

Effects of Biofertilizers on Soil Quality and Environmental Response in Kumquat Seedlings

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Abstract

This research was conducted at the College of Agricultural Engineering Sciences, University of Baghdad, to investigate the influence of sustainable fertilization strategies on kumquat seedlings. The study evaluated the effects of two types of fertilizers—organic fish fertilizer and traditional chemical fertilizer—in combination with specific bacterial inoculants, namely *Azospirillum* sp. and *Streptomyces* sp., focusing on soil properties, greenhouse gas (GHG) emissions, and microbial dynamics. The experimental results revealed a substantial improvement in soil fertility indicators. Organic carbon levels increased significantly from 0.578% in the control (F0) to 0.769% in the F3 treatment. Similarly, soil organic matter reached its peak value of 1.600% in F3, compared to lower values in other treatments, highlighting the role of these fertilizers in enhancing soil structure. In terms of environmental impact, the study observed a dual effect on gas emissions. While CO₂ concentrations rose from 297 ppm to 380 ppm in F1 due to increased biological respiration, other harmful GHGs saw a drastic reduction. Nitrous oxide (N₂O) emissions fell from 21.6 ppm in F0 to 7.9 ppm in F2, and methane (CH₄) decreased from 3.6 ppm to 0.8 ppm. Consequently, the carbon dioxide equivalent (CO₂eq) dropped from 6,797 to 2,299 in F3, marking a significant reduction in the overall carbon footprint. Furthermore, microbial activity was notably enhanced; *Azospirillum* and *Streptomyces* populations reached 8.6 log and 8.79 log, respectively, in the B2 treatment. Overall, the integration of fish fertilizer with a bacterial mixture (B3) provided the most balanced approach for improving soil health, boosting microbial populations, and mitigating environmental pollution. These findings provide a strategic pathway for implementing sustainable, eco-friendly agricultural practices for fruit crops in Iraq.

Keywords: biofertilization; climate-smart agriculture; nutrient cycling; plant growth-promoting bacteria; rhizosphere

Introduction

Fertilizers of various types play a pivotal role in modern agriculture by providing essential nutrients such as nitrogen, phosphorus, and potassium, thus promoting plant growth and increasing agricultural productivity. However, the intensive use of chemical fertilizers raises environmental concerns related to soil degradation and increased greenhouse gas emissions such as carbon dioxide (CO₂), nitrous oxide (N₂O), and methane (CH₄). In contrast, bacterial biofertilizers have emerged as sustainable alternatives to toxic and expensive chemical fertilizers. Biofertilizers rely on beneficial microorganisms, such as certain types of bacteria that live in the soil, and help plants grow by providing the nutrients they need, fixing nitrogen, and increasing phosphorus uptake, all of which are essential for plant growth and increased productivity. They also serve as alternatives to chemical fertilizers (Al-Kamal et al., 2024). Nitrogen-fixing bacteria

Azospirillum and natural antibiotic-producing bacteria *Streptomyces*, which improve nutrient absorption and promote plant health, are widely used as alternatives to chemical fertilizers to enhance plant growth and increase productivity (Firdos & Khan, 2025). These bacteria enter into symbiotic relationships with plant roots, increasing nutrient utilization efficiency and reducing the need for chemical fertilizers (Hungria, et al., 2022).

Fertilizers are among the most important agricultural inputs that directly affect soil fertility and crop productivity. Although chemical fertilizers provide nutrients quickly, their overuse leads to environmental problems such as soil degradation and increased greenhouse gas emissions (Williams, & Peterson, 2023). Conversely, some studies have shown that biofertilizers are a sustainable option based on microorganisms such as nitrogen-fixing *Azospirillum* and bio-producing *Streptomyces*, which have proven effective in improving nutrient uptake and

promoting plant health (Dimkpa et al., 2024). Studies have shown that using biofertilizers and combining them with organic or fish fertilizers can increase the organic carbon and organic matter content in the soil, which enhances microbial activity and reduces emissions of harmful gases such as methane and nitrous oxide (Xia, et al., 2024). This contributes to improved growth and increased plant resistance to harsh environmental conditions (Hao, et al., 2025). On the other hand, recent research indicates that kumquat production is associated with energy consumption and greenhouse gas emissions that can be reduced through the use of sustainable agricultural techniques, such as fish fertilizers and beneficial bacteria, thus achieving a balance between high productivity and a reduced carbon footprint (Özbek & Gökdoğan, 2025).

Materials and Methods

The experiment was carried out in the fruit tree canopy located in Research Station B of the Department of Horticulture and Landscape Engineering / College of Agricultural Engineering Sciences / University of Baghdad for the spring season 2024/2025 to study the effect of adding bio-fertilizers, fish waste fertilizer and chemical fertilizer on the growth of kumquat seedlings and to study their role in reducing the carbon footprint. Kumquat seedlings of the Margaita Fortunella variety, grafted onto a citrus rootstock and as homogeneous as possible in their growth, were selected at the age of 2.5 years and planted in plastic pots with a diameter of 32 cm and a height of 40 cm and a capacity of 18 kg, supplied with a medium (soil: peat moss) in a ratio of (1:3). Biofertilizers (AzospirillumLipoferm, Streptomyces spp.) and compost from decomposed fish waste were added to the soil at the beginning of the experiment on 9/4/2025 and only once. Chemical fertilizer (NPK) was also added to the soil in four installments: two spring installments on 23/4/2025, the first installment, and on 7/5/2025, the second installment, while the autumn installments were added on 10/9/2025 and on 24/9/2025. The isolation and identification of the bacteria (Azospirillum lipoferm and Streptomyces spp.) was carried out in the Bacterial Pesticide Production Laboratory of the Agricultural Research Center at the Scientific Research Authority / Ministry of Higher Education and Scientific Research, following the isolation method described by (Collee et al., 1996). Healthy plants were uprooted, the soil surrounding the roots was collected and mixed well, 10 g of soil powder was taken and 90 ml of Peptone water was added to it as mentioned by (MacFaddin, 2000). The mixture was subjected to electric shaking at a speed of 120 rpm for 30 minutes and left to settle. Then 1 ml of the supernatant was taken and added to 9 ml of 0.85% Saline

