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Effect of Glyphosate and 2,4-D Herbicides and Bacterial Inoculation on Soil Properties, Growth, and Yield of Maize (*Zea mays* L.)

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Abstract

Weed-control using herbicides can inhibit weed growth but can also affect soil properties and soil microbial activity, ultimately impacting plant growth and productivity. A field experiment was carried out in Najaf Governorate within the Agricultural Research Station at the University of Kufa during the autumn season of 2024-2025 in a sandy-loam soil to study the effect of glyphosate and 2,4-D herbicides and bacterial inoculation on some soil characteristics, growth, and yield of yellow corn (*Zea mays* L.). The experiment was conducted using a randomized complete block design with two factors: herbicides at three concentrations (half the recommended, full recommendation, and double the recommended) and bacterial inoculation. The results showed that the interaction treatment between bacterial inoculation (*A. chroococcum*) and the use of glyphosate at the full recommended concentration, as well as the control treatment, achieved the highest values in the studied characteristics. The highest concentration of available nitrogen in the soil reached 35.67 mg N/kg soil, and available phosphorus reached 26.67 mg P/kg soil. In contrast, the degree of soil reaction remained within the appropriate limits for plant growth. The electrical conductivity decreased to 1.22 dS/m. Soil bacterial counts also increased to 37.00 × 10^6 CFU/g dry soil, which was reflected in an improvement in total yield to 15.57 mg/ha.

Keywords: 2,4-D, *A. chroococcum,* glyphosate, microbial activity, pesticide residues, *Zea mays* L.

Introduction

Maize (*Zea mays* L.) is a global staple food crop, highly productive and highly nutritious. With the increasing need to intensify agricultural production, weed control in fields, along with other complementary agronomic practices, has become critical to maintaining crop quality and increasing yields. Standard weed-control practices use herbicides such as glyphosate and 2,4-D to inhibit weed growth. However, these chemicals can affect soil properties and microbial activity, ultimately impacting plant growth and productivity. Bacteria that fix nitrogen and provide nutrients to plants are among the classes of plant growth-promoting bacteria. Bacterial inoculation of plants is known to promote plant growth. Therefore (Alfalahi et al., 2015). Its importance lies in its multiple uses, both directly in human food and indirectly in animal feed and various food industries, due to its high content of carbohydrates, protein, oil, minerals, and vitamins, which gives it significant nutritional and economic value. In Iraq, maize is cultivated on approximately 359,549 dunums (35,954.9 ha), with an average yield of 14.97 t/ha (Central Statistical Organization of Iraq, 2023). Despite its importance, maize is a soil-stressing crop due to its high nutrient depletion, which makes it highly responsive to mineral fertilization. However, excessive use of fertilizers has adverse effects on the environment and human health (Dikici & Dündar, 2006). The low organic matter content and high alkalinity in Iraqi soils reduce nutrient availability.

Weed interference is one of the most

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significant challenges facing maize cultivation, resulting in yield losses of up to 50% or more due to competition for water, nutrients, light, and space (Ayana et al., 2023). This problem is exacerbated by climate change, the spread of weed resistance to herbicides, and the difficulty of finding effective alternatives, making chemical control the most popular choice among farmers. Herbicides have proven effective in reducing damage caused by weed competition (Al-Jubouri et al., 1985; Al-Tharwani et al., 2024). However, their intensive and repeated use has led to serious environmental and agricultural problems, including the accumulation of pesticide residues in soil and other ecosystems. It is worth noting that the persistence of herbicides in the soil varies with herbicide type, concentration, soil characteristics, and biological and climatic conditions, and may range from weeks to several years (Al-Budairy & Al- Taweel, 2025; Curran, 1998). The main aim of this study was to evaluate the potential applicability of *B. subtilis* as a sustainable biological pest control agent for long-term use in integrated weed management, by assessing its effects on the growth and productivity of maize (*Zea mays* L.). This study specifically described how *B. subtilis* infection influences the development of competitive plants by secreting biologically active metabolites, volatile organic compounds, and enzymes, among other substances. These pathways secrete substances that inhibit the growth of competitive plants. The current study also sought to determine how *B. subtilis* infection influences nutrient uptake in maize, promotes plant growth through phytohormone production, and induces resistance to biotic and abiotic stressors. This study was designed to investigate the effects of herbicides and bacterial inoculation, and their interactions on soil properties, maize growth, and productivity. This study will synthesize and integrate mechanisms to develop evidence-based strategies that, in practice, reduce reliance on chemical herbicides, minimize environmental pollution, and improve soil fertility, thereby increasing maize yield and quality. Thus, it will be an approach to sustainable agricultural development and food security amid climate change.

