

The Effect of Bio Inoculation of *Azotobacter chroococcum* and *Glomusae mosseae* and Irrigation Periods on some Vital Characteristics of the Rhizosphere of the Maize Crop *Zea mays* L.

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Abstract

Biological inoculation with the bacteria *Azotobacter chroococcum* and the fungus *Glomus mosseae* effects on the growth and yield of *Zea mays* L. variety (Baghdad 3) under different levels of water stress was investigated in this study. It was conducted in the autumn season of 2020 in Diwaniyah Governorate - Afak county fields. According to the Randomized Complete Block Design (R.C.B.D) design, the experiment was designed with three replications, and the different treatments were distributed randomly on the experimental units. Three levels of irrigation were used in the experiment, namely (I_4) irrigation every four days, (I_7) irrigation every seven days, (I_{10}) irrigation every ten days, and inoculation levels. The inoculation with the bacteria at two different levels (B_1) inoculation with bacteria *A-chroococcum*, (B_0) without inoculation. Fungal inoculation with *G-mosseae* at two inoculation levels (F_1) and without inoculation with (F_0). In addition to the overlapping treatment between fungus and (B_1F_1) bacteria. The treatments' mean values were compared using the least significant difference (L.S.D) test at a significant level of 5%. The results present that the treatments inoculated with fungi or bacteria, or both, significantly increased the number of *Azotobacter chroococcum* for flowering and end-of-season periods and *Glomusae mosseae* infection rates. The number of bacteria and the rate of infection increased for seven days, the treatments ($10^6 \times 9.14$ and 7.11×10^6 , 70.3% and 53.0%) respectively for the flowering and end of season periods increased compared to the control (no application + irrigation every ten days) that resulted in ($10^6 \times 3.97$, 2.87×10^6 , 27.0% and 17.7%) respectively for the flowering and end of season periods.

Keywords: *Zea mays* L.; *Azotobacter chroococcum*; *Glomus mosseae*, Bio-fertilizers; Fungus inoculation, Bacterial inoculation

Introduction

Zea mays L. is a crucial crop worldwide. Because there are many variations, North America is its origin. Maize is significant since it is used as animal feed and fodder in all its grains and vegetative sections. It also plays a part in paper production and the extraction of different oils from its seeds and starch. It is a concentrated feed because it contains carbohydrates, proteins, and oil (81, 10.6, and 2) %. It is cultivated in two seasons throughout the world and is known for its adaptability to various environmental conditions. It is the third most productive crop after wheat and barley. For the year 2012, the world's planted land is (182) million hectares, with a grain production of (824) million/tons. The average production is (4527.50) kg.ha⁻¹, (2012, F.A.O). The cultivated area in Iraq of the crop is (125,000) hectares in 2012 (Arab Organization for Agricultural Development, 2012).

Biofertilizers are one of the most critical fertilizers in agricultural systems. Organic fertilizers include several microorganisms that can provide the plant with the essential elements and nutrients in a readily available and affordable manner. As a result, they lower or eliminate the use of chemical fertilizers, which reduces pollution and avoids the use of mineral fertilizers and chemical pesticides, which leads to lower agricultural costs (Mishra et al., 2013).

Azotobacter inoculation is one of the most critical vaccines used. This bacterium is a member of the Azotobactereacea tribe, whose members are free-living, compulsive, heterotrophic, and present in neutral soils, with a cell count that seldom exceeds (104,105) (D Aljawasim et al. 2020). As a carbon supply, *A-chroococcum* bacteria utilize the following organic acids, sugar, and alcohol derivatives: glucose, sucrose, fructose, methyl, and formate. They live in soil, water, and on plant root surfaces to benefit from the root secretions of sugars, amino acids, and vitamins (Abd El-Fattah, 2013). In 1885, the German researcher Frank coined the term microscopic (mushroom + root) to explain the symbiotic relationship between mushrooms and roots.

