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Effect of Herbicides and *Azotobacter chroococcum* Bacteria on Soil Bacterial Growth, Growth, and Yield of Maize (*Zea mays* L.)

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Abstract

To obtain high yield, herbicides are applied to food crops, including maize. It is important to adopt sustainable agricultural practices to enhance soil fertility by bacterial inoculation. Two experiments were conducted at the Faculty of Agriculture, University of Kufa, Iraq, to investigate the effects of glyphosate and 2,4-D herbicides on soil bacterial activity, growth, and maize (*Zea mays* L.) yield. A laboratory experiment, using a completely randomized design, evaluated the response of *Azotobacter chroococcum* and *Bacillus subtilis* to varying herbicide concentrations. Bacterial populations were quantified after 24 hr of incubation to determine the minimum inhibitory concentration within the nutrient medium. Subsequently, a field experiment was conducted in a sandy loam soil during the 2024–2025 autumn season using a randomized complete block design; the factors included bacterial inoculation and herbicide application at three rates: half, full (recommended), and double the recommended dose. Results indicated that high concentrations of glyphosate and 2,4-D significantly reduced total bacterial counts to 3.50 × 10^6 CFU/g dry soil. Conversely, inoculation with *A. chroococcum* increased the population to 18.83 × 10^6 CFU/g, with the interaction between *A. chroococcum* and the recommended glyphosate dose achieving the highest density of 37.00 × 10^6 CFU/g. Regarding plant physiology, inoculation increased total chlorophyll content from 1.33 to 1.68 mg/g and leaf area from 415.34 to 879.71 cm^2 per plant. Leaf nutrient concentrations also improved significantly; nitrogen rose from 1.18% to 2.08%, phosphorus from 0.17% to 0.23%, and potassium from 1.26% to 1.50%. These improvements were reflected in vegetative growth and productivity; the dry weight of the vegetative system increased from 199.25 g in uninoculated controls to 278.36 g in the full-recommended glyphosate treatment with inoculation. Total grain yield ranged from 12.31 to 16.29 t/ha, with *A. chroococcum* inoculation alone reaching a mean yield of 15.57 t/ha.

Keywords: biological treatment, bioremediation, chlorpyrifos degradation, herbicide detoxification, maize, soil microbiology

Introduction

Maize (*Zea mays* L.) is a staple crop in global agriculture, ranking third in production and food consumption for humans and animals, after wheat and rice. Maize is a significant source of numerous byproducts, including glucose, starch, and oils. It is also used in animal feed and various food industries due to its high content of carbohydrates, protein, oil, minerals, and vitamins, giving it significant nutritional and economic value (Alfalahi et al., 2015). In Iraq, maize is grown on vast areas, estimated at approximately 359,549 dunums, with an average yield of 1,497 kg per dunum, reflecting its importance for local food security (Central Statistical Organization of Iraq, 2023).

Maize is a soil-demanding crop that consumes large quantities of nutrients during its various growth stages, making it highly

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responsive to mineral fertilizers. Excessive use of chemical fertilizers has adverse effects on the environment and human health (Dikici & Dündar, 2006). The low organic matter content and high soil pH in many Iraqi soils reduce the availability of nutrients to plants. This calls for adopting sustainable agricultural practices to enhance soil fertility and the efficiency of plant nutrition, such as the use of organic fertilizers, balanced fertilization, and crop rotation.

The spread of weeds and pests is one of the most significant challenges facing corn production, directly impacting yields by competing with the crop for essential resources such as water, nutrients, light, and space. Losses resulting from this competition can exceed 50% in some cases (Ayana et al., 2023). The problem is exacerbated by climate change and the spread of resistance to traditional herbicides, making reliance on chemical control the most popular option among farmers. Herbicides have proven effective in reducing weed competition and controlling weed spread. However, the intensive and repeated use of the same herbicide has led to the accumulation of residues in the soil and other ecosystems, causing environmental and agricultural problems such as soil and water pollution, decreased beneficial microbial activity, and negative impacts on future crops (Al-Jabouri et al., 1985; Ofosu et al., 2023). The persistence of herbicides in soil varies with herbicide type, concentration, soil characteristics, and biological and climatic conditions, and may last from weeks to several years (Curran, 1998; Ghazi et al., 2023). While a long duration of herbicide helps control weeds during the growing season, the continued presence of herbicide after the end of the growing season may negatively affect subsequent crops, especially sensitive ones, due to several factors, including chemical and photodegradation, absorption from the soil or plants, volatilization, and leaching by irrigation water or rain. Accordingly, there is an urgent need to adopt integrated and sustainable weed management strategies, with a focus on improving soil fertility and the efficiency of plant nutrition. This will ensure high maize yields and improved crop quality, thereby contributing to both local and global food security amid accelerating environmental and climate change. Despite the known importance of soil health, a significant research gap exists regarding the synergistic effects of herbicide application and bacterial inoculation on soil microbial dynamics and crop productivity. Consequently, this study aims to evaluate the impact of bacterial soil treatments and herbicide applications on maize growth and grain yield.

