The Effect of Glyphosate on Nitrogen-Fixing Bacteria *Azospirillum* Spp

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Abstract

Due to the effect of herbicide on the soil, the natural fluorescence changes, and prevents the growth of bacteria that fix nitrogen an imbalance occurs between the organisms that live in the soil. Harmful organisms present in the soil increase until they become pests. Studying the effects of glyphosate on nitrogen-fixing bacteria like Azospirillum normally includes a combination of laboratory and field trials. An overarching framework for conducting such studies is outlined below. The average number of Azospirillum cells at each concentration and in the control group is shown by the mean values. There were more total counts of Azospirillum in the control group, suggesting a higher mean (8200.00) without glyphosate. There appears to be a dose-dependent effect of glyphosate on Azospirillum count, with the mean values decreasing as the concentration of glyphosate rises from 5% to 20%. The p-values give us an idea of how significant the results are. A p-value of 0.00001 for the 5% concentration level implies an extremely low likelihood of achieving the observed results if there were no meaningful effects. Work on the research began on 12/26/2022 in the laboratories affiliated with the College of Education for Pure Sciences, University of Mosul, and the work and writing of the research was completed within 7 months.

Keywords: glyphosate , nitrogen fixation , Azospirillum

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تأثير الغليفوسات على البكتيريا المثبتة للنيتروجين Azospirillum spp.

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الخلاصة:

نتيجة لتأثير مبيدات الأعشاب على التربة يؤدي الى منع نمو البكتريا المثبتة للنيتروجين ويحدث خلل بين الكائنات الحية التي تعيش في التربة وبذلك تزداد الكائنات الضارة الموجودة في التربة حتى تصبح آفة. تتضمن هذه الدراسة تأثيرات الغليفوسات على البكتيريا المثبتة للنيتروجين مثل *Azospirillum* عادةً مجموعة من التجارب المختبرية. اظهرت النتائج ان متوسط عدد خلايا *Azospirillum* في كل تركيز وفي مجموعة السيطرة موضحة بالقيم المتوسطة، كان هناك عدد أكبر من بكتريا *Azospirillum* في المجموعة الضابطة، مما يشير إلى ان اعلى متوسط (٨٢٠٠,٠٠) بدون الغليفوسات، هناك علاقة عكسية بين تركيز مبيد الغليفوسات ونمو بكتريا *Azospirillum*، اذ تنخفض القيم المتوسطة مع ارتفاع تركيز الغليفوسات من ٥٪ إلى ٢٠٪. تعطينا القيم p فكرة عن مدى أهمية النتائج، اذ تشير القيمة p البالغة ٠,٠٠٠٠١ لمستوى التركيز ٥٪ إلى احتمالية منخفضة للغاية لتحقيق النتائج المرصودة إذا لم يكن هناك تأثير معنوي. بدأ العمل بالبحث بتاريخ ٢٠٢٢/١٢/٢٦ في المختبرات التابعة لكلية التربية للعلوم الصرفة جامعة الموصل وتم الانتهاء من العمل وكتابة البحث خلال ٧ اشهر.

Introduction

Soil microorganisms are just one example of the many non-target creatures that might be negatively impacted by the widely used herbicide glyphosate.[1] Nitrogen-fixing bacteria of the genus Azospirillum are abundant in herbicide the rhizosphere of many plant species.[2,3] In order for plants to flourish, nitrogen-fixing bacteria must first fix atmospheric nitrogen gas ($N2$) into ammonia ($NH3$) or other nitrogen compounds. [4] Multiple factors contribute to the complexity of glyphosate's effect on Azospirillum and other nitrogen-fixing bacteria.[5,6] Glyphosate as a Chelator: Glyphosate is known to form complexes with many metal ions, including critical micronutrients like manganese (Mn) and iron (Fe).[7,8] Enzymes in nitrogen fixation rely on these metal ions for proper functioning.[9] Chelation of these ions by glyphosate can reduce the activity of these enzymes, which may have consequences for nitrogen fixation.[10] Soil microbial communities may be altered by glyphosate, which may have an impact on the abundance of nitrogen-fixing bacteria like Azospirillum.[11] It's possible that this will alter the competitive dynamics among the soil microorganisms.[12] Glyphosate's side effects on plant health may have an indirect effect on nitrogen fixation.[13] It is well-documented that glyphosate can harm plants in both direct and indirect ways.[14] Root exudates are released from unhealthy plants less frequently, which can starve nitrogen-fixing microorganisms.[15] The number and activity of these bacteria can be affected by changes in root exudates.[3] Strains Resistant to Glyphosate May Evolve Some research has shown that some strains of Azospirillum may evolve resistance to glyphosate.[6] These glyphosate-resistant variants may still be able to perform nitrogen fixation.[3] It's worth noting that glyphosate's effect on Azospirillum and other nitrogen-fixing bacteria varies depending on factors including the concentration of glyphosate, the type of soil, the presence of other chemicals, and the races of bacteria involved.[8] Soil microbial communities, such as nitrogen-fixing bacteria, are an important topic of study in agronomy and environmental science, and research into the impacts of glyphosate on these populations is ongoing.