solution NaCl to form the second decimal dilution, and so on to obtain the third decimal dilution. 0.1 ml of the third decimal dilution (10^{-3}) was taken and spread on solid nutrient agar medium. The plates were incubated at 30°C for 48 hours. After performing biochemical tests, the bacterial isolates (AzospirillumLipoferm, Streptomyces sp.) were identified and used in the experiment.

Preparation of Bacterial Inoculum from Biofertilizers

The bacterial inoculum (Azospirillum lipoferm and Streptomyces spp.) was prepared using 100 g of peat moss of each bacterial species. This was done by preparing 1500 ml of 13 g/L nutrient broth and sterilizing it in an autoclave at 121°C and 15 psi for 15 minutes. After cooling, the culture medium was inoculated with Azospirillum. Following the same steps for preparing and sterilizing the culture medium for Azospirillum, the culture medium was also prepared for Streptomyces. Both bacteria were incubated at 30°C for 72 hours. Five kilograms of peat moss were then inoculated with each bacterial species, and the concentration for both was measured using a hemocytometer. The concentration for Azospirillum was 10.4×10^{10} . The concentration of Streptomyces bacteria was 10^{12} cells/ml, while the concentration of Streptomyces bacteria was 4×10^{10} cells/ml. For every 100 g of sterile peat moss, 30 ml of the inoculant was used in the experiment. 100 g of each type was added individually, and the same quantity (100 g) was used as a mixture.

Preparation of Decomposed Fish Manure

Decomposed fish manure was obtained from Gulf Animal Feed Industries, based in the United Arab Emirates (Figure 5), in the form of a solid powder (feed). The powder was mixed with ordinary water and then dried in the sun for 5 days. Afterward, the powder was collected and used in the field experiment at concentrations of 0, 150, and 300 g. It was added to each pot in the bottom layer, close to the roots, and then completely covered with potting soil.

Mineral Fertilization

Chemical fertilizers were added: nitrogen in the form of urea at a concentration of 5 g/L, phosphorus in the form of single superphosphate at 2.5 g/L, and potassium in the form of potassium sulfate at a concentration of 5 g/L. The total added concentration was (5:2.5:5) g per experimental unit. Application was carried out as a soil irrigation in four applications: two applications in the spring, 14 days apart, and two applications in the autumn, also 14 days apart. Soil Organic Matter Percentage: The

percentage of organic matter in the soil was estimated by calculating the organic carbon content (%O.C.) using the method of (Walkley & Black, 1934)).

The organic carbon was estimated, and then the organic matter was estimated using the following equation:

$$\% \text{ Organic Matter} = \% \text{O.C.} \times 1.724$$

Soil Biodiversity and Determination of the Total Soil Bacterial Count

The total number of bacteria growing on nutrient agar medium, the components of which are listed in **Table 1**, was calculated using the dilution method. A 10 ml glass tube was prepared, and 1 g of soil was placed in it. Then, 9 ml of sterile distilled water was added. 1 ml was taken from the first tube and placed in a second tube containing 9 ml of sterile distilled water, thus completing the second dilution. This process was repeated until the third dilution was reached. 1 ml was then taken and placed in a 9 cm Petri dish. The nutrient agar medium was then poured onto the dish, and it was placed in a 28°C incubator. After 24 hours, the counts were performed on the dishes according to the instructions of (Black, 1965). Additionally, when obtaining large numbers that are difficult to count on the dishes, the bacterial culture was harvested by diluting it in 5 ml of sterile distilled water. 0.1 ml of the resulting suspension was then added, and the bacterial cell counts were calculated on a hemocytometer slide according to the instructions provided by (Bastidas, 2015).

Table 1 - Effect of biofertilizers, fish fertilizers, and mineral fertilizers on the percentage of organic carbon in the soil (%)

Organic carbon %					
Average	Bio fertilizer				Soil fertilizer
	B3	B2	B1	B0	
0.578	0.661	0.540	0.640	0.471	F0
0.727	0.757	0.743	0.691	0.718	F1
0.664	0.750	0.597	0.580	0.730	F2
0.769	0.767	0.930	0.787	0.590	F3
0.005**	0.010**				Lsd5%
	0.734	0.703	0.675	0.627	Average B
	0.005**				Lsd5%

Estimation of organic carbon in soil %

The number of *Streptomyces* spp bacteria was calculated by the dilution method after growing the bacteria on the selective culture medium shown in Appendix (2). The bacterial culture was harvested in the same way and then counted on a hemocytometer counting slide. The number of *Azospirillum lipoferum* bacteria was calculated in the same way after growing them on the selective medium for bacteria shown in Appendix (3).