Materials and Methods

The theoretical model of the research addresses the relationship between the independent variables: herbicides (glyphosate, 2,4-D) and bacterial inoculation, and the dependent variables: soil properties (organic matter, available nitrogen, microbial activity), corn growth (height, dry weight), and yield. We hypothesize that (1) the use of herbicides glyphosate and 2,4-D leads to significant changes in soil properties compared to the control sample; (2) bacterial inoculation will improve maize growth and yield by improving soil properties and increasing nutrient availability; (3) Herbicides interact with bacterial inoculation in affecting maize growth and yield.

The study was conducted during the autumn season of 2024-2025 at the Research Station of the College of Agriculture, University of Kufa, on maize (*Zea mays* L). The field experiment evaluated the effects of glyphosate and 2,4-D concentrations, as well as bacterial inoculation, on maize growth and yield. The study used a randomized complete block design with three replicates. The bacterial inoculum was prepared under sterile conditions using nutrient broth medium, and the samples were incubated at 37 °C for 24 hr. Maize seeds were sown on July 14, 2024, after being soaked in the bacterial inoculum in 3 × 4 m experimental plots. The soil was prepared by plowing, and samples were taken to a depth of 0–30 cm. These samples were then sieved through a 2 mm sieve to determine their physical and chemical properties. Plant samples were collected after 60 days to measure soil nitrogen (N) and phosphorus (P) levels. Harvesting was carried out on December 15, 2024, after the plants had reached physiological maturity. HPLC was used to estimate pesticide residues in soil after extraction using specialized methods. Total bacterial counts were also calculated using the dilution method. The results were subjected to statistical analysis using ANOVA and the least significant difference (LSD) test at a 5% significance level (Table 1).

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Table 1

Physical and Chemical Properties of the Field Soil Before Planting

Soil properties		Value	Unit
	pH 1:1	7.57	-
	Ec 1:1	4.45	ds/m
Cation	Ca^{+2}	320	Meq/mol
	Mg^{+2}	33	
	Cl^{-1}	11.93	
Anion	SO_4^{-2}	1914	
	HCO_3^{-1}	5.85	
	O.M	12.30	mg/kg soil
	N	20.30	
	P	21.14	
	K	314.90	
	Texture	Sandy loam	

Experimental Factors

Chemical Herbicides

Two types of herbicides were used in this study: glyphosate and 2,4-D (2,4-dichlorophenoxyacetic acid) at four concentrations: glyphosate at 0, 2, and 4 kg/ha, and 2,4-D at 0, 1, and 2 kg/ha. Herbicides should be applied to pre- or post-emergent weeds according to the manufacturer's recommended application method, taking necessary safety and environmental precautions.

Bacterial Biofertilizer

The bacterial biofertilizer used in the experiment was obtained from the Plant Protection Department at the Faculty of Agriculture, University of Kufa. It included two bacterial isolates: *A. chroococcum* and *B. subtilis*. These isolates were prepared under sterile laboratory conditions for later use in preparing the culture medium and conducting biological treatments. *Azotobacter chroococcum* was used in the field experiment due to its high efficiency in nitrogen fixation. Application methods included pre-coating seeds with bacterial inoculants before planting, spraying them onto the soil surrounding the roots (106 CFU dry soil), and reapplication intervals.

Operating Specifications

Cultivation conditions (irrigation, lighting, temperature, and soil type) were controlled to maintain consistency between treatments. Experimental design: a completely randomized design with replications to ensure the reliability of the results. Soil and plant variables were measured at specified time intervals. Available nitrogen was extracted with potassium chloride (KCl), and the nitrogen content was determined using a Kjeldahl apparatus, following the method described by Page et al. (1982). Available soil phosphorus was extracted using sodium bicarbonate ($NaHCO_3$), and the extract was color-developed using ammonium molybdate and ascorbic acid solutions. Phosphorus was measured using a spectrophotometer at 882 nm, following the method described by Page et al. (1982). The soil pH was estimated from a 1:1 extract using a pH meter, according to the technique of Black (1965). The electrical conductivity was measured using the saturated paste extract with an EC meter, as described by Black (1965).