Many plant roots have a symbiotic relationship with mycorrhizae. Nevertheless, for the cruciferous tribe, 90 percent of the recognized families are marine, brackish, or desert species. The mycorrhizal fungus influences clusters' formation because of its symbiosis that causes significant changes in the root, where the fungus has a mycelium of extended strings that increase soil aggregation and stability. Mycorrhizae fungus increases the amounts of the growth regulators released in the growth medium (cytokinin, gibberellin, and auxin), which have an active role in the growth of root hairs (Bori, 2008). The relationship between the fungus and the plant is symbiotic because it benefits the fungus and the plant and the fungus's role in preparing the plant with major and minor elements. It also protects the plant from diseases found in the soil and increases its capacity to survive stress and drought conditions (Read and Smith, 2008). Many studies have shown that water stress causes many phenotypic and physiological changes in plants. It reduces plant height and leaf area and the chlorophyll content of leaves and their relative content, resulting in a decrease in photosynthesis rates and the accumulation of dry matter in the plant (Bori, 2008).

Irrigation intervals are critical for plant growth and efficiency, and they have expressed in-field plant characteristics, influencing yield components. Previous research has presented that the flowering periods and the end of the season are susceptible to water stress due to the decrease in photosynthesis outputs, affecting the growth and formation of arbors due to part of the male and female inflorescence (Al-Aboudi et al., 2010; and Alawsy, W. et al. 2018).

This study aimed to:

1. The impact of *Azotobacter chroococcum* bacteria on yellow maize growth and yield.
2. Examine the impact of the *Glomeas mosseae* fungus on maize drought tolerance, development, and yield.
3. Investigate the impact of irrigation cycles on yellow maize growth and yield.
4. Investigate the relationship of *Glomus mosseae* and *Azotobacter chroococcum* and the effects of irrigation cycles on maize growth and yield.

Methods and materials

In the fall of 2020, a field experiment was performed in Qadisiyah Governorate/Afak county to cultivate the yellow corn crop *Zea mays*. L of the cultivar (Baghdad 3) in loam sandy soil. The field soil is of high quality and ideal for agriculture. Homogeneous soil sample for processing was collected at random from the soil's surface horizon and distributed to several regions. Soil's physical, chemical, and biological properties before planting, the soil was air-dried and sifted with a sieve with a diameter of (2) mm aperture. After that, the physical, chemical, and biological properties were measured.

Seeds preparation and inoculation for planting: In the field trial, maize seeds *Zea mays*. L (Baghdad 3) were grown. Mycorrhizae inoculation was used, which included (spores, contaminated roots, and dry soil). *Glomus mosseae* was brought from the Agricultural Research Service, Ministry of Science and Technology, Zafarania. *Azotobacter chromium* inoculation was also used. After surface sterilization with mercury chloride and 95 percent alcohol, the seeds were inoculated with the bacterial vaccine *A chroococcum*. Removing alcohol and suspended matter, the seeds were washed many times with purified water. They were air-dried before being dipped in prepared Arabic gum in a 1:10 (gum-water) ratio for eight minutes to ensure that the bacterial inoculum adhered to the washed seeds. They were inoculated with previously prepared bacterial inoculum by mixing 50% of the liquid culture *A_chroococcum* and left for (15) minutes to ensure seed pollination (Bashan et al., 1995). To prevent contamination, uninoculated seeds were sown first, in the shade, away from the sunlight. As a control treatment, seeds were kept uninoculated.

Preparation of the fungal vaccine: *Glomus_mosseae* (spores, polluted roots, and dry soil) was added to the seedbed before sowing.

Experimental design

The study was constructed with three replications using the Randomized Complete Block Design R.C.B.D. The experimental treatment was as follows: mycorrhiza inoculation G, inoculation with *A. azotobacter*, overlap between fungi and bacteria A + G, control for C₀, with three irrigation periods of (4, 7, and 10) days. The treatments were assigned randomly to the experimental units. The experimental field was

split into three replications of 36 experimental units. The area of one experimental unit is $4 \times 4 = 16 \text{ m}^2$, leaving 1.5 m between each experimental unit and the next and 2 m between each replication and the next to monitor the irrigation phase. The experimental unit was split into five lines. The gap among lines is 75 cm and between plants within a line is 25 cm. The cultivated field soil was prepared for planting from plowing, smoothing, and leveling. *Mycorrhizae* inoculation was added and dispersed under the seeds, 5 cm wide and (5) cm thick, with the seeds being inoculated with the *Azotobacter bacteria* an hour before planting to ensure the adhesion of the most significant number of bacterial cells to the seeds. "July 29th" 2020 was the day of the planting of the crops. To compensate for the failure, three seeds were planted in one seedbed and grafted together a week later. Crop service and manual weeding were also conducted to eliminate the bushes continuously.