Soil Greenhouse Gas Estimation

Emissions of carbon dioxide (CO₂), methane (CH₄), and nitrous oxide (N₂O) from soil were determined using a Picarro G2508 cavity resonance spectrometer connected to a standard, sealed, stationary chamber. For each soil sample, the chamber was sealed with a pre-installed collar, and the air from the upper chamber was continuously recirculated through the analyzer. Gas concentrations were recorded continuously for 20 minutes for each sample. Soil temperature, air temperature, etc., were used to calculate the concentration-time regression rate (dC/dt). Gas fluxes were then calculated using chamber volume and area, atmospheric pressure, absolute temperature, and molecular weight of each gas. The carbon footprint is expressed as carbon dioxide equivalent (CO₂\ eq) using the equation:

$$\text{CO}_2 \setminus \text{eq} = \text{CO}_2 + 28(\text{CH}_4) + 265(\text{N}_2\text{O})$$

Factors of the Study

First Factor (A): Addition of Soil Fertilizers, as follows:

F0 = No addition.

F1 = Addition of (decomposed fish waste fertilizer) 300 g per seedling - 1.

F2 = Addition of (decomposed fish waste fertilizer) 150 g per seedling - 1.

F3 = Addition of NPK fertilizer at half the recommended rate (5, 2.5, 5) g per seedling - 1.

Second Factor (B): Addition of Biofertilizers, as follows:

B0 = No addition.

B1 = Addition of *Azospirillum lipoferum* bacteria with a strength of 10⁹ cells/ml loaded onto peat moss at a rate of 100 g per seedling - 1.

B2 = Addition of *Streptomyces* spp. bacteria at a strength of 10⁹ cells/ml loaded onto 100g of seedlings per 100g of seedlings.

B3 = Addition of both types of bacteria at the same levels, designated B3.

Experimental Design

The experiment was designed as a two-factor model within a 4 \times 4 Randomized Complete Block Design (RCBD) with three replications and two seedlings per experimental unit, resulting in a total of 96 seedlings. The study results were statistically analyzed using the GENSTAT software, and the means were compared using the LSD test at a probability level of 0.05% (Al-Sahouki & Wahib, 1990).

Results and Discussion

Table (1) shows that the percentage of organic carbon in the soil was significantly affected by the addition of bacterial bio fertilizer. Treatment (B3) (*A. lipoferum* + *Streptomyces* spp.) significantly outperformed

the other bacterial fertilizer treatments, yielding the highest concentration of organic matter in the soil at 0.734%, compared to treatment B0 (without addition), which yielded the lowest percentage of organic carbon at 0.627%. The same table also shows that the addition of NPK chemical fertilizer to the potting soil significantly affected the percentage of organic carbon in the soil. Treatment F3 was observed to be superior, with an average of 0.769%, while the control treatment F0 gave the lowest average at 0.578%. Regarding the interaction between the two study factors, the results in **Table 2** show that treatment F3B2 exhibited the highest interaction between the chemical fertilizer and *Streptomyces* spp. bacteria, yielding the highest average at 0.930%, while the control treatment F0B0 had the lowest average of 0.471%.

Table 2 - Effect of biofertilizers, fish fertilizers, and mineral fertilizers on the percentage of organic matter in the soil (%)

Organic matter %					
Average	Bio fertilizer				Soil fertilizer
	B3	B2	B1	B0	
0.978	1.130	0.920	1.090	0.771	F0
1.294	1.250	1.260	1.420	1.244	F1
1.146	1.280	1.047	0.990	1.267	F2
1.327	1.283	1.600	1.350	1.073	F3
0.006**	0.013**				Lsd5%
	1.236	1.207	1.213	1.089	Average B
	0.006**				Lsd5%

- Decomposition of organic matter in the soil %:

Table 2 shows that the concentration of organic matter in the soil was significantly affected by the addition of bacterial bio fertilizer. Treatment (B3) (*A. lipoferm* + *Streptomyces* spp.) significantly outperformed the other treatments by giving the highest concentration of organic matter in the soil, reaching 1.236%, compared to treatment B0 without the addition, which gave the lowest concentration of organic matter, reaching 1.089%. The same table also shows that the addition of NPK chemical fertilizer to the potting soil significantly affected the concentration of organic matter in the soil. Treatment F3 was observed to be superior with an average of 1.327%, while the control treatment F0 gave the lowest average of 0.978%. As for the interaction between the two factors of the study, the results in **Table 3** showed the superiority of treatment F3B2 in the interaction between the mineral fertilizer and the *Streptomyces* spp. bacteria. B gave the highest average of 1.600%, followed by the F1B1 treatment (fish fertilizer concentration 300 g and *A. liporerm* bacteria) which gave an average of 1.420%, while the F0B0 control treatment gave the lowest average of 0.771%.

Table 3 shows that the rate of increase in carbon

Table 3 - Effect of biofertilizers, fish fertilizers and mineral fertilizers on carbon dioxide

CO ₂ ppm					
Average F	Bio fertilizer				Soil fertilizer
	B3	B2	B1	B0	
306.50	320.00	308.00	301.00	297.00	F0
359.75	370.00	369.00	352.00	348.00	F1
365.75	380.00	379.00	364.00	340.00	F2
387.25	390.00	388.00	386.00	385.00	F3
1.92**	3.83**				Lsd5%
	365.00	361.00	350.75	342.50	Average B
	1.92**				Lsd5%

Estimate the amount of carbon dioxide in the soil in mg/m³

dioxide emissions was significantly affected by the addition of bacterial bio fertilizer. Treatment (B3) with *A. lipoferm* + *Streptomyces* spp. significantly outperformed the other bacterial fertilizer treatments, giving the highest emissions at 365.00 ppm, compared to treatment B0 without the addition, which showed the smallest difference at 342.50 ppm. The same table also shows that the addition of NPK chemical fertilizer to the potting soil significantly affected emissions. Treatment F3 was observed to be superior, with an average of 387.25 ppm, while the control treatment F0 gave the lowest average at 306.50 ppm. Regarding the interaction between the two study factors, the results in **Table 4** show that treatment F3B3, in terms of the interaction between the chemical fertilizer and the combined bacterial fertilizer (containing two types of bacteria), gave the highest average. 390.00 ppm, while the comparison treatment F0 B0 gave the lowest average of 297.00 ppm.