Total bacterial counts (CFU/g dry soil)

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were estimated by dilution and plate counting, as described by Black (1965). Field soil samples were collected at the end of the experiment, and a series of dilutions of the soil suspension was prepared on Nutrient Agar, ranging from 10^1 to 10^7. The dilution was poured into sterile Petri dishes, and 1 ml of the 10 dilution was added. The dishes were then incubated at 28 °C for 48 hr. Viable bacterial cells were calculated by multiplying the colony count by the reciprocal of the dilution.

The yield per plant was calculated in grams, and the total grain yield was calculated in tons at a standard moisture content of 15.5% according to the following equation (Al-Sahouki, 1990). Pesticide residue in soil was measured in ppm. The first pesticide, glyphosate, was extracted from the soil with a sodium borate solution, then thermally derivatized, and the product was stabilized with HCl. After filtration, the sample was injected into a SYKAM HPLC system using a C18 column and a mobile phase of 1% acetic acid in methanol (60:40), with detection at 254 nm (Association of Analytical Communities, 2000).

For 2,4-D, the sample was extracted with distilled water, followed by a liquid-liquid extraction with ethyl acetate. The organic phase was then dried, condensed, and dissolved in methanol. The sample was stored until analysis on an HPLC-DAD using a C18 column and a mobile phase of acetonitrile/water (75:25) with 0.2% formic acid, with detection at 230 and 280 nm-the active compounds of maize leaf extract were analyzed using an Agilent 1260 Infinite Series high-performance liquid chromatography (HPLC), equipped with a UV detector tuned to a wavelength of 210 nm to identify the target compounds accurately. To achieve optimal separation results, we used a C18 (octadecylsilane) column with dimensions of 250 × 4.6 mm and a particle size of 5 µm (Sherma, 2016; Environmental Protection Agency, 1996).

The mobile phase was a 70:30 (v/v) mixture of methanol and distilled water. The device was operated under isothermal conditions at a flow rate of 1.0 ml/min, while maintaining a constant temperature of 25 ± 1 °C. An injection volume of 20 µl was used for each sample, and the separation time ranged from 10 to 20 min, depending on the type of active compound.

The device was calibrated using pure standard solutions prepared at known concentrations (10–100 µg/ml). A standard curve was drawn, linking each active compound's concentration to its absorbance, to estimate the concentrations in the samples. The linear correlation coefficient (R^2) for all standard curves was ≥0.999, confirming the accuracy and validity of the calibration.

The limits of detection (LOD) and quantification (LOQ) were determined using signal-to-noise ratios (S/N) of 3 and 10, respectively. The repeatability and accuracy of the results were confirmed by analyzing three replicates of each sample, and the relative standard deviation (RSD%) was calculated, which did not exceed 2%, demonstrating high reliability.

The active compounds in the samples were identified by comparing the retention time and wavelength absorbance values with those of standard reference solutions. The results were expressed in mg/g dry matter. The instrument's analysis software (ChemStation Software, Agilent Technologies) was used to extract quantitative and qualitative values (Šaponjac et al., 2019).

Statistical Analysis

The laboratory experiment was analyzed using a factorial design. In contrast, the field experiment was implemented using a randomized complete block design (RCBD) with three replicates, following a factorial design. The results obtained 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) test (Al-Rawi & Khalaf Allah, 1980).

Many agricultural researchers consider three replications of field experiments acceptable under conditions of high environmental variability or when resources for experimentation are

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limited. According to Cochran and Cox (1957), discussed earlier, fewer replications are sufficient when the degrees of freedom (dfE) for variance are large, thereby increasing the relevance of statistical tests and reducing the influence of environmental variance on the results. However, Filazzola and Cahill (2021) noted that the number of replications depends on the total number of treatments and the variance distribution within the experiment (Filazzola & Cahill, 2021). In some cases, three replications may be sufficient to demonstrate statistically significant effects, for example, when treatment differences are large and variances within treatments are minor (Filazzola & Cahill, 2021).

