasing in agronomic parameter referred to the action of bio-fertilizers. It's well known that considerable number of bacterial species associated with plant rhizosphere are able to exert a beneficial effect upon plant growth and yield such as improvement might be attributed to N₂fixing and phosphate solubilizing capacity of bacteria as well as the ability of these microorganisms to produce growth promoting substances [1]. As well as, AM fungi might affect plant and soil microbial activity by stimulating the productivity of root exudates, phytoalexins, and phenolic compounds [5]. Also it has been found that AM fungi can protect host plants against determining effects caused by drought stress [14], and improved acquisition of phosphorus, nitrogen and other growth promoting nutrients [15]. AM fungi can also reduce the impact of environmental stresses such as salinity [16].

Our results of Tables-(2,3,4 and 5) revealed that *Glomus mosseae* and *Azotobacter chroococum* has improved wheat growth parameters in all treatments. These findings suggest that mycorrhizal fungus and *Azotobacter chroococum* used in this study act as agents for promoting plant growth regulators of wheat plants as reported by many papers, this due to many reasons; production of phytohormones such as IAA and gibberellins by *Azotobacter chroococum* [10]. AM fungus improved plant growth parameters by increase in the processes of absorption and transport of nutrients such as N, P, K, Ca, S, Mg, Zn, Cu, Fe, Mo, Mn, among others [17], improve productivity in soils of low fertility [18], and it can also improve absorption of N from NH₄⁺ -N mineral fertilizers, transporting it to the plant [19], secretion of antibiotics and support of a community that competes or antagonizes pathogenic microorganisms, thus aiding in disease suppression [20], increased production of plant growth hormones such as cytokines and gibberellins compare to non-inoculated plants [21]. Therefore, their use as bio-fertilizers for agriculture improvement has been a focus of numerous researchers for a number of years.

Finally, it can be concluded from this study that the interaction of *Azotobacter chroococum* and AM fungus *Glomus mosseae* under field conditions showed that all tested bio agents separately or in combination significantly increased most wheat growth parameters (shoot length, the flag leaf area, chlorophyll content and fresh weight of the total vegetative).

Conclusion

Water deficit decreases the plant height, flag leaf area, flag leaf chlorophyll content and the fresh weight of total vegetative of wheat. Nevertheless, as a result of it, some of these changes could be compensated for through the introduction of combined bio-fertilizer treatments. The findings of the present study indicate that improved on some growth parameter of *Triticum aestivum* were some tenable mechanisms for PGR-induced water deficit in wheat plants.

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