Methodology

Studying the effects of glyphosate on nitrogen-fixing bacteria like Azospirillum normally includes a combination of laboratory and field trials. An overarching framework for conducting such studies is outlined below.

Bacterial Isolation and Culture:

Obtaining pure isolates of Azospirillum from the culture collection or removing Azospirillum strains from soil rich in the roots of leguminous plants. 15 cm of this soil was removed and samples were taken from near the roots of the plants. After that, a series of decimal thinnings were made and they were planted on the media and diagnosed.

Glyphosate Exposure:

Glyphosate is a commonly employed herbicide, particularly effective against seasonal broadleaf weeds that pose competition to agricultural crops. It is a substance that belongs to the class of organophosphorus compounds. American scientist John Franz made the discovery at Monsanto in 1970. Glyphosate is distinguished by its efficacy in eradicating weeds while being non-detrimental to agricultural crops and its affordability. This insecticide was utilised in various concentrations of 5%, 10%, 15%, and 20%.

Testing the ability of bacteria to fix nickel using Nestler's reagent(1)

Soil and Plant Interactions:

Researching glyphosate's indirect impacts on nitrogen-fixing bacteria requires the inclusion of plants. Stress and nutritional deficiencies in plants can be detected by observing how they react.

Chemical Analysis:

Apply analytical chemistry methods such as high-performance liquid chromatography (HPLC) or mass spectrometry, and screen soil and plant samples for glyphosate residues. This study was carried out in the laboratories of the University of Baghdad, College of Agriculture

Statistical Analysis:

Use proper statistical procedures to analyses the data, such as analysis of variance (ANOVA) and post-hoc tests to find significant differences between treatment groups.

Results and discussion

Table 1 effect of glyphosate on total count of Azospirillum

	Mean	Std. Deviation	Std. Error	p value
5%	4500.00	500.000	223.607	0.00001
10%	3000.00	707.107	316.228	
15%	2400.00	547.723	244.949	
20%	1400.00	547.723	244.949	
control	8200.00	836.660	374.166	
Total	3900.00	2491.653	498.331	

The average number of Azospirillum cells at each concentration and in the control group is shown by the mean values. There were more total counts of Azospirillum in the control group, suggesting a higher mean (8200.00) without glyphosate. The number of Azospirillum exhibits a dose-dependent response to glyphosate, whereby the mean values decrease as the concentration of glyphosate increases from 5% to 20%. P-values provide an indication of the level of significance of the results. A p-value of 0.00001 at the 5% concentration level suggests an extremely little likelihood of obtaining the observed results if there is no significant effect. This suggests that the presence of glyphosate at this concentration has a highly discernible impact. There is a correlation between the dosage and the effect, since the average outcomes tend to decline as the concentrations of glyphosate increase (from 5% to 20%). This supports the idea that increased exposure to glyphosate at higher concentrations will result in a more pronounced impact on azospirillum. The impact of high concentration on bacteria can be elucidated by its influence on the biosynthesis of the bacterial cell wall, as well as its ability to impede metabolic processes.