Results and Discussion

Available Nitrogen (mg N/kg soil)

The results in Table 2 showed that the addition of glyphosate and 2,4-D at different concentrations significantly affected the available nitrogen concentration in the rhizosphere. The glyphosate treatment at the full recommended concentration achieved the highest average of 30.00 mg N/kg soil, while the 2,4-D treatment at a concentration twice the recommended concentration recorded the lowest average of 16.17 mg N/kg soil. The results also showed that inoculation with *A. chroococcum* led to a significant increase in available nitrogen concentration compared with the non-inoculated control. The average in the inoculated treatment was 26.29 mg N/kg soil, compared to 18 mg N/kg soil in the uninoculated treatment. As for the two-way interaction between chemical Herbicides and bacterial inoculation, the results also showed a significant effect, as the interaction between glyphosate at the full recommended concentration and inoculation with *A. chroococcum* recorded the highest average of 35.67 mg N/kg soil, while the interaction between 2,4-D at a concentration twice the recommended concentration and no inoculation gave the lowest average of 13.67 mg N/kg soil.

2,4-D helps solubilize phosphorus by stimulating certain bacteria that produce organic acids that release phosphorus from its insoluble compounds. Glyphosate works differently; it occupies adsorption sites on clay, iron, and aluminum particles in the soil, temporarily reducing the availability of some elements. However, its effect varies with dose and soil type (Al-Rajab & Al-Hakimi, 2014). The increased phosphate solubilization observed with 2,4-D may be associated with changes in microbial activity and increased secretion of organic acids. Meanwhile, glyphosate, at the full recommended dose, may strike a balance between weed

Table 2

Effect of Different Concentrations of Glyphosate and 2,4-D Herbicides and Inoculation with A. chroococcum Bacterium on Available Nitrogen

Herbicides	Available Nitrogen (mg N/kg soil)		Available N with pesticide (mg N/kg soil)
	Control (no bacterial inoculation)	*A. chroococcum* inoculation	
Without and with bacterial inoculation	17.00	28.67	22.83
Glyphosate (50% recommended dose)	20.67	31.67	26.17
Glyphosate (recommended dose)	24.33	35.67	30.00
Glyphosate (2x recommended dose)	15.67	22.33	19.00
2,4-D (50% recommended dose)	23.67	24.67	24.17
2,4-D (recommended dose)	15.00	20.00	17.50
2,4-D (2x recommended dose)	13.67	18.67	16.17
Bacterial inoculation rate	18.33	26.29	
LSD 0.05	Herbicides = 0.70	Bacteria = 0.35	Integration = 0.99

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control and reduced nutrient competition, enabling plants to respond optimally to bacterial inoculation (Luiz et al., 2012).

Available Phosphorus (mg P/kg soil)

The results in Table 3 showed that the addition of glyphosate and 2,4-D at different concentrations significantly increased the available phosphorus concentration in the rhizosphere compared to the control. The 2,4-D treatment at a concentration twice the recommended level achieved the highest average of 46.33 mg P/kg soil, while the glyphosate treatment at a concentration twice the recommended level recorded the lowest average of 16.17 mg P/kg soil. The results also showed that inoculation with *A. chroococcum* bacteria led to a significant increase in available phosphorus concentration compared to the uninoculated treatment, with an average of 33.29 mg P/kg in the inoculated treatment. In comparison, the uninoculated treatment recorded the lowest average of 24.62 mg P/kg soil. As for the two-way interaction between herbicides and bacterial inoculation, a significant effect was found; specifically, the interaction between 2,4-D at twice the recommended concentration and inoculation with *A. chroococcum* recorded the highest average of 51.00 mg P/kg soil. In comparison, the interaction between glyphosate at twice the recommended concentration and no inoculation yielded the lowest average, 11.00 mg P/kg soil.

The use of herbicides must be balanced with biological applications in agricultural systems management. In our experience, we observed differences in soil microbial activity (nitrogen fixers and phosphate solubilizers) when using glyphosate and 2,4-D at their recommended concentrations. This may be due to the direct effects of both herbicides on rhizosphere microorganisms (disrupting enzymatic processes responsible for nitrogen fixation and phosphorus solubilization). Although scientifically recommended doses reduce the inhibitory effect, they do not eliminate their negative impact on microbial diversity (Zabaloy et al., 2010). Therefore, it is essential to combine herbicides with bacterial inoculants to reduce this effect and maintain the effectiveness of biological processes in the soil. Herbicides can alter the microbial balance in soil by reducing beneficial bacteria, especially those attached to plant roots. Other researchers have also found this, reporting that herbicides can alter soil microbial balance (Cycoń et al., 2015).