Estimation of *Azotobacter chroococcum* numbers for the flowering period and end of season: Tit was estimated according to the (Black 1965b) method. Reducing and counting the dishes was used by making a series of loosening the soil suspension, which includes from 1-10 to 7-10. The nutrient nitric medium N.A was used. The nutrient medium was put into ready-to-sterilized Petri dishes and took 1 ml of dilution 10-5, 10-6, 10-7 with three replications. Petri dishes were placed in the incubator for five days at 28°C. After vaccination, bacteria were counted in the plates.

Estimation of infestation by *Glomusae mosseae* for the flowering and end of season periods: The ratio is estimated according to the method of (Kormanik et al. 1980) according to the following steps: Acid fuchsin tincture is prepared from: acetic acid 875 ml, Glycerol 63 ml, and Distilled water 63 ml.

Dye powder 0.1 ml

The root system of plants was washed and taken randomly from each treatment well under a continuous stream of water to get rid of the soil attached to the root. Root hairs were taken 1 cm long and placed in glass test tubes. Potassium hydroxide solution K.O.H. at a concentration of 10% was applied to the root capillaries in the test tubes and then placed in a water bath of 90°C for 10-15 minutes and washed with distilled water. Root capillaries were shortened with 10% base H_2O_2 for 15-60 seconds. H.C.L. was applied at a concentration of 10% for 3 minutes. An F.A.A solution (Formalin Aceto Alcohol) was used until staining was performed to preserve the fungal structures from any morphological change. The prepared Acid fuchsin dye was applied to the samples, and the stained samples were placed in a water bath of 90°C for 10-15 minutes. Samples were taken out from the dye solution, and Lactic acid was applied to them. The samples were examined microscopically using a glass slide after selecting ten pieces of stained root capillaries of 1 cm length for each sample randomly. The infestation percentage was extracted according to the following equation:

$$\text{Root infestation rate} = \text{Number of affected plots} / \text{Total root plots (10)} \times 100$$

Table (1): Chemical and physical properties of the soil before planting.

Trait	Value	Unit	References
PH	7.71	-----	Mclean, 1982
EC	14.24	DesiSmens.M ⁻¹	Richard,1954
CEC	10.17	Cml.charge.kg ⁻¹ .soil	Rhoades,1982
Soil texture	Sandy Loam		Richards,1954
Sand	765.6	g.kg ⁻¹ Soil	
Clay	34.7		
Silt	199.7		
Organic matter O.M	8.7		Walkley,1947 and F.A.O.,1974
N	40.18	Mg.kg ⁻¹ Soil	Bremner and Mulvaney,1982
P	20.37		Olsen et al.,1954
K	224.45		
Ca	1200	Cmol _c . L ⁻¹	Richards,1954
mg	697.84		
CO ₃	0		
HCO ₃	0.492		
SO ₄	4.739		Williams and Stenbergs,1959
Total bacteria	2.90×106	CFU gm dry soil ⁻¹	Black 1965, b
Total fungi	1.81×103	CFU gm dry soil ⁻¹	Kormanik et al.1980,

Results and discussion

Effect of *A.chroococcum* and *G.mosseae* applications and irrigation periods on bacteria numbers in the flowering period Cfu/dry/soil

Table (2) presents that the bacterial inoculation with *A.chroococcum* bacteria resulted in a significant increase in the number of *A.chroococcum* bacteria in the flowering period. The inoculation treatment yielded (7.83×10^6) Cfu/dry/soil compared to the control (non-pollination), which gave the most negligible value (6.15×10^6) Cfu/dry/soil. This increase is because inoculation with *A.chroococcum* bacteria was successful and achieved an increase in the number of these bacteria (Bin Mahmoud and Iman, 2016). Inoculation with *G.mosseae* fungus significantly increased the number of *A.chroococcum* bacteria and the flowering period. Inoculation treatment yielded (106×7.40) Cfu/dry/soil compared to the comparison treatment (6.58×10^6) Cfu/dry/soil. It is attributed to the secretions of the fungus that help increase the number of bacteria and that the roots of plants inoculated with the microscopic droplets have more bacterial numbers in them compared to non-inoculated plants (Ayad Kadhim Ali Al-Husseni et al 2021; Khaeim, H. et al. 2019).