Materials and Methods

Two experiments were conducted at the College of Agriculture, University of Kufa, to investigate the impact of glyphosate and 2,4-D herbicides on soil bacterial activity, growth, and maize (*Zea mays* L.) yield. The laboratory experiment was conducted using a completely randomized design to assess the response of *A. chroococcum* and *B. subtilis* bacteria to varying concentrations of herbicides. Their numbers were measured after 24 hr to determine the minimum inhibitory concentration (MIC) of the herbicides in the nutrient medium. The field experiment was conducted according to a completely randomized block design at the Agricultural Research Station, University of Kufa, during the autumn season of 2024-2025 in a sandy mixed soil. The experiment had two factors: bacterial inoculation and herbicide type and concentration (half-recommendation, full recommendation, and double recommendation).

The *A. chroococcum* bacterial inoculum was prepared under sterile conditions using nutrient broth medium, and the samples were incubated at 37 °C for 24 hr. Yellow maize seeds, 'Australian' variety, were sown on July 14, 2024, after being soaked in the bacterial inoculum *A. chroococcum*, in 3 m × 4 m experimental plots. The soil was prepared by plowing and sampling at 0–30 cm depth, then sieved through a 2 mm sieve to determine its physical, chemical, and biological properties. Plant samples were collected after 60 days to measure the N, P, K, and chlorophyll contents. Harvesting was carried out on December 15, 2024, after the plants reached physiological maturity (Figure 1B). Herbicide

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Figure 1

Maize Crops at the Vegetative (A) and Full Maturity Stage (B) at University of Kufa, Iraq

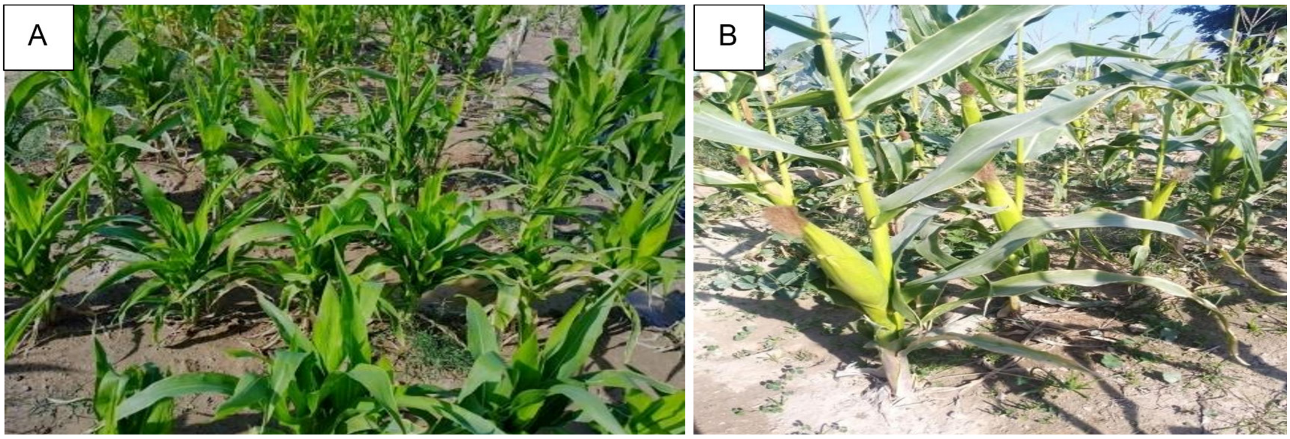

Table 1

Soil Physical and Chemical Properties Before Planting

Soil properties	Values
pH 1:1	7.57
EC 1:1	4.45 ds/m
Ca^{+2}	320 meq/100g
Mg^{+2}	33 meq/100g
Cl^{-1}	11.93 meq/100g
SO_4^{-2}	1914 meq/100g
HCO_3^{-1}	5.85 meq/100g
Organic matter	12.3%
N	12.3 mg/kg
P	20.3 mg/kg
K	314.9 mg/kg
Texture	Sandy loam

residues in the soil were determined using HPLC after extraction with specialized methods. Total bacterial counts were also calculated using the dilution method (Bastakoti & Shrestha, 2025). The data were subjected to statistical analysis using ANOVA and the least significant difference (LSD) test at a 5% probability level (Table 1).