The concept of complementarity, as applied in this study, refers to the interaction between the bacterial inoculant and the herbicide, whereby the inoculant helps offset the herbicide’s potential negative effects by activating microbial enzymes and secreting growth promoters. However, moderate or excessive herbicide use, regardless of the rate, will lead to long-term decline in microbial activity. Therefore, an Integrated Waste Management (IWM) program using biofertilizers is recommended, as it provides optimal weed control while maintaining soil health (Singh & Singh, 2021).

The greater P-solubilization observed with 2,4-D can be attributed to its indirect stimulation of soil microbial activity and to changes in root exudation patterns, which favor the secretion of low-molecular-weight organic acids capable of chelating Ca, Fe, and Al ions that bind phosphate in soil. Such changes increase bioavailable phosphorus via microbially mediated solubilization pathways (Kremer & Means, 2009; Rose et al., 2013).

On the other hand, at the full recommended dose, glyphosate seems to have a more ‘balanced’ effect on the soil–plant system. While glyphosate may temporarily affect rhizosphere microbial communities, properly applied rates remove weed competition for nutrients without drastically disrupting beneficial microbial functions. This allows optimal nutrient uptake by the plant and, consequently, a better response to bacterial inoculation, manifested as improved utilization rather than excessive solubilization of phosphorus (Luiz et al., 2012; Manisankar et al.,2024; Zablotowicz & Reddy, 2007).

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Table 3

Effect of Different Concentrations of Glyphosate and 2,4-D Herbicides and Inoculation with A. chroococcum Bacterium on Available Phosphorus

Herbicides	Available Phosphorus (mg P/kg soil)		Average
	Control (no bacterial inoculation)	*A. chroococcum*	
Without and with bacterial inoculation	21.00	32.33	26.67
Glyphosate (50% recommended dose)	23.67	35.67	29.67
Glyphosate (recommended dose)	16.33	26.67	21.50
Glyphosate (2x recommended dose)	11.00	22.33	16.67
2,4-D (50% recommended dose)	27.67	17.00	22.33
2,4-D (recommended dose)	35.33	49.00	42.17
2,4-D (2x recommended dose)	41.67	51.00	46.33
Bacterial inoculation rate	24.62	33.29	
LSD 0.05	Herbicides = 0.98	Bacteria = 0.49	Integration = 1.38

Soil pH

The results in Table 4 showed that the application of glyphosate and 2,4-D herbicides at different concentrations significantly affected soil pH compared with the control treatment. The glyphosate treatment at half the recommended concentration recorded the highest pH value of 7.89, while the 2,4-D treatment at half the recommended concentration achieved the lowest value of 7.18. It was also found that inoculation with *A. chroococcum* bacterium led to a significant increase in pH compared to the non-inoculated treatment, with the inoculated treatment recording the highest average of 7.64, compared to 7.49 in the non-inoculated treatment. Two-way interactions between herbicides and bacterial inoculation showed significant differences, particularly between glyphosate at the full recommended concentration and inoculation with *A. chroococcum,* which achieved the highest value of 7.86. In contrast, the 2,4-D treatment at half the recommended concentration, without inoculation, recorded the lowest value of 7.10.

Electrical Conductivity (Soil EC) (dS/m)

The results in Table 5 showed that applying glyphosate and 2,4-D at different concentrations significantly reduced soil electrical conductivity (EC) compared with the control treatment. The 2,4-D treatment at half the recommended concentration recorded the lowest value of 1.25 dS/m, while the 2,4-D treatment at twice the recommended concentration achieved the highest value of 2.94 dS/m. It was also shown that inoculation with *A. chroococcum* reduced electrical conductivity compared to non-inoculated treatments, with inoculated treatments recording lower rates. The value in the 2,4-D treatment at half the recommended concentration with inoculation reached 1.36 dS/m, compared to 1.87 dS/m in the uninoculated treatment. As for the two-way interaction between Herbicides and bacterial inoculation, a significant effect also appeared; the interaction treatment between 2,4-D at a concentration twice the recommended level and no inoculation recorded the highest value of 4.37 dS/m, while the treatment with 2,4-D at a concentration half the recommended level and inoculation achieved the lowest value of 1.22 dS/m.