Table 4 - Effect of biofertilizers, fish fertilizers, and mineral fertilizers on nitrous oxide emissions.

N ₂ O					
Average F	Bio fertilizer				Soil fertilizer
	B3	B2	B1	B0	
20.35	16.90	18.40	21.60	24.50	F0
16.33	15.10	17.20	16.60	16.40	F1
11.28	10.20	11.70	11.80	11.40	F2
7.90	6.30	7.30	8.40	9.60	F3
0.42**	0.83**				Lsd5%
	12.13	13.65	14.60	15.48	Average B
	0.42**				Lsd5%

Estimation of the amount of nitrous oxides in the soil (mg/m³)

Table 4 shows that the rate of increase in nitrous oxide emissions was significantly affected by the addition of bacterial bio fertilizer. The treatment *A. lipoferm* + *Streptomyces* spp.) (B3) significantly outperformed the other bacterial fertilizer treatments, exhibiting the smallest difference in emissions, with an average of 12.13 ppm, compared to treatment B0 without the addition, which produced the highest average emission at 15.48 ppm. The same table also shows that the addition of NPK chemical fertilizer to the potting soil significantly affected the nitrous oxide emission rate. Treatment F3 recorded the lowest

emission, with an average of 7.90 ppm, while the control treatment F0 produced the highest average at 20.35 ppm. Regarding the interaction between the two study factors, the results in **Table 5** show that the F3B3 treatment, representing the interaction between the chemical fertilizer and the combined bacterial fertilizer (containing two types of bacteria), significantly impacted the nitrous oxide emission rate. b. It gave the lowest emission with an average of 24.50 ppm, while the comparison treatment F0B0 gave the highest nitrous oxide gas emission with an average of 24.50 ppm.

Table 5 - Effect of biofertilizers, fish fertilizers, and mineral fertilizers on methane gas emissions.

CH ₄					
Average F	Bio fertilizer				Soil fertilizer
	B3	B2	B1	B0	
3.60	2.50	3.10	3.40	5.40	F0
1.95	2.00	2.20	2.10	1.50	F1
0.80	0.00	0.90	1.00	1.30	F2
0.00	0.00	0.00	0.00	0.00	F3
0.11**	0.22**				Lsd5%
	1.13	1.55	1.63	2.05	Average B
	0.11**				Lsd5%

Estimating the amount of methane in the soil in mg/g²

Table 5 shows that the methane emission rate was significantly affected by the addition of bacterial bio fertilizer. The treatment (A. *lipoferm* + *Streptomyces* spp.) (B3) significantly outperformed the other bacterial fertilizer treatments, exhibiting the lowest emission rate, with an average of 1.13 ppm, compared to treatment B0 without the addition, which had the highest emission rate, averaging 2.05 ppm. The same table also shows that the addition of NPK chemical fertilizer to the potting soil significantly affected the nitrous oxide emission rate. Treatment F3 recorded the lowest emission rate, averaging 0.00 ppm, while the control treatment F0 had the highest average, averaging 3.60 ppm. Regarding the interaction between the two study factors, the results in **Table 6** show that treatment F3B3, due to the interaction between the mineral fertilizer and the combined bacterial fertilizer (containing two types of bacteria), resulted in the lowest emission rate and zero emissions. The emission from the soil was at an average of 0.00 ppm, which is similar to the interference coefficients F2B3, F3B0, F3B1, F3B2, while the comparison treatment F0B0 gave the highest emission at an average of 5.40 ppm.

Table 6 shows that the carbon footprint was significantly affected by the addition of bacterial bio fertilizer. Treatment (B3) (A. *lipoferm* + *Streptomyces* spp.) significantly outperformed the other bacterial fertilizer treatments, giving the smallest difference in carbon footprint value, with an average of 3512.63 ppm,

Table 6 - Effect of biofertilizers, fish fertilizers, and mineral fertilizers on the carbon footprint.

CO ₂ /eq					
Average F	Bio fertilizer				Soil fertilizer
	B3	B2	B1	B0	
5636.75	4630.00	5158.80	5961.20	6797.00	F0
4566.15	4311.00	4845.60	4568.00	4540.00	F1
3256.93	3040.00	3452.70	3268.00	3267.00	F2
2483.25	2069.50	2322.50	2612.00	2929.00	F3
4.81**	9.63**				Lsd5%
	3512.63	3944.90	4102.30	4383.25	Average B
	4.81**				Lsd5%

Estimating the carbon footprint (ppm) in the soil.

compared to treatment B0 without the addition, which gave the highest carbon footprint with an average of 4383.25 ppm. The same table also shows that adding NPK chemical fertilizer to the potting soil significantly affected the carbon footprint. Treatment F3 recorded the lowest footprint with an average of 2483.25 ppm, while the control treatment F0 gave the highest average of 5636.75 ppm. Regarding the interaction between the two study factors, the results in **Table 7** show that treatment F3B3 showed the interaction between the mineral fertilizer and the combined bacterial fertilizer treatment. The two types of bacteria, B, gave the lowest fingerprint with an average of 2069.50 ppm, while the control treatment, F0B0, gave the highest fingerprint with an average of 6797.00 ppm.

Table 7 - Effect of bio fertilizers, fish fertilizers, and mineral fertilizers on the number of *Azospirillum* bacteria.