Experimental Factors

Synthetic Herbicides

Two herbicides were used in this study: glyphosate and 2,4-D (2,4-Dichlorophenoxyacetic acid) at four concentrations: 0, half the recommended concentration, full concentration, and double the recommended concentration.

Commonly recommended application rates are:

a. Glyphosate (N-phosphonomethylglycine): usually applied at 1000-2160 g/ha (equivalent to approximately 3-5 L/ha of a 41% formulation).
b. 2,4-D (2,4-dichlorophenoxyacetic acid): usually applied at 500-1,000 g/ha (equivalent to approximately 1-2 L/ha of an ester or amine formulation).

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Bacterial Biofertilizer

The isolates used were *A. chroococcum* and *B. subtilis* bacteria (CFU/g dry soil), originally isolated from maize field soil in Najaf Governorate and deposited at the Microbial Collection of the Faculty of Agriculture at the University of Kufa, *A. chroococcum* is known for its ability to fix atmospheric nitrogen and produce plant growth regulators, while *Bacillus subtilis* is known for its ability to produce antibiotics, stimulate root growth, and protect plants from pathogens. These isolates were selected for their ability to enhance soil microbial activity and reduce the negative effects of herbicides. Previous domestic and international studies have confirmed their effectiveness in improving plant growth and productivity (Table 2).

Studied Traits

The number of *A. chroococcum* and *B. Subtilis* bacteria (CFU/g dry soil) was determined by dilution and plate counting, as described by Black (1965). Field soil samples were taken at the end of the experiment, and a series of dilutions of the soil suspension was prepared using nutrient agar from 10 to 10. The dilution was poured into sterile petri dishes, and 1 ml of the 10 dilution was added. The dishes were then placed in an incubator at 28 °C for 48 hr. The number of live bacterial cells was calculated by multiplying the number of colonies by the dilution factor. Total bacterial counts (CFU/g dry soil) were estimated by dilution and plate counting according to Black (1965).

Chlorophyll measurement (mg/g plant): one gram of leaf was taken at the sprouting stage and cut into several small pieces using clean, sterile scissors. The pieces were crushed in a ceramic mortar with 5–10 ml of 85% acetone, depending on the sample weight. The filtrate was then separated from the precipitate by centrifugation for 10 min. The extraction process was repeated until the precipitation was free of green pigment. The extract was collected in 10-25 ml tubes covered with opaque paper to block light from the chlorophyll and prevent photooxidation of the pigment. The volume was completed by adding acetone. The optical density of the filtrate was measured using a

Table 2

Weeds in the Cultivated Field

Field observation notes	% Cover	Dry biomass (g/m^2)	Fresh biomass (g/m^2)	Weed density (plants/m²)	Weed species (local name + scientific name)
Clustered growth in scattered spots; dense around the irrigation system	15	40	180	8	Sweet clover (*Melilotus indicus*)
Established plants; density increases in the lower parts of the field	40	95	450	22	Mesquite (*Prosopis farcta*)
Severe infestation across most of the field; plants are young (seedlings)	20	55	250	12	Lambsquarters (*Chenopodium album*)
Strong root growth (tubers); heavily spreading within the crop rows	10	25	100	5	Nut sedge (*Cyperus rotundus*)
Intermingled with the main crop (wheat/barley); difficult to separate	115	290	1330	62	Wild oats
Total density and biomass for all weed species found in the square meter	115	290	1330	62	(*Avena fatua*)
					Total weeds

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spectrophotometer at wavelengths of 645 and 663 nm using the following equations: according to the concentration of chlorophyll a, b, and total in plant leaves based on mg/g of fresh plant tissue.

Leaf area per plant (cm^2 per plant) was calculated according to the following equation: Leaf area per plant = leaf length^2 × 0.75 (Elsahookie et al., 2021). Nitrogen percentage was determined using the Kjeldahl method using a micro-Kjeldahl apparatus and according to the process described in (Black, 1965). Phosphorus percentage was estimated using ammonium molybdate according to the method of Jones et al. (1978). Potassium percentage was determined in the digested samples by flame photometer according to the method described in Association of Official Analytical Chemists (1970). Dry vegetative weight (g per plant): ten plants were harvested and dried. The plants were then weighed using a sensitive balance, and the average was calculated. Grain yield (t/ha) was calculated in grams using a sensitive balance, and the total grain yield was calculated in tons at a standard moisture content of 15.5% according to the following equation (Elsahookie et al., 2021).