Soil Bacterial Count (10^6 CFU/g dry soil)

The results in Table 6 showed that the addition of glyphosate and 2,4-D herbicides at

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Table 4

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

Herbicides	Soil pH		Average
	Control (no bacterial inoculation)	*A. chroococcum*	
Comparison	7.07	7.72	7.40
Glyphosate (50% recommended dose)	7.92	7.86	7.89
Glyphosate (recommended dose)	7.82	7.86	7.84
Glyphosate (2x recommended dose)	7.73	7.24	7.46
2,4-D (50% recommended dose)	7.27	7.10	7.18
2,4-D (recommended dose)	7.40	7.84	7.62
2,4-D (2x recommended dose)	7.62	7.78	7.70
Bacterial inoculation rate	7.49	7.64	
LSD 0.05	Herbicides = 0.19	Bacteria = 0.09	Integration = 0.26

Table 5

Effect of Different Concentrations of Glyphosate and 2,4-D Herbicides and Inoculation With A. chroococcum Bacteria on Electrical Conductivity EC

Herbicides	Bacterial inoculation		Average pesticide
	Control (no bacterial inoculation)	*A. chroococcum*	
Without and with bacterial inoculation	1.38	1.32	1.35
Glyphosate (50% recommended dose)	1.44	1.34	1.39
Glyphosate (recommended dose)	1.39	1.34	1.36
Glyphosate (2x recommended dose)	1.53	1.35	1.44
2,4-D (50% recommended dose)	1.28	1.22	1.25
2,4-D (recommended dose)	2.23	1.44	1.84
2,4-D (2x recommended dose)	4.37	1.50	2.94
Bacterial inoculation rate	1.87	1.36	
LSD 0.05	Herbicides = 0.001	Bacteria = 0.004	Integration = 0.012

different concentrations significantly decreased bacterial counts in the rhizosphere compared to the control treatment. The control treatment recorded the highest average of 21.00 × 10^6 CFU/g dry soil, while the 2,4-D therapy at the recommended concentration achieved the lowest average of 3.50 × 10^6 CFU/g dry soil. It was also demonstrated that inoculation with *A. chroococcum* bacteria resulted in a significant increase in bacterial counts compared to non-inoculation. The average in the inoculated treatment was 18.83 × 10^6 CFU/g dry soil, compared to 2.50 × 10^6 CFU/g dry soil in the non-inoculated treatment. Regarding the two-way interaction between herbicides and bacterial inoculation, the results showed a significant effect the control treatment with *A. chroococcum* inoculation achieved the highest average of 37.00 × 10^6 CFU/g dry soil, while the lowest value of 0.00 × 10^6 CFU/g dry soil was

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recorded in both the glyphosate treatment at a concentration twice the recommended level and the 2,4-D treatment at a concentration twice the recommended level without adding bacterial inoculation.

Maize Yield

The results in Table 7 showed the effects of different concentrations of glyphosate and 2,4-D herbicides, and of inoculation with *A. chroococcum*, on the total yield of maize plants (ton/ha). The addition of herbicides had a significant effect on total yield, with the glyphosate treatment at the full-rate concentration achieving the highest rate of 16.29 ton/ha. In comparison, the 2,4-D treatment at the full-recommendation concentration recorded the lowest rate, at 13.35 ton/ha. The results also showed that inoculation with *A. chroococcum* significantly increased total yield, with the inoculated treatment recording the highest value of 15.57 ton/ha, compared to the uninoculated treatment at 13.30 ton/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 ton/ha, while the 2,4-D treatment recorded 2,4-D at the full recommendation concentration without inoculation had a minimum average concentration of 12.31 ton/ha.

Pesticide Residues in Soil (mg/kg)

The results shown in Table 8 illustrate the effects of different glyphosate and 2,4-D herbicide concentrations and *A. chroococcum* bacterial inoculation on pesticide residues in the soil (mg/kg). The various treatments demonstrated that herbicide concentrations significantly affected pesticide residue levels (mg/kg) in the soil. The glyphosate treatment at a concentration twice the recommended level recorded the highest residue of 51.40 mg/kg, compared to the control treatment, which recorded the lowest value of 0 mg/kg. The results also showed that inoculation with *A. chroococcum* led to a significant reduction in accumulation, with the inoculated treatment recording the lowest residue rate of 8.43 mg/kg.

In comparison, the uninoculated treatment recorded the highest rate of 30.21 ton/kg. The data on the two-way interaction between herbicides and bacterial inoculation showed apparent and significant effects. The glyphosate treatment at a concentration twice the recommended level, without inoculation, resulted in the highest average residue, 80.23 mg/kg. In comparison, the control treatment with bacterial inoculation zero.