Table 2 ANOVA table of effect of glyphosate on total count of Azospirillum

ANOVA

Azospirillum

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	140800000.000	4	35200000.000	85.854	.000
Within Groups	8200000.000	20	410000.000		
Total	149000000.000	24			

The outcomes of an ANOVA for the variable "Azospirillum" are displayed in Table 2. There are statistically significant variations in the groups' means, as indicated by the F-statistic of 85.854, with a corresponding p-value of very low (significant at conventional levels). Differences between groups likely account for a sizeable fraction of the total variability, as the sum of squares for Between Groups (140,800,000) is significantly higher than the sum of squares for Within Groups (8,200,000). The results indicate that Azospirillum numbers vary significantly between the groups. More research is needed to determine which groups have differences that contribute to this overarching relevance; post hoc testing may be useful in this regard**.**

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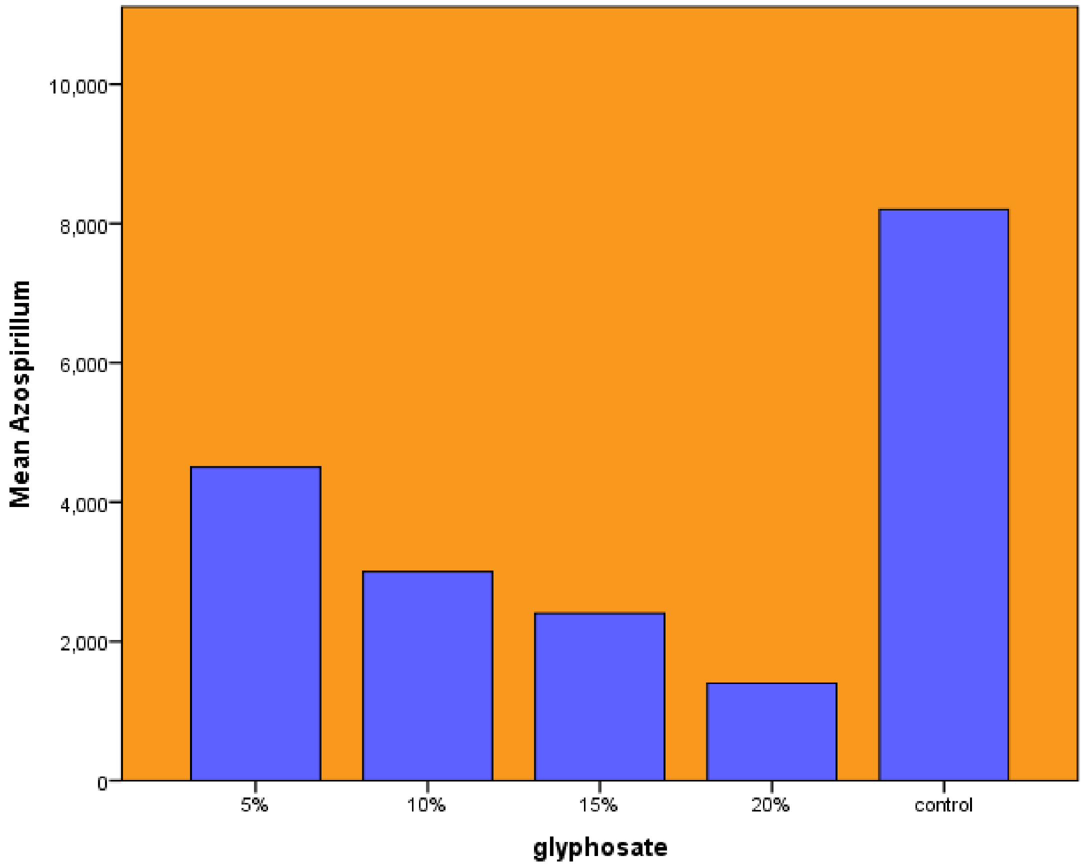

Figure 1 effect of glyphosate on total count of Azospirillum

Table 3 effect of glyphosate on nitrogen fixation from Azospirillum bacteria

	concentration of nitrogen fixation mg/l			
	Mean	Std. Deviation	Std. Error	p value
5%	4.500	1.2247	.5477	0.0001
10%	3.280	.1304	.0583	
15%	2.420	.4438	.1985	
20%	1.400	.4000	.1789	
control	7.020	.6979	.3121	
Total	3.724	2.0743	.4149	

Nitrogen fixation, on average, decreases from 5% to 20% as glyphosate concentrations increase. The average level of nitrogen fixation is higher in the control group. Therefore, it appears that increased glyphosate concentrations may inhibit nitrogen fixation by Azospirillum bacteria. Nitrogen fixation was significantly different from the control at the 5% concentration level (p0.0001). This implies that nitrogen fixation by Azospirillum is significantly impacted at a glyphosate concentration of 5%. For the remaining concentrations (10%, 15%, 20%), the p-values are not provided or are not significant, indicating that the observed differences may not be statistically significant at the specified significance level (typically set at 0.05.)