Statistical Analysis

The laboratory experiment was set up using a factorial design, whereas the field experiment used a randomized complete block design with three replicates. The results from the two experiments were statistically analyzed using analysis of variance (ANOVA), and the differences between the arithmetic means were tested at the 5% level using the least significant difference (LSD).

Results and Discussion

A. chroococcum and *B. subtilis* Bacteria in the Culture Medium After 24 Hr

Effect of synthetic herbicides and different concentrations on the numbers of *A. chroococcum* and *B. subtilis* bacteria in the culture medium after 24 hr of treatment. The results in Table 3 showed that adding synthetic herbicides to the culture medium significantly affected bacterial counts for both *A. chroococcum* and *B. subtilis* after 24 hr of treatment. The control treatment (without addition) recorded the highest bacterial count rate of 329.33 × 10^6 CFU/ml, while the treatment with glyphosate herbicide at a concentration twice the recommended level recorded the lowest count rate of 89.83 × 10^6 CFU/ml. The results also showed significant differences between the two bacterial types, with *A. chroococcum* showing a substantial superiority in numbers compared to *B. subtilis*, with the highest count rate for *A. chroococcum* reaching 337.96 × 10^6 CFU/ml. In contrast, the lowest count rate was observed for *B. subtilis,* reaching 96.21 × 10^6 CFU/ml. Regarding the two-way interaction between the herbicide type and its concentration with the bacterial type, the results showed a significant effect, as observed in both the control treatment and the addition of *A. chroococcum* bacterial inoculum recorded the highest average bacterial count of 520.00 × 10^6 CFU/ml, while the glyphosate treatment with a concentration twice the recommended and the addition of *B. subtilis* bacterial inoculum recorded the lowest average of 50.67 × 10^6 CFU/ml.

Soil Bacterial Count (10^6 CFU/g Dry Soil)

The results of Table 4 show that the control treatment significantly outperformed the total number of bacteria in the rhizosphere, recording the highest rate of 21.00 × 10^6 CFU/g dry soil, while the 2,4-D therapy at the recommended concentration led to an apparent decrease in the number of bacteria, recording the lowest value of 3.50 × 10^6 CFU/g dry soil. The results of the same table show the superiority of the inoculation treatment with *A. chroococcum* in enhancing the number of bacteria in the soil, as it reached 18.83 × 10^6 CFU/g compared to the non-inoculation treatment, which recorded 2.50 × 10^6 CFU/g, indicating the positive role of nitrogen-fixing bacteria in supporting biological activity in the root zone. The results of the same table showed that the two-way interaction between herbicides

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and bacterial inoculation had a significant effect on bacterial counts, as the interaction between the control and bacterial inoculation significantly outperformed, with the highest value of 37.00 × 10^6 CFU/g. In contrast, the bacterial counts decreased to the lowest level in the interaction between glyphosate at a concentration twice the recommended concentration and 2,4-D at a concentration twice the recommended concentration, without bacterial inoculation, reaching 0.00 × 10^6 CFU/g.

Maize Leaf Total Chlorophyll

Table 5 shows the effects of different glyphosate and 2,4-D herbicide concentrations and *A. chroococcum* bacterial inoculation on total chlorophyll content in maize leaves. The data showed that the addition of herbicides led to a significant increase in the total chlorophyll content compared to the control treatment, where the glyphosate treatment at the full recommended concentration recorded the highest rate of

Table 3

Effects of Different Concentrations of Glyphosate and 2,4-D on the Numbers of A. chroococcum and B. subtilis Bacteria in the Culture Medium After 24 Hr

Herbicide rates	Medium bacterial counts (106 CFU/ml)		Average
	Control (no bacterial inoculation)	*A. chroococcum*	
Comparison	138.67	520.00	329.33
Glyphosate at 50% of the recommended rate	121.33	385.00	253.17
Glyphosate at the recommended rate	60.67	420.33	240.50
Glyphosate at double the recommended rate	50.67	129.00	89.83
2,4-D at 50% of the recommended rate	98.67	380.33	239.50
2,4-D at the recommended rate	73.00	128.67	100.67
2,4-D at double the recommended rate	88.00	220.67	154.33
Bacterial inoculation rate	96.21	337.96	
LSD 0.05	Herbicides = 0.54	Bacteria = 0.27	Integration = 0.77

Table 4

Effect of Different Concentrations of Glyphosate and 2,4-D Herbicides and Inoculation with A. chroococcum Bacteria on Soil Bacterial Counts