AZOSPIRILLUM LOG					
Average F	Bio fertilizer				Soil fertilizer
	B3	B2	B1	B0	
7.223	8.560	6.480	8.670	5.180	F0
7.450	8.600	6.350	8.320	6.530	F1
7.408	8.520	6.210	8.380	6.520	F2
7.503	8.810	6.280	8.660	6.260	F3
0.019**	0.038**				Lsd5%
	8.623	6.330	8.508	6.123	Average B
	0.019**				Lsd5%

Azospirillum *Lipoferum* bacteria counts, logarithm of colony formation unit g-1. Soil.

The results in Table 7 show that the addition of bacterial bio fertilizer significantly affected the logarithm of the total number of *Azospirillum* bacteria in the soil, especially the treatment with the addition of two types of bacteria (B3), which gave the highest average logarithm of bacteria, which reached 8.623 logarithms of colony-forming units per 1 g of soil. The addition of *Azospirillum* bacteria alone also significantly affected the mentioned trait, as the treatment B1 was superior in giving the highest average logarithm of bacteria, which reached 8.508 logarithms of colony-forming units per 1 g of soil, compared to the non-additional treatment B0, which gave 6.123 logarithms of colony-forming units per 1 g of soil. As can be seen from the same table, the interaction between the two study factors significantly affected the

bacterial logarithm, as the interaction treatment F3B3 was observed to be superior, giving the highest average bacterial logarithm of 8.810 logarithms of colony-forming units (CFU) of soil g⁻¹ compared to the interaction treatment for comparison F0B0, which gave the lowest average of 5.180 logarithms of CFU/g⁻¹ soil.

Table 8 shows that the addition of bacterial bio fertilizer significantly affected the log₁₀ total bacterial count in the soil, particularly the treatment with the addition of two B3 bacteria types, which yielded the highest average total bacterial count log₁₀ of 8.643 ¹² colony-forming units. The addition of *Azospirillum* bacteria alone also significantly affected this trait, with treatment B1 resulting in the highest average total bacterial count log₁₀ of 8.570 ¹² colony-forming units compared to treatment B0, which yielded an average of 8.398 ¹² colony-forming units. As can be seen from the same table, the F3 mineral fertilizer gave the highest average of 8.708 compared to the non-added F0, which had the lowest average of 8.363 logarithms of colony-forming units (CFU) per 1 g of soil. The interaction between the two study factors significantly affected the logarithm of the total bacterial count, as the F3B3 interaction treatment was observed to be superior, giving the highest average total bacterial count of 9.050 logarithms of CFU per 1 g of soil, compared to the F0B0 interaction treatment, which gave the lowest average of 8.180 logarithms of CFU per 1 g of soil.

Table 8 - Effect of biofertilizers, fish fertilizers, and mineral fertilizers on the total bacterial count.

Total bacteria LOG					
Average F	Bio fertilizer				Soil fertilizer
	B3	B2	B1	B0	
8.363	8.460	8.370	8.440	8.180	F0
8.403	8.380	8.290	8.410	8.530	F1
8.550	8.680	8.320	8.960	8.240	F2
8.708	9.050	8.670	8.470	8.640	F3
0.004**	0.008**				Lsd5%
	8.643	8.413	8.570	8.398	Average B
	0.004**				Lsd5%

Total bacterial count (log₁₀¹² colony-forming units) in soil.

Table 9 shows that the addition of bacterial bio fertilizer significantly affected the CFU log₁₀ of *Streptomyces spp.* in the soil, particularly the treatment with the addition of two bacterial types, B3, which yielded the highest average CFU log₁₀ of 8.800 g⁻¹. The addition of *Streptomyces spp.* alone also significantly affected this trait, with treatment B2 exhibiting the highest average CFU log₁₀ of 8.623 g⁻¹ compared to treatment B0, which yielded 6.100 g⁻¹. As shown in the same table, factor F3, which represents mineral fertilization, gave the highest average, reaching 7.540 logarithms of colony-forming units per 1 g of soil, compared to the absence

Table 9 -Effect of biofertilizers, fish fertilizers, and mineral fertilizers on the number of *Streptomyces* bacteria.

Streptomyces bacteria Log					
Average	Bio fertilizer				Soil fertilizer
	B3	B2	B1	B0	
7.405	8.700	8.920	6.240	5.760	F0
7.410	8.650	8.600	6.280	6.110	F1
7.498	8.960	8.520	6.330	6.180	F2
7.540	8.890	8.450	6.470	6.350	F3
0.026**	0.053**				Lsd5%
	8.800	8.623	6.330	6.100	Average B
	0.026**				Lsd5%

Streptomyces spp. colony-forming units (CFU) log₁₀ g⁻¹ in soil.

of addition, which reached 7.405 logarithms of colony-forming units per 1 g of soil. The interaction between the two study factors significantly affected the bacterial logarithm, as the interaction treatment of 300 g of fish fertilization and bacterial bio fertilizer, represented by two types of bacteria (F2B3), was observed to give the highest average bacterial logarithm, reaching 8.960 logarithms of colony-forming units per 1 g of soil, compared to the interaction treatment for comparison, F0B0, which gave the lowest average, reaching 5.760 logarithms of colony-forming units per 1 g of soil.

The results showed that organic carbon increased from 0.578% in F0 to 0.769% in F3, while organic matter reached its highest value at 1.600% in F3. This increase confirms that combining fish fertilizers with chemical fertilizers enhances soil fertility, which is consistent with a study conducted at the University of Diyala on wheat, where bio fertilization with fertilizers led to a significant increase in growth and grain yield (Suhail, 2022). The study also demonstrated that the use of *Azotobacter* and *Em1* improved soil fertility and increased morphological growth (Al-Omar et al., 1996).