The study results showed that inoculating the soil with *A. chroococcum* bacteria effectively improved soil fertility and increased nutrient availability. Nitrogen-fixing bacteria, such as *A. chroococcum*, increased the concentration of nitrogen available to plants through biofixation, converting atmospheric nitrogen into forms directly absorbable by plants (Song et al., 2021; Song et al., 2024). Simultaneously, phosphate-solubilizing organisms increased phosphorus availability by secreting organic acids that solubilize insoluble phosphate compounds in the soil, thereby enhancing nutrient uptake and promoting plant growth (Sharma et al., 2013; Sharma et al., 2024; Song et al.,2024). This dual mechanism of nitrogen fixation and phosphate solubilization synergistically enhances soil fertility, leading to improved maize growth and higher productivity (Khoso et al., 2024; Kumar et al., 2025).

Herbicide and inoculation treatments were also observed to influence the soil's chemical properties. Bacterial inoculation reduced electrical conductivity compared with non-inoculated soils, indicating restoration of ionic balance and stimulation of microbial activity in the rhizosphere (Chennappa et al., 2025; Gurikar et al., 2016). The increase in the population of beneficial bacteria following inoculation supports nutrient cycling, suppresses soil-borne pathogens, and improves soil structure, collectively enhancing fertility and plant performance (Al-budairy & Al-Taweel, 2025; Kaur et al., 2024).

The integration of herbicide treatments with bacterial inoculation had a profound effect

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Table 6

Effect of Different Concentrations of Glyphosate and 2,4-D Herbicides and Inoculation with A. chroococcum on Bacterial Colony Forming Unit)

Herbicides	Bacterial Colony Forming Unit (CFU per g dry soil)		Average
	Control (no bacterial inoculation)	*A. chroococcum*	
Comparison	5.00	37.00	21.00
Glyphosate (50% recommended dose)	4.00	16.00	10.00
Glyphosate (recommended dose)	2.00	21.67	11.83
Glyphosate (2x recommended dose)	0.00	8.00	4.00
2,4-D (50% recommended dose)	3.00	15.00	9.00
2,4-D (recommended dose)	1.00	6.00	3.50
2,4-D (2x recommended dose)	0.00	10.00	5.00
Bacterial inoculation rate	2.50	18.83	
LSD 0.05	Herbicides = 0.17	Bacteria = 0.09	Integration = 0.24

Table 7

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

Herbicides	Maize total yield (t/ha)		Average pesticide
	Control (no bacterial inoculation)	*A. chroococcum*	
Without and with bacterial inoculation	12.59	15.15	13.87
Glyphosate (50% recommended dose)	14.53	16.10	15.32
Glyphosate (recommended dose)	14.91	17.67	16.29
Glyphosate (2x recommended dose)	12.45	14.90	13.67
2,4-D (50% recommended dose)	13.75	16.11	14.92
2,4-D (recommended dose)	12.31	14.39	13.35
2,4-D (2x recommended dose)	13.30	15.11	14.21
Bacterial inoculation rate	13.30	15.57	
LSD 0.05	Herbicides = 0.019	Bacteria = 0.01	Integration = 0.03

on maize yield. The application of glyphosate at the full recommended concentration was more effective at controlling weeds than 2,4-D, thereby reducing competition for essential nutrients, water, and light and allowing maize plants to allocate more energy to growth and production (Manisankar et al., 2022; Manisankar et al., 2024). Remarkably, the combination of glyphosate application and *A. chroococcum* inoculation resulted in the highest yields, demonstrating the additive benefits of reducing competition, enhancing nutrient uptake, and stimulating plant growth through microbial interactions (Kaur et al., 2024; Kumar et al., 2025).

Glyphosate achieved effective weed control while permitting the activity of beneficial bacteria in the rhizosphere, as bacterial inoculants were applied to produce maximum

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maize yield under the fully recommended dose, thereby forming a functional balance between the two activities. Glyphosate has been reported to transiently reduce certain microbial populations, but there is no long-term suppression of soil microorganisms experienced when glyphosate is applied at agronomically recommended rates. Therefore, apart from reducing nutrient, water, and light competition from weed pressure, it ensures that inoculated beneficial bacteria efficiently support plant growth. In addition, the glyphosate degradation products, mainly aminomethylphosphonic acid (AMPA), could also serve as nutrient sources for selected soil microorganisms, thereby supporting a quicker recovery of microbes and microbial functions within the rhizosphere, enhancing nutrient solubilization and uptake by inoculated plant growth-promoting bacteria, and leading to better crop productivity. On the other hand, insufficient or excessive herbicide doses that fail to effectively control weeds may cause greater disruption of microbes, reducing synergistic effects with bacterial inoculation. These results agree with earlier work, which reports that at recommended doses, glyphosate can maintain the functions of soil microbes while improving crop performance by reducing weed competition (Luiz et al., 2012).