The irrigation periods (4-7-10) had a significant effect on the number of *A. chroococcum* bacteria and the flowering period. The treatment of the irrigation period every four days

the highest value (8.03×10^6) Cf/dry/soil compared to the irrigation period every seven days that resulted in (7.91×10^6) Cf/dry/soil and the irrigation period every ten days that gave the lowest value (106×5.02) Cf/dry/soil.

The bilateral interaction between the biological vaccines and the irrigation periods significantly affected *A.chroococcum* number and the flowering period. The inoculation treatment B_1I_7 resulted in the highest value (106×9.14) Cf/dry/soil compared to the comparison treatment B_0I_{10} that resulted in the lowest value (4.41×106) Cf/dry/soil. The triple overlap between study factors, treatment $B_1F_1I_7$, significantly affected the number of *A. chroococcum* bacteria and the flowering period. It produced (106×9.42) Cf/dry/soil compared to the comparison treatment $B_0F_0I_{10}$, which gave the lowest value (106×3.97) Cf/dry/soil.

Table (2): Effect of *A.chroococcum* and *G.mosseae* application and irrigation periods on numbers of *A.chroococcum* in the flowering period.

Bacteria	Fungi	Irrigation periods /day			Mean of Binary overlap F x B
		I ₄	I ₇	I ₁₀	
B ₀	F ₀	7.09 × 10 ⁶	5.64 × 10 ⁶	3.97 × 10 ⁶	5.56 × 10 ⁶
	F ₁	7.63 × 10 ⁶	7.73 × 10 ⁶	4.86 × 10 ⁶	6.74 × 10 ⁶
B ₁	F ₀	8.38 × 10 ⁶	8.86 × 10 ⁶	5.52 × 10 ⁶	7.59 × 10 ⁶
	F ₁	9.02 × 10 ⁶	9.42 × 10 ⁶	5.75 × 10 ⁶	8.07 × 10 ⁶
LSD .05		0.64 × 10 ⁶			0.37 × 10 ⁶
Bi-interaction I x B					
Bacteria	Irrigation periods /day			Mean	
	I ₄	I ₇	I ₁₀		
B ₀	7.36 × 10 ⁶	6.68 × 10 ⁶	4.41 × 10 ⁶	6.15 × 10 ⁶	
B ₁	8.70 × 10 ⁶	9.14 × 10 ⁶	5.64 × 10 ⁶	7.83 × 10 ⁶	
LSD .05		0.45 × 10 ⁶			0.26 × 10 ⁶
Bi-interaction O x F					
Fungi	Irrigation periods /day			Mean	
	I ₄	I ₇	I ₁₀		
F ₀	7.73 × 10 ⁶	7.25 × 10 ⁶	4.74 × 10 ⁶	6.58 × 10 ⁶	
F ₁	8.33 × 10 ⁶	8.58 × 10 ⁶	5.31 × 10 ⁶	7.40 × 10 ⁶	
LSD .05		0.45 × 10 ⁶			0.26 × 10 ⁶
I mean		8.03 × 10 ⁶	7.91 × 10 ⁶	5.02 × 10 ⁶	
LSD .05		0.32 × 10 ⁶			

Effect of *A. chroococcum* and *G.mosseae* application and irrigation periods on bacterial numbers at the end of season Cf/dry/soil

Table (3) showed that the bacterial inoculation with *A.chroococcum* bacteria resulted in a significant increase in the number of *A.chroococcum* bacteria during the end of the season period. The inoculation treatment yielded (5.97×106) Cf/dry/soil compared to the comparison treatment (non-pollination), which gave the least value (4.99×106)

Cfu/dry/soil. The reason is attributed to the success of the inoculation process with *A.chroococcum* bacteria and the lack of competition between the applied bacteria and the bacteria that colonize the soil. These results are consistent with (Bashir 2003). The addition of *A.chroococcum* as a vaccine grows well in the rhizosphere. Inoculation with *G.mosseae* resulted in a significant increase in the number of *A. chroococcum* bacteria during the end-of-season period. The inoculation treatment resulted in (106×5.82) Cfu/dry/soil compared to the control (5.13×106) Cfu/dry/soil. The reason is that the fungus *G.mosseae* secretes growth-regulating substances that cause an increase in the number of bacteria (Bashir, 2003).