N₂O emissions decreased from 21.6 ppm in F0 to 7.9 ppm in F2, and CH₄ emissions decreased from 3.6 ppm in F0 to 0.8 ppm in F2. These results support the findings of (Abobatta & El-Azazy, 2020) regarding the role of bio fertilizers in reducing environmental pollution and emissions. The CO₂ equivalent decreased from 6797 in F0 to 2299 in F3, consistent with the (Özbek & Gökdoğan, 2025) study demonstrating the potential for reducing the carbon footprint of kumquat production.

Azospirillum recorded the highest value at B1 (8.6 log), while *Streptomyces* reached 8.79 log at B2. These results are consistent with (Dimkpa et al., 2024) regarding the role of *Azospirillum* in improving nutrient uptake. The fishery treatment (F2) reduced N₂O from 21.6 ppm to 7.9 ppm. This aligns with a recent study showing that combining organic and nitrogen fertilizers reduces nitrous oxide emissions by improving soil nitrogen use efficiency (Xia, et al., 2024). CH₄ also decreased from 3.6 ppm in F0

to 0.8 ppm in F2. Bio fertilizers reduce methane emissions by promoting the activity of beneficial bacteria that contribute to the carbon cycle and its storage in the soil.

The CO₂ equivalent also decreased from 6797 in F0 to 2299 in F3, indicating a reduction in the carbon footprint. Agree that using modern fertilizer technologies such as bio fertilizers or nano fertilizers reduces carbon emissions and achieves higher productivity with a smaller environmental impact. Therefore, F2 fish fertilizer was the most effective in reducing N₂O and CH₄. F3 chemical fertilizer contributed to reducing the CO₂ equivalent but was less efficient at reducing N₂O and CH₄ compared to bio fertilizers. The F2 fish fertilizer treatment with B3 bacterial mixture was the most effective in reducing greenhouse gas emissions. N₂O emissions decreased from 21.6 ppm in F0 to 7.9 ppm in F2, and CH₄ decreased from 3.6 ppm in F0 to 0.8 ppm in F2. These results are consistent with (Xia, et al., 2024) stating that combining organic and nitrogen fertilizers reduces nitrous oxide emissions by improving nitrogen use efficiency, also demonstrated that Bio- and nano-fertilizers reduce methane and carbon dioxide emissions by promoting microbial activity in the soil.

The F3 chemical fertilizer treatment showed good results in increasing organic carbon to 0.769% and organic matter to 1.600%, in addition to reducing the CO₂ equivalent from 6797 in F0 to 2299 in F3. These results are consistent with the study by (Özbek & Gökdoğan, 2025), which demonstrated that improved fertilizer management reduces the carbon footprint of kumquat production. Although chemical fertilizer is effective in improving soil fertility, its effect on reducing N₂O and CH₄ was less than that of fish fertilizers.

Treatment F1 showed moderate improvement, with CO₂ concentration rising to 380 ppm, but it did not significantly reduce N₂O or CH₄ compared to F2. This suggests that fish fertilizers are more efficient at reducing harmful emissions, while chemical fertilizers are more effective at increasing organic matter.

Azospirillum bacteria recorded the highest value in treatment B1 at 8.6 log, compared to 5.18 log in B0. This reflects *Azospirillum*'s ability to fix atmospheric nitrogen and increase its availability to plants, thus improving growth and reducing the need for chemical fertilizers (Dimkpa et al., 2024). (Xia, et al., 2024) also showed that inoculating plants with *Azospirillum* leads to increased biomass and improved nutrient uptake. As for *Streptomyces* bacteria, the highest value was recorded at B2 at 8.79 log, while it was lower at B0 and B1 at around 6.1. *Streptomyces* bacteria are known for producing natural antibiotics that reduce root diseases and promote plant health. Their

high density also reflects a healthy soil environment rich in beneficial microorganisms (Abo El-Ala, 1998). The total bacterial count values ranged between 8.36 and 8.46 log in all treatments, indicating the stability of the soil bacterial community. This stability enhances the soil's ability to resist environmental changes and increases the efficiency of the nutrient cycle (Özbek & Gökdoğan, 2025).

The logarithmic data for bacterial counts clearly show a significant advantage when using bio inoculators loaded onto peat moss. The success of microbial colonization is attributed to the physical properties of the carrier material, which provided chemical and biological protection for *Azospirillum* strains against unfavorable soil conditions. Peat moss not only acted as a carrier but also provided a moisture-rich and porous microenvironment, allowing bacteria to maintain high population densities exceeding the critical threshold required for effective inoculation. This was confirmed by (Herrmann & Lesueur, 2013), who stated that organic carriers ensure the survival of inoculated microbes with high physiological activity in the rhizosphere. The results showed a strong correlation between increased *Azospirillum* bacteria populations and higher levels of available nitrogen, particularly at level B1. The effectiveness of these bacteria in fixing atmospheric nitrogen and converting it into mineral forms (NH₄) was enhanced by the presence of organic matter (fish manure and peat moss), which provided the necessary carbon sources for energy production required by the nitrogenase enzyme. Furthermore, the secretion of growth regulators by the bacteria improved the root system, thus increasing the efficiency of nitrogen uptake in the soil. This aligns with the findings of (Fukami, 2015), who indicated the role of *Azospirillum* bacteria in enhancing nitrogen availability. A significant increase in *Streptomyces* bacteria and the total bacterial count led to a substantial improvement in soil fertility. This microbial community, thanks to its high capacity for producing hydrolytic enzymes, mineralized complex organic matter, releasing organically bound sulfur and phosphorus. The numerical dominance of *Streptomyces* at balanced fertilization levels reflects the soil's ability to naturally cycle nutrients and biologically control pathogens, a finding corroborated by (Olanrewaju et al., 2019) regarding the role of this bacterium in enhancing plant growth and nutrient availability.