Regarding herbicide residues, *A. chroococcum* has been shown to facilitate the biodegradation of glyphosate in soil, leading to a gradual reduction in its concentration until it disappears over a relatively short period (Al-Tharwani et al., 2023; Al-Tharwani et al., 2024). This biodegradation occurs due to microbial enzymatic pathways that cleave glyphosate molecules, transforming them into less harmful compounds, thereby reducing environmental contamination and mitigating risks to soil health and groundwater quality. Nevertheless, intensive or irrational herbicide use can lead to the accumulation of residues or their degradation products, negatively impacting the physical, chemical, and biological properties of the soil and potentially entering the food chain (Chennappa et al.,2025; Sumbul et al., 2020).

Some studies indicate that *A. chroococcum* exhibits relative tolerance to glyphosate at recommended doses; however, exposure to high concentrations can inhibit its respiratory activity, reduce nitrogen fixation, impair protein synthesis, and disturb cell volume, which ultimately diminishes its growth-promoting effects (Santos & Flores, 1995; Santos & Flores, 2025). The continuous monitoring of microbial health and the optimization of inoculation strategies are therefore critical for maintaining the efficacy of biofertilizers in agricultural systems.

Additionally, recent studies have highlighted the potential of other microbial inoculants and biofertilizers, including *Pseudomonas aeruginosa* combined with organic wastes and nano-fertilizers, to enhance soil enzyme activities, growth characteristics, and crop yield (Al-budairy & Al-Taweel, 2025). Collectively, these findings support the adoption of integrated approaches that combine beneficial microbes and optimized agrochemical management to achieve sustainable crop production and restore soil health (Guardado-Fierros et al., 2025; Santos & Flores, 2025).

Bacillus subtilis suppresses weed growth by secreting secondary compounds and volatiles that inhibit weed seed germination and impair weed growth. It also produces enzymes and lipopeptides that harm seedling tissue, reducing seedling competitiveness in the soil. *Bacillus subtilis* also promotes corn growth by stimulating nutrient uptake, producing plant growth regulators, and activating resistance systems. This enables plants to compete more effectively with weeds and reduces their negative impact. This is reflected in increased vegetative growth and improved corn yield. A recent study demonstrated that soil inoculation with *Bacillus subtilis* improved the growth, yield, and nutritional value of corn grown in acidic soils (Diaz-Chuquizuta et al., 2025; Khoso et al., 2024).

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Table 8

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

Herbicides	Pesticide Residues in Soil (mg/kg)		Average
	Without adding	*A. chroococcum*	
Without and with bacterial inoculation	0.00	0.00	0.00
Glyphosate (50% recommended dose)	36.03	0.00	18.02
Glyphosate (recommended dose)	50.73	19.03	34.88
Glyphosate (2x recommended dose)	80.23	22.57	51.40
2,4-D (50% recommended dose)	7.62	3.04	5.35
2,4-D (recommended dose)	22.52	10.80	16.66
2,4-D (2x recommended dose)	44.50	12.02	28.26
Bacterial inoculation rate	30.21	8.43	
LSD 0.05	Herbicides = 0.36	Bacteria = 0.18	Integration = 0.50

Conclusions

Biofertilization with *Azotobacter chroococcum* increased nitrogen availability and enhanced the uptake of macro- and micronutrients, resulting in significant improvements in physiological growth traits and yield components. Integration of biofertilization with herbicide application at recommended doses improved plant productivity by balancing biological nitrogen fixation with effective weed control, thereby reducing competition for nutrients, water, and light. Herbicides can never selectively eliminate pathogenic microbes. Sensible and rational use of herbicides involving their application at scientifically recommended doses in combination with biofertilizer application will result in the minimal inhibition effect on beneficial soil microorganisms because microbial activity due to nutrient cycling processes helps reduce persistence as well as toxicity pesticide residues within the soil, hence Integrated Nutrient plus Weed Management Program adopting *A.chroococcum* biofertilization is proposed towards enhancement of crop productivity concurrently with negative impacts on biological health of soils.

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