Herbicides	Soil bacterial counts (106 CFU/g dry soil)		Average
	Control (no bacterial inoculation)	*A. chroococcum*	
Comparison	5.00	37.00	21.00
Glyphosate at 50% of the recommended rate	4.00	16.00	10.00
Glyphosate at the recommended rate.	2.00	21.67	11.83
Glyphosate at double the recommended rate.	0.00	8.00	4.00
2,4-D at 50% of the recommended rate	3.00	15.00	9.00
2,4-D at the recommended rate	1.00	6.00	3.50
2,4-D at double the recommended rate.	0.00	1.00	5.00
Bacterial inoculation rate	2.50	18.83	
LSD 0.05	Herbicides = 0.17	Bacteria = 0.09	Integration = 0.24

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1.68 mg/g plant, while the 2,4-D treatment at the full recommended concentration recorded the lowest rate of 1.35 mg/g plant. The same results also showed that the *A. chroococcum* bacterial inoculation significantly outperformed the total chlorophyll content compared to the no-herbicide treatments, with the concentration in the inoculation treatment reaching 1.62 to 1.43 mg/g plant, compared to 1.43 mg/g plant without inoculation. The results indicated that the two-way interaction between synthetic herbicides and bacterial inoculation significantly affected total chlorophyll content, as evidenced by the glyphosate treatment at the full recommended concentration with *A. chroococcum* added. Outperformed and recorded the highest average of 1.76 mg/g plant, while the 2,4-D treatment at the full recommended concentration without the addition of *A. chroococcum* gave the lowest average of 1.32 mg/g plant.

Maize Leaf Area

The results of Table 6 show the effect of different concentrations of glyphosate and 2,4-D herbicides, as well as *A. chroococcum* bacterial inoculation, on the average leaf area of maize plants. It was found that the addition of synthetic herbicides significantly affected the leaf area of the plant, as the glyphosate treatment at the full recommended concentration outperformed and recorded the highest average leaf area of 713.08 m^2 per plant, while the 2,4-D treatment, at the full recommended concentration, recorded the lowest average of 304.15 m^2 per plant. The results also showed that the addition of *A. chroococcum* biofertilizer led to a significant increase in leaf area, as the bacterial inoculation treatment achieved the highest average of 587.17 m^2 per plant, compared to the non-inoculation treatment, which recorded only 412.36 m^2 per plant. The two-way interaction between herbicide type and bacterial inoculation had a significant effect, as evidenced by the glyphosate treatment at the full recommendation concentration with inoculation, which recorded the highest average leaf area of 879.71 m^2 per plant. In comparison, the 2,4-D treatment at the full recommendation concentration without inoculation recorded the lowest average of 245.45 m^2 per plant.

Maize Leaf Nitrogen Concentration (%)

The results in Table 7 show the effects of different glyphosate and 2,4-D herbicide concentrations, as well as inoculation with *A. chroococcum* bacteria, on the total nitrogen concentration (%) in the leaves of yellow corn plants. The results showed significant differences between the treatments, with

Table 5

Effect of Different Concentrations of Glyphosate and 2,4-D Herbicides and Inoculation with A. chroococcum on Maize Leaf Total Chlorophyll Content

Herbicides	Leaf chlorophyll content (mg/g)		Average
	Control (no bacterial inoculation)	*A. chroococcum*	
Comparison	1.39	1.68	1.54
Glyphosate at 50% of the recommended rate	1.51	1.73	1.62
Glyphosate at the recommended rate.	1.60	1.76	1.68
Glyphosate at double the recommended rate	1.42	1.55	1.48
2,4-D at 50% of the recommended rate	1.48	1.71	1.60
2,4-D at the recommended rate	1.32	1.35	1.33
2,4-D at double the recommended rate.	1.37	1.50	1.43
Bacterial inoculation rate	1.43	1.62	
LSD 0.05	Herbicides = 0.014	Bacteria = 0.007	Integration = 0.019

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the treatment using the full recommended concentration of glyphosate herbicide being significantly superior and recording the highest rate of nitrogen concentration in the leaves, at 1.72%. In contrast, the treatment with the full recommended concentration of 2,4-D herbicide recorded the lowest rate, at 1.01%. The results showed that bio-inoculation with *A. chroococcum* bacteria led to a significant increase in leaf nitrogen content compared to the treatment without bio-inoculation, with the highest rate reaching 1.55%, while the lowest rate was 1.18% in the treatment without bio-inoculation. The results in the same table also indicated a significant interaction between the two-way effect of synthetic herbicides and inoculation with *A. chroococcum* bacteria on nitrogen concentration in yellow corn leaves, where the treatment of glyphosate herbicide at the full recommendation concentration with bacterial inoculation recorded the highest average of 2.08%, while the treatment of 2,4-D herbicide at the full recommendation concentration and without adding bacterial inoculation recorded the lowest average of 0.99%.