The interaction between levels of bio fertilizer (B) and mineral and fish fertilizer (F) created a balanced nutrient regime. The mineral component addressed immediate deficiencies, while the bio fertilizer (carried on peat moss) ensured the sustainability of soil fertility through

the continuous release of nutrients and enhanced rhizosphere activity. These results strongly support the use of integrated fertilization techniques to reduce reliance on chemical fertilizers and achieve sustainable agricultural productivity, consistent with the scientific principles established by (Brady & Weil, 2016) regarding nutrient cycling in agricultural ecosystems.

It is noteworthy that the treatments with the highest levels of *Streptomyces* bacteria were the same ones with the highest concentrations of available potassium. Fish manure acts as a rich energy source for decomposing bacteria (such as *Streptomyces*), which in turn accelerates the breakdown of organic matter and the release of associated potassium. Furthermore, increased dissolved organic matter increases the cation exchange capacity (CEC), preventing potassium fixation and making it available in the soil solution. This aligns with (Sultani et al., 2020) finding that the use of liquid organic waste (such as fish extracts) acts as a biofuel for microbes, thus accelerating nutrient cycling in the soil.

The bacterium *Azospirillum* recorded the highest value in treatment B1 at (8.6 log) compared to (5.18 log) in B0. This reflects *Azospirillum*'s ability to fix atmospheric nitrogen and increase its availability to the plant, thus improving growth and reducing the need for chemical fertilizers (Dimkpa et al., 2024). (Xia, et al., 2024) also showed that inoculating plants with *Azospirillum* leads to an increase in biomass and improved nutrient uptake. As for the bacterium *Streptomyces*, it also recorded the highest value in treatment B2 at (8.79 log), while it was lower in treatment B0 and B1 at around 6.1. *Streptomyces* is known for producing natural antibiotics that reduce root diseases and promote plant health. Its high density also reflects a healthy soil environment rich in beneficial microorganisms (Abo El-Ala, 1998). *Azospirillum* recorded the highest value in treatment B1 (8.6 log), while *Streptomyces* 8.79 log in B2, the highest value. These results are consistent with what (Dimkpa et al., 2024) stated regarding the role of *Azospirillum* in improving nutrient absorption.

The total bacterial count ranged from 8.36 to 8.46 log in all treatments, indicating the stability of the soil bacterial community. This stability enhances the soil's resistance to environmental changes and increases the efficiency of the nutrient cycle (Özbek & Gökdoğan, 2025). Increased numbers of *Azospirillum* and *Streptomyces* were associated with a clear improvement in kumquat seedling growth. The bacterial mixture (B3) provided a balanced environment that enhanced soil fertility and microbial community stability. These results are

consistent with literature confirming that high bacterial density is an indicator of the health and sustainability of the agricultural system [19]. The significant effects of the biological treatments (B), mineral fertilization, and fish manure (F) on increasing the concentration of CO₂ emitted from the soil. Treatment B3 (co-inoculation with *Azospirillum* and *Streptomyces*) recorded the highest averages compared to single inoculation or the control treatment. This increase is attributed to microbial synergism, whereby *Streptomyces* bacteria break down complex organic compounds through their hydrolytic enzyme secretions, creating a carbon-rich environment for *Azospirillum* bacteria. This intensive metabolic activity directly translates to increased soil respiration rates and CO₂ release. Regarding fertilization levels (F), the results showed that the use of fish manure (F1, F2) led to a significant increase in carbon emissions. This is explained by the fact that fish manure represents an organic substrate rich in readily available carbon and nitrogen, which stimulates the added and original microbial communities in the soil to engage in their biological activity. This is scientifically known as the stimulatory effect, where the addition of readily available organic matter stimulates microbes to attack and oxidize the organic matter in the soil (Lashammari, 2021).

The superior interaction between F3B3, which recorded the highest value (390.00 ppm), is consistent with the fact that the high availability of organic matter and organic carbon in these interactions created a large energy reserve. This reserve was utilized very efficiently by the bio-inoculation mixture, thus accelerating the carbon cycle in the rhizosphere. These results are consistent with the findings of (Al-Khafaji, 2020) and (Jassim, 2022), who concluded that the integration of organic fertilizers and bio stimulants increases microbial metabolic efficiency, thereby enhancing soil fertility and biological activity as measured by CO₂ emissions. Table 1 shows a significant advantage for treatment B3F3 in CO₂ emissions, reaching a value of 390.00 parts per million. This advantage is attributed to the complementary role of NPK mineral fertilization and the combined bio inoculation. The addition of mineral fertilizer at concentrations of 5, 2.5, and 5 g provided the essential elements (nitrogen, phosphorus, and potassium) required by the added microorganisms (*Azospirillum* and *Streptomyces*) for cell building and enzyme activation. The effect of two factors (F and B) on CO₂ emissions or concentration. Based on the data, the following can be concluded: Effect of factor (F): We observe an increase in CO₂ emissions. The average CO₂ values continued to increase as we moved

from level F0 to F3. Level F0 recorded the lowest average (306.50), while level F3 recorded the highest average (387.25). This increase indicates that higher levels of the F coefficient (which may represent fertilization or moisture levels) directly stimulate biological activity in the soil, thus increasing microbial respiration rates and CO₂ emissions. The effect of coefficient B: The results show a clear dominance of level B3 in all interactions with coefficient F. The overall average for B3 is the highest (365.00), while B0 is the lowest (342.50). This indicates a positive relationship between B levels and increased gas concentration. Interaction: The highest interaction value was recorded at F3/B3 (390.00 ppm), reflecting the synergistic effect. These results are consistent with scientific literature indicating that the addition of amendments or fertilizers (which may be denoted by treatments F and B) increases the availability of organic and mineral carbon in the soil. Regarding the effect of fertilization and microbial activity, studies confirm that the addition of organic matter or nitrogen fertilizers increases the rate of decomposition of the original organic matter in the soil by microbes, leading to an increase in CO₂ emissions (Al-Rawi, 2020).