The irrigation periods (7-10-4) had a significant effect on the number of *A.chroococcum* and the end-of-season period. The treatment of the four-day irrigation period has the highest value (6.68×106) Cfu/dry/soil compared to the irrigation period every seven days (6.15×106) Cfu/dry/soil and the irrigation period every ten days (106×3.60) Cfu/dry/soil.

The bilateral interaction between the vital vaccines and the irrigation periods significantly affected *A.chroococcum* number and the end of the season period.

The inoculation treatment B_1I_4 resulted in the highest value (106×7.03) Cfu/dry/soil compared to the comparison treatment B_0I_{10} , which produced the lowest value (3.24×106) Cfu/dry/soil. The triple interaction between study treatments $B1F1I4$ produced a significant increase in the number of *A. chroococcum* bacteria during the end of season period (106×7.26) Cfu/dry/soil compared to the control $B_0F_0I_{10}$ (106×2.87) Cfu/dry/soil.

Table (3): The effect of *A. chroococcum* and *G.mosseae* application and irrigation periods on bacterial numbers for the end of season period.

Bacteria	Fungi	Irrigation periods /day			Mean of Binary overlap F x B
		I ₄	I ₇	I ₁₀	
B ₀	F ₀	6.03 × 10 ⁶	4.62 × 10 ⁶	2.87 × 10 ⁶	4.51 × 10 ⁶
	F ₁	6.61 × 10 ⁶	6.20 × 10 ⁶	3.61 × 10 ⁶	5.47 × 10 ⁶
B ₁	F ₀	6.80 × 10 ⁶	6.68 × 10 ⁶	3.80 × 10 ⁶	5.76 × 10 ⁶
	F ₁	7.26 × 10 ⁶	7.11 × 10 ⁶	2.87 × 10 ⁶	6.17 × 10 ⁶
LSD .05		0.42 ×10 ⁶			0.24 × 10 ⁶
Bi-interaction I x B					
Bacteria	Irrigation periods /day			Mean	
	I ₄	I ₇	I ₁₀		
B ₀	6.32 × 10 ⁶	5.41 × 10 ⁶	3.24 × 10 ⁶	4.99 × 10 ⁶	
B ₁	7.03 × 10 ⁶	6.70 × 10 ⁶	3.97 × 10 ⁶	5.97 × 10 ⁶	
LSD .05		0.29 × 10 ⁶			0.17 × 10 ⁶
Bi-interaction O x F					
Fungi		Irrigation periods /day			Mean

	I₄	I₇	I₁₀	
F₀	6.42×10^6	5.65×10^6	3.33×10^6	5.13×10^6
F₁	7.03×10^6	6.90×10^6	3.97×10^6	5.82×10^6
LSD .05	0.29×10^6			0.17×10^6
I mean	6.68×10^6	6.15×10^6	3.60×10^6	
LSD .05	0.21×10^6			

Effect of *A.chroococcum* and *G.mosseae* application and irrigation periods on infection rate in the flowering period (%)

Table (4) showed the significant effect of *A.chroococcum* inoculation on the infection rate for the flowering period. The inoculation treatment yielded a value of (55.17)% compared to the comparison treatment that gave (46.39)%, and the reason is due to the success of the bacterial inoculation process. Inoculation with *G.mosseae* fungus resulted in a significant increase in infection rate and flowering period. The inoculation treatment achieved the highest value (61.9)% compared to the comparison treatment (39.6%). The reason is due to the success of the inoculation process is causing infection as well as the ability of the mycorrhizae fungus to penetrate the root hairs and form tree structures called dendrites and vesicles that grow after the dendrites in the form of terminal swellings of the fungal strands extending between or inside the cells. It extends from the affected root in the peripheral root zone and in the distant soil areas to provide the plant with nutrients, especially phosphorus and zinc (Al-Kartani et al., 2016).

The effect of irrigation periods was significant in infection rate and flowering period. The irrigation period every seven days resulted in the highest value (55.5) % compared to the irrigation period every four days (53.4%) and the irrigation period every ten days (43.4%).