Maize Leaf Phosphorus Concentration (%)

The results shown in Table 8 showed that the addition of herbicides and inoculation with *A. chroococcum* bacterium significantly affected the total phosphorus concentration (%) in maize leaves. It was found that the herbicides had a significant effect, as the 2,4-D herbicide treatment at half the recommended concentration recorded the highest average phosphorus concentration of 0.27%, while the glyphosate herbicide treatments at full and double the recommended concentrations recorded the lowest average of 0.15%. The results also showed that bio-inoculation with *A. chroococcum* bacteria led to a significant increase in leaf phosphorus concentration compared to the non-addition treatment, with the highest rate at the highest inoculation level reaching 0.23%. In comparison, the lowest average reached 0.17% in the non-inoculation treatment. The results also indicated a significant two-way interaction between the herbicide type and bacterial inoculation, as evidenced by the interaction treatment between the herbicide 2,4-D at a concentration half the recommended level and *A. chroococcum*, which outperformed, recording the highest average phosphorus concentration of 0.28%. In contrast, treating glyphosate at a concentration twice the recommended level without inoculation resulted in the lowest average of 0.13%.

Table 6

Effect of Different Concentrations of Glyphosate and 2,4-D Herbicides and Inoculation with A. chroococcum on the Maize Leaf Area

Herbicides	Leaf area (cm^{-2})		Average
	Control (no bacterial inoculation)	*A. chroococcum*	
Comparison	466.18	577.01	521.59
Glyphosate at 50% of the recommended rate	498.01	786.06	642.04
Glyphosate at the recommended rate.	546.44	879.71	713.08
Glyphosate at double the recommended rate.	384.26	462.17	423.21
2,4-D at 50% of the recommended rate	440.63	589.84	515.23
2,4-D at the recommended rate	245.45	362.85	304.15
2,4-D at double the recommended rate.	251.76	462.60	357.18
Bacterial inoculation rate	412.36	587.17	
LSD 0.05	Herbicides = 6.45	Bacteria = 3.23	Integration = 9.12

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Potassium Concentration in Maize Leaves (%)

The results shown in Table 9 indicate that different concentrations of glyphosate and 2,4-D herbicides, in conjunction with inoculation with *A. chroococcum* bacterium, significantly affected the potassium concentration (%) in maize leaves. The results showed that adding herbicides significantly increased potassium concentration in the leaves. The treatment with glyphosate herbicide at the full recommended concentration was significantly superior, recording the highest average of 1.85%. In comparison, the treatment with 2,4-D herbicide at the full recommendation concentration recorded the lowest average of 0.15%. The results also showed that bacterial inoculation with *A. chroococcum* led to a significant increase in potassium concentration compared to the non-inoculation treatment, with the highest average reaching 1.50% in the inoculation treatment, compared to 1.26% in the no herbicide treatment. Regarding the two-way interaction between the herbicide type and bacterial inoculation, the results showed a clear and significant effect, as evidenced by the interaction treatment between the glyphosate herbicide at the full recommended concentration and *A. chroococcum* was significantly superior, recording the highest average potassium concentration of 2.13%, while the 2,4-D herbicide treatment at the full recommendation concentration without inoculation recorded the lowest average of 0.98%.

Maize Shoot Dry Weight (g)

The effect of glyphosate, 2,4-D, and inoculation with *A. chroococcum* on the dry weight of maize shoots (g). The results in Table 9 showed that the addition of synthetic herbicides significantly affected the dry weight of maize shoots. Treatment with glyphosate at the full recommended concentration yielded the highest average dry weight of 278.36 g, significantly higher than the other treatments. In contrast, the lowest average dry weight was recorded when using 2,4-D at the same concentration, reaching 199.25 g, indicating a difference in effect between the two types of herbicides, as well as the effect of bio-inoculation with *A. chroococcum*, it was found that the use of bacterial inoculum contributed to enhancing the dry weight of the plant, as the highest average was reached in the inoculated treatment, at 266.40 g, compared to 221.66 g in the non-inoculated treatment, which reflects the effective role of bacteria in improving vegetative growth (Table 10).