Effect of Treatments (F) and Mineral Fertilization

We observed that the highest methane emission was in the control treatment F0 (no fertilization) at 3.60 ppm, while this value gradually decreased with the addition of fish manure (F1, F2) until it was completely nullified (0.00) in the mineral treatment F3 (NPK). NPK mineral fertilizer (especially nitrates) acts as an alternative electron acceptor for oxygen, thus inhibiting the activity of anaerobic methanogens. In kumquat soils, balanced fertilization appears to have improved soil aeration and reduced the redox potential to levels that inhibit methane formation (Al-Hadithi, 2021).

Treatment B3 (*Azospirillum* + *Streptomyces*) reduced methane emissions to 1.13 ppm compared to 2.05 ppm in the control treatment B0. This is attributed to the presence of *Azospirillum* and *Streptomyces*, which promotes rhizosphere health and increases nitrogen uptake efficiency, thus reducing anaerobic conditions that produce methane. *Streptomyces*, being an aerobic filamentous bacterium, competes with anaerobic methanogenic bacteria and shifts the microbial balance in favor of aerobic processes. The significant decrease in methane emissions when using the bio-mineral combination. This decrease in kumquat soil is attributed to the role of mineral fertilization F3 and bio fertilization B3 in improving the chemical and physical properties of the soil, which led to the inhibition of the methanogenesis process between the two factors in stimulating carbon

emissions.

Kumquats, as a type of citrus fruit, thrive in well-drained soils. Therefore, the addition of fish fertilizer and bio stimulants has been shown to increase stable organic matter, improving soil structure and reducing anaerobic micro sites responsible for methane production. These findings align with (Al-Saadi, 2021) study, which confirmed that adding bio-inoculations to fruit trees reduces anaerobic gas emissions by stimulating aerobic organisms in the root zone. (Aziz, 2023), in his research on kumquats, also indicates that balanced fertilization reduces soil environmental stress, which in turn reduces the carbon footprint of greenhouse gases such as methane.

The results demonstrated that kumquat (*Citrus japonica*) seedlings exhibited a superior environmental response when mineral or fish fertilizer was combined with double bio-inoculation (B3). The sharp decrease in methane (CH₄) emissions from 5.40 in the control to 0.00 in the advanced treatments is attributed to the role of *Azospirillum* and *Streptomyces* bacteria in improving rhizosphere aeration and increasing methane oxidation, which is consistent with what Fukami & Nakajima (2013) concluded in their thesis on the role of microbes in controlling gaseous cycles of horticultural crops.

Regarding nitrous oxide (N₂O), the results showed a significant reduction from 24.50 to 6.30. This decrease is explained by the increased nitrogen uptake efficiency of kumquat seedlings aided by the bio-inoculation, which reduced the denitrification processes that release the gas. This finding supports the study by (Bakshi, et al., 2023), which confirmed that biofertilizers in citrus act as a biological regulator preventing nitrogen gas loss, as well as what (Abdelkarim, 2023) indicated in his thesis on the role of bacterial inoculation in reducing the carbon load of citrus seedlings.

Despite the significant increase in CO₂ concentration per ppm (from 297.00 to 390.00), the scientific discussion clarifies that this increase is a positive indicator of active root respiration and a flourishing microbial biomass. (Borden et al., 2021) confirm this. (Al-Dulaimi, 2022) demonstrated in their thesis that organic fertilizers, such as fish manure, stimulate healthy microbial decomposition, thereby increasing respiratory CO₂ while reducing more harmful gases (CH₄, N₂O).

Ultimately, this synergy resulted in a reduction of the total carbon footprint (CO₂/eq (from 6797.00 to 2069.50). These findings align with the (IPCC, 2022) report, which emphasizes that managing the interaction between fertilization and microbial activity in orchards is key to

transforming these systems into carbon sinks. The study also demonstrates that 2.5-year-old kumquat seedlings possess high carbon neutrality potential when following a double pollination strategy (B3), which is consistent with the global trends in climate-smart agriculture discussed by (Wang et al., 2024).

Conclusion

In conclusion, the findings of this study provide compelling evidence that integrating organic fish manure with microbial bio-fertilizers represents a transformative shift toward sustainable pomology. By achieving a significant reduction in the carbon footprint—lowering the CO₂ equivalent from 6797.00 to 2069.50—this research underscores the viability of biological alternatives in mitigating greenhouse gas emissions without compromising seedling vigor. The synergistic interaction between *Azospirillum* and *Streptomyces* not only stabilized the soil's microbial community but also optimized nutrient uptake efficiency, as evidenced by the enhanced organic matter and carbon levels. Consequently, adopting the F2B3 treatment strategy offers a dual-benefit framework: promoting robust growth for kumquat seedlings while aligning agricultural practices with global climate-smart objectives. These results advocate for a policy shift toward bio-based fertilization to ensure long-term soil health and environmental sustainability in citrus orchards.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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