Table 4 ANOVA table of effect of glyphosate on nitrogen fixation from Azospirillum bacteria

ANOVA

concentration of nitrogen fixation mg/l

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	93.822	4	23.455	49.673	.000
Within Groups	9.444	20	.472		
Total	103.266	24			

The table shows the outcomes of an ANOVA performed on the nitrogen fixation concentration (mg/l) for each group. There are statistically significant variations between the groups' means, as indicated by the F-statistic of 49.673 and the correspondingly small p-value (significant at the customary thresholds). Differences across groups likely account for a sizeable fraction of the total variability, since the across Groups sum of squares (93.822) is significantly bigger than the Within Groups sum of squares (9.444). According to the results, the levels of nitrogen fixation differ significantly between the groups ..

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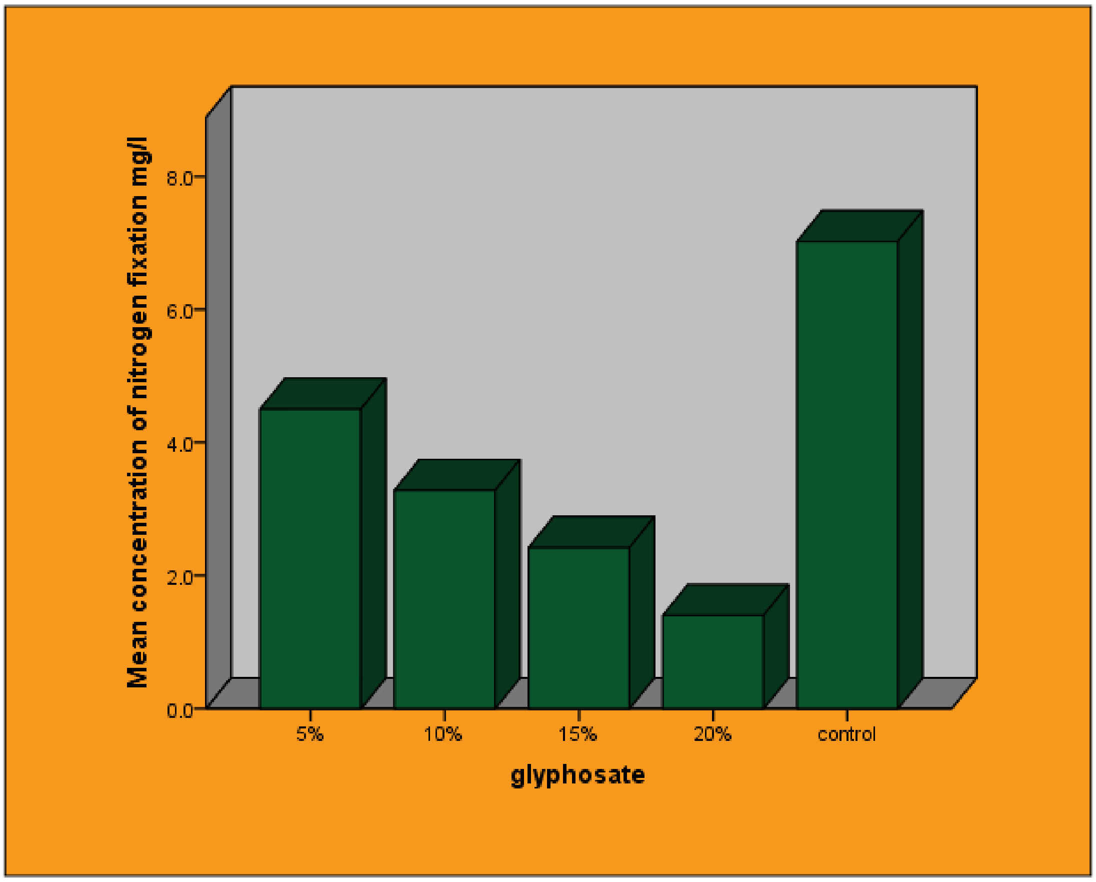

figure 2 effect of glyphosate on nitrogen fixation from Azospirillum bacteria

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