Table 7

Effect of Different Concentrations of Glyphosate and 2,4-D Herbicides and Inoculation with A. chroococcum on Maize Leaf Nitrogen Concentration

Herbicides	Leaf N (%)		Average
	Control (no bacterial inoculation)	*A. chroococcum*	
Comparison	1.28	1.57	1.42
Glyphosate at 50% of the recommended rate	1.16	1.96	1.56
Glyphosate at the recommended rate.	1.36	2.08	1.72
Glyphosate at double the recommended rate.	1.04	1.12	1.08
2,4-D at 50% of the recommended rate	1.26	1.77	1.52
2,4-D at the recommended rate	0.99	1.04	1.01
2,4-D at double the recommended rate.	1.05	1.30	1.17
Bacterial inoculation rate	1.18	1.55	
LSD 0.05	Herbicides = 0.003	Bacteria = 0.002	Integration = 0.004

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Total Yield

Table 11 showed the effects of different concentrations of glyphosate and 2,4-D herbicides, and of inoculation with *A. chroococcum* bacteria, on the total yield of maize. The addition of herbicides showed a significant effect on total yield, with the glyphosate treatment at full recommendation concentration achieving the highest rate of 16.29 t/ha. In comparison, the 2,4-D treatment at the full recommendation concentration recorded the lowest rate of 13.35 t/ha. The results also showed that inoculation with *A. chroococcum* led to a significant increase in total yield, with the inoculated treatment recording the highest rate of 15.57 t/ha compared to the uninoculated treatment, which recorded 13.30 t/ha. As for the two-way interaction between herbicides and bacterial inoculation, an apparent significant effect was observed, as the glyphosate treatment at the full recommendation concentration with the addition of bacterial inoculation achieved the highest average total yield of 17.76 t/ha, while the 2,4-D treatment recorded 2,4-D at the full recommendation concentration without inoculation had a minimum

Table 8

Effect of Different Concentrations of Glyphosate and 2,4-D Herbicides and Inoculation with A. chroococcum Bacteria on Maize Leaf Phosphorus Concentration

Herbicides	Leaf P (%)		Average
	Control (no bacterial inoculation)	*A. chroococcum*	
Comparison	0.18	0.30	0.24
Glyphosate at 50% of the recommended rate	0.15	0.17	0.16
Glyphosate at the recommended rate.	0.14	0.16	0.15
Glyphosate at double the recommended rate.	0.13	0.16	0.15
2,4-D at 50% of the recommended rate	0.25	0.28	0.27
2,4-D at the recommended rate	0.19	0.25	0.22
2,4-D at double the recommended rate.	0.18	0.23	0.21
Bacterial inoculation rate	0.17	0.23	
LSD 0.05	Herbicides = 0.003	Bacteria = 0.002	Integration = 0.004

Table 9

Effect of Different Concentrations of Glyphosate and 2,4-D Herbicides and Inoculation with A. chroococcum Bacteria on Potassium Concentration in Maize Leaves

Herbicides	Leaf K (%)		Average
	Without adding	*A. chroococcum*	
Comparison	1.37	1.52	1.44
Glyphosate at 50% of the recommended rate	1.43	1.66	1.54
Glyphosate at the recommended rate.	1.56	2.13	1.85
Glyphosate at double the recommended rate.	1.22	1.12	1.17
2,4-D at 50% of the recommended rate	1.16	1.78	1.47
2,4-D at the recommended rate	0.98	1.02	1.00
2,4-D at double the recommended rate.	1.01	1.27	1.14
Bacterial inoculation rate	1.26	1.50	
LSD 0.05	Herbicides = 0.17	Bacteria = 0.01	Integration = 0.024

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average concentration of 12.31 t/ha (Table 11).

Regarding the two-way interaction between the herbicide type and bacterial inoculum, the results showed apparent significant differences between the treatments (Table 8). The glyphosate treatment at the full-recommendation concentration and the bacterial inoculum achieved the highest average dry weight of 299.00 g. In comparison, the 2,4-D treatment at the full recommendation concentration without inoculum recorded the lowest average of 152.18 g, highlighting the positive effect of the interaction between chemical and biological treatments on vegetative growth characteristics (Figure 1A).

The results of Table 4 indicate that glyphosate and 2,4-D herbicides significantly inhibited bacterial growth at high concentrations. Glyphosate inhibits EPSPS, preventing the synthesis of aromatic amino acids required for protein synthesis and cell growth, while 2,4-D generates oxidative stress that damages proteins, DNA, and cell membranes. *Azotobacter*

Table 10

Effect of Different Concentrations of Glyphosate and 2,4-D Herbicides and Inoculation with A. chroococcum Bacteria on the Maize Shoot Dry Weight

Herbicides	Shoot dry weight (g)		Average
	Control (no bacterial inoculation)	*A. chroococcum*	
Comparison	266.44	259.67	243.05
Glyphosate at 50% of the recommended rate	251.50	281.64	266.57
Glyphosate at the recommended rate.	257.71	299.00	278.36
Glyphosate at double the recommended rate.	202.62	268.37	235.49
2,4-D at 50% of the recommended rate	246.13	263.00	254.58
2,4-D at the recommended rate	152.18	246.31	199.25
2,4-D at double the recommended rate.	210.34	254.58	232.46
Bacterial inoculation rate	221.66	266.40	
LSD 0.05	Herbicides = 4.17	Bacteria = 2.09	Integration = 5.90