The bilateral interaction between the biological vaccines and the irrigation periods was significant in the infection rate for the flowering period. The treatment F₁I₇ produced the highest value of interference (68.0) % compared to the comparison treatment F₀I₁₀, which produced the most negligible value for interference (33.0%). The triple overlap between study factors was also significant in infection percentage and flowering period. The treatment B₁F₁I₇ achieved the highest value of interference (70.3%) than the comparison treatment B₀F₀I₁₀, which gave the lowest value of interference (27.0%).

Table (4): The effect of *A.chroococcum* and *G.mosseae* application and irrigation periods on the incidence of the flowering period (%).

Bacteria	Fungi	Irrigation periods /day			Mean of Binary overlap F x B
		I₄	I₇	I₁₀	
B₀	F ₀	37.7	33.3	27.0	32.7
	F ₁	63.0	65.7	51.7	60.1
B₁	F ₀	48.0	52.7	39.0	46.6
	F ₁	65.0	70.3	56.0	63.8

LSD .05	3.0			1.7
Bi-interaction I x B				
Bacteria	Irrigation periods /day			Mean
	I ₄	I ₇	I ₁₀	
B ₀	50.3	49.5	39.3	46.39
B ₁	56.5	61.5	47.5	55.17
LSD .05	2.1			1.2
Bi-interaction O x F				
Fungi	Irrigation periods /day			Mean
	I ₄	I ₇	I ₁₀	
F ₀	42.8	43.0	33.0	39.6
F ₁	64.0	68.0	53.8	61.9
LSD .05	2.1			1.2
I mean	53.4	55.5	43.4	
LSD .05	1.5			

The effect of *A.chroococcum* and *G.mosseae* plication and irrigation periods on infection rate for the end of season period (%)

Table (5) presented a significant effect of *A. chroococcum* inoculation on infection rate and for the end of season period. The inoculation treatment yielded (39.3)% compared to the control (33.6%). The reason is the efficiency of the bacterial inoculum used and the response of the plant host to the inoculation with the fungus that causes root injury (Al-Fahdawi, 2016). The inoculation with *G.mosseae* produced a significant increase in infestation rate and for the end-of-season period. The inoculation treatment achieved the highest value (45.2%) than the control (27.7%). The reason is the efficiency of the fungus in causing infection and forming a group of fungal strings that extend to remote areas to absorb nutrients beneficial to the plant. The effect of irrigation periods was significant on infection rate and for the end of season period.

The irrigation period every seven days produced the highest value of (40.5)% compared to the irrigation period every four days (38.6%), and the irrigation period every ten days produced the lowest value (30.3%).

The bilateral overlap between the vital inoculation and the irrigation periods was significant in the infection rate and the end-of-season period. The treatment F₁I₇ produced the highest value of interference (51.2)% compared to the comparison treatment F₀I₁₀, which resulted in the most negligible value for interference, which was (21.7)%. The triple overlap between the study factors was also significant in the injury rate and the end-of-season period. The treatment B₁F₁I₇ achieved the highest value for interference (53.0%) than the comparison treatment for B₀F₀I₁₀, which gave the lowest value for interference (17.7%).

Table (5): The effect of *A. chroococcum* and *G.mosseae* application and irrigation periods on infection rate for the end of the season (%).

Bacteria	Fungi	Irrigation periods /day			Mean of Binary overlap F x B
		I ₄	I ₇	I ₁₀	
B ₀	F ₀	30.0	23.3	17.7	23.7
	F ₁	44.3	49.3	36.7	43.4
B ₁	F ₀	33.0	36.3	25.7	31.7
	F ₁	47.0	53.0	41.0	47.0
LSD .05		2.9			1.7
Bi-interaction I x B					
Bacteria		Irrigation periods /day			Mean
		I ₄	I ₇	I ₁₀	
B ₀		37.2	36.3	27.2	33.6
B ₁		40.0	44.7	33.3	39.3
LSD .05		2.0			1.2
Bi-interaction O x F					
Fungi		Irrigation periods /day			Mean
		I ₄	I ₇	I ₁₀	
F ₀		31.5	29.8	21.7	27.7
F ₁		45.7	51.2	38.8	45.2
LSD .05		2.0			1.2
I mean		38.6	40.5	30.3	
LSD .05		1.4			

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