Table 11

Effect of Different Concentrations of Glyphosate and 2,4-D Herbicides and Inoculation with A. chroococcum Bacteria on the Maize Total Yields

Herbicides	Maize yields (tons/ha)		Average
	Control (no bacterial inoculation)	*A. chroococcum*	
Comparison	12.59	15.15	13.87
Glyphosate at 50% of the recommended rate	14.53	16.10	15.32
Glyphosate at the recommended rate.	14.91	17.67	16.29
Glyphosate at double the recommended rate.	12.45	14.90	13.67
2,4-D at 50% of the recommended rate	13.75	16.11	14.92
2,4-D at the recommended rate	12.31	14.39	13.35
2,4-D at double the recommended rate.	13.30	15.11	14.21
Bacterial inoculation rate	13.30	15.75	
LSD 0.05	Herbicides = 0.019	Bacteria = 0.01	Integration = 0.03

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chroococcum is particularly sensitive to these effects because it relies on nitrogenase activity, which is easily disrupted under chemical stress. However, at lower herbicide concentrations, *A. chroococcum* demonstrated higher tolerance, likely due to the production of exopolysaccharides (EPS) and antioxidant enzymes that mitigate the severity of stress, whereas *Bacillus subtilis* appeared more susceptible to the toxic effects (Santos & Linardi, 2004; Ubogu & Akponah, 2022). Furthermore, herbicide application contributed to a reduction in weed density in maize fields, decreasing competition for nutrients, water, and light, which positively impacted the abundance of beneficial rhizosphere bacteria and increased macronutrient (NPK) concentrations in both soil and plant tissues, enhancing nutrient uptake efficiency (Bastakoti & Shrestha, 2025; Kommireddy & Poojitha, 2017).

Inoculation with *A. chroococcum* further strengthened these effects by fixing atmospheric nitrogen and converting it into bioavailable ammonium, as well as producing organic acids and enzymes that solubilize phosphate and potassium compounds, thereby increasing NPK content in maize leaves (Muhamed & Nuni, 2024; Sharma et al., 2013; Singh & Sharma, 2022). The improved nutrient availability was reflected in physiological traits such as higher chlorophyll content, expanded leaf area, and enhanced photosynthetic efficiency, resulting in increased carbohydrate production, plant dry weight, and total yield (Chennappa et al., 2016; Song et al., 2022; Wei et al., 2024). The integration of herbicide application and bacterial inoculation proved even more effective, as herbicides minimized nutrient depletion by weeds, while inoculation enhanced nitrogen fixation and phosphorus and potassium uptake, collectively increasing leaf macronutrient content, yield, and its components (Al-Budairy & Al-Taweel, 2025; Rejali & Baghery, 2021).

Although high herbicide concentrations can inhibit beneficial microorganisms by reducing metabolic and enzymatic activities, the use of recommended doses, combined with inoculation using *A. chroococcum*, mitigates these adverse effects by promoting microbial activity and maintaining soil fertility (Al-budairy & Al-Taweel, 2025; Ganie & Jhala, 2021; Tomkiel & Kwiatkowska, 2023; Wei et al., 2024). Studies also indicate that moderate glyphosate application does not significantly inhibit microbial activity and may even improve nutrient absorption efficiency, whereas 2,4-D may negatively affect some beneficial enzymes at high or repeated doses (Bastakoti & Shrestha, 2025; Santos & Linardi, 2004; Ubogu & Akponah, 2022). Overall, these findings demonstrate that combining moderate herbicide use with *A. chroococcum* inoculation provides an effective strategy to control weeds, maintain soil microbial health, enhance nutrient availability, and significantly improve maize growth and productivity (Bastakoti & Shrestha, 2025; Chennappa et al., 2016; Muhamed & Nuni, 2024; Song et al., 2022).

Conclusions

The study demonstrated that the application of biofertilizer increased maize N, P, and K concentrations, indicating improved nutrient uptake efficiency. High concentrations of herbicides were observed to inhibit the activity of *Azotobacter chroococcum*, reducing its nitrogen-fixation ability and limiting its contribution to plant growth. These results highlight the importance of applying herbicides at scientifically recommended doses to preserve beneficial microbial activity. Integrating biofertilizer with measured herbicide application offers a dual benefit: supporting plant nutrition and controlling weeds, thereby enhancing crop growth and yield in a sustainable manner.

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