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ROLE OF ORGANIC, BIO AND MINERAL FERTILIZERS IN ENVIROMENTAL SUSTAINABILITY AND ENHANCING LETTUCE PRODUCTIVITY

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Abstract

This field experiment was conducted during the fall season of 2023 at Research Station 1, an affiliate of the College of Agriculture, University of Anbar. Three factors were used: organic fertilizer at two levels 1 and 2%, bacterial isolates (*Bacillus megaterium* + *Azotobacter chroococcum* and *Stenotrophomona maltopilia),* and mineral fertilizer at levels of 100, 75, 50 and 25%, in a randomized complete block design (RCBD) with three replicates. The results demonstrate the capability of bacterial isolates along with organic material to increase NPK availability in soil as well as optimize the yields and production of lettuce. Interaction treatment exceeded others by increasing N and P concentrations, with total yields and number of phosphate-dissolving and nitrogen-fixing bacteria attaining 133.61 mg N kg^{-1}, 29.67 mg P kg^{-1}, 61.38 Mg. h^{-1}, 7.843 log cfu. g^{-1} soil, and 6.962 log cfu g^{-1} soil, respectively. In addition to providing the lettuce plant with nutrition it reduced the amount of added mineral fertilizers by 50% from the recommended amount. It also achieved a positive role in reducing the concentration of nitrates in the lettuce leaves.

Keywords: Bio fertilizers, Organic fertilizers, Mineral fertilizers, Environmental sustainability, Lettuce.

دور الاسمدة العضوية والحيوية والمعدنية في الاستدامة البيئية وتحسين انتاجية الخس

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الخلاصة

أجريت تجربة حقلية في محطة ابحاث 1 لكلية الزراعة/ جامعة الأنبار الواقعة على خطي عرض 33.41 وطول 43.3 التابعة خلال الموسم الخريفي 2023 باستعمال ثلاثة عوامل: الأول السماد العضوي اضيف بمستوين 1 و2% للتربة والعامل الثاني عزلات بكتيرية *Bacillus megaterium* + *Azotobacter chroococcum* و *Stenotrophomona maltopilia* اما العامل الثالث فهو التسميد المعدني اضيف بأربع مستويات من التوصية السمادية (100% و75% و50% و25%)، نظمت تجربة عامليه بتصميم القطاعات العشوائية (RCBD) وبثلاث مكررات. اظهرت النتائج قدرة العزلات البكتيرية والمادة العضوية زيادة جاهزية عناصر NPK في التربة وتحسين نمو وانتاج نبات الخس، تفوقت معاملة التداخل الثلاثي بأفضل النتائج بزيادة تركيز النتروجين والفسفورفي التربة والحاصل الكلي وعدد البكتريا المذيبة للفوسفات والمثبتة للنتروجين اذ بلغت (133.61 ملغم N كغم تربة$^{-1}$، 29.67 ملغم P كغم تربة$^{-1}$، 61.38 ميكاغرام هـ$^{-1}$، 7.843 لوغاريتم وحدة تكوين مستعمرة غم تربة$^{-1}$، 6.962 لوغاريتم وحدة تكوين مستعمرة غم تربة$^{-1}$) على التتابع. اظهرت النتائج ايضا قدرة العزلات البكتيرية والاسمدة العضوية بتجهيز نبات الخس بالعناصر المغذية مع خفض كمية الاسمدة المعدنية المضافة بنسبة 50% من التوصية السمادية، كما حققت دور ايجابي في اختزال تركيز النترات في اوراق نبات الخس.

كلمات مفتاحية: التسميد الحيوي، الاستدامة البيئية، اسمدة عضوية، سماد المعدني، الخس.

Introduction

Excessive use of chemical fertilizers threaten the health of ecosystems and living organisms and can affect the diversity of beneficial plant organisms. It has also caused groundwater and soil pollution and increased greenhouse gas emissions (17). Research will help uncover sustainable solutions to increase crop production while promoting a sustainable environment. Various solutions have been proposed, including the use of organic fertilizers, which is one of the promising and innovative technologies for reducing the use of mineral fertilizers, improving nutrient absorption, promoting the productive and physiological state of the plant, overcoming obstacles, and enhancing interactions between the soil, microbes, and the plant (24).

Biofertilizers have proven the ability to restore environmental balance and achieve a healthy environment, as well as contribute to crops having good and healthy attributes and free of chemical pollutants. Many fertilizers are found in sustainable agricultural fields and have achieved improvements in the physical, chemical, fertility and biological characteristics of the soil by stabilizing some elements, such as nitrogen. Nitrogen helps in the dissolution of compounds containing phosphorus and potassium, facilitates nutrients necessary for plant nutrition, and secretes many enzymes, organic acids, growth regulators, chelating substances, and are bioinhibitors of some microscopic diseases (7, 16, 17 and 26). *Azotobacter* bacteria are used in biofertilization due to their ability to fix atmospheric nitrogen and increase its readiness for plants, with the rates of nitrogen fixed in the soil by them ranging from 0.3 to 15 kg N ha^{-1} annually, as well as reducing the amounts of added nitrogen fertilizers (1 and 23). The use of *B. megaterium* bacteria in biofertilization is due to their ability to dissolve phosphorus compounds and convert them into forms that are easy for the plant through multiple mechanisms. They also discharge organic acids in the rhizosphere that contribute to lowering pH, produce organic compounds and secrete growth regulators that reflect positively on plant growth and productivity (2). It was found that the bacteria *Stenotrophomonas maltopilia* colonized the vicinity of the plant tissue and its roots in the rhizosphere of the soil, fixed atmospheric nitrogen freely, and worked to dissolve phosphate compounds as a result of its secretion of particular organic acids. It produces phosphatase and phytase enzymes and form chelating compounds with humic acids resulting from their decomposition of organic matter in the environment of the plant roots. This reflects positively in plant productivity (3).

Lettuce are among the crops that are sensitive to nitrogen fertilization, as production increases with increasing fertilization. However, the use of nitrogen fertilizers in large quantities may lead to the accumulation of nitrates in the plant, creating various health issues for users, in addition to increasing the loss of nitrogen from the soil due to washing that reaches the separators and the resulting damage to the environment (13). Therefore, this study aimed to promote sustainable agricultural development by regulating the addition of chemical fertilizers through the use of organic and microbial fertilizers and benefiting from their interactions in achieving good productivity, reducing nitrate accumulation in lettuce plants, and achieving balanced and beneficial microbial diversity in the soil.

Materials and Methods

A field experiment was carried out during the 2023 fall season at Research Station 1 of the College of Agriculture in the city of Ramadi of Anbar Governorate. It involved adding organic fertilizer and biofertilizers (*chroococcum Bacillus megaterium* and *Azotobacter, Stenotrophomonas melophilia*) to the lettuce plants using soil with a sandy mixture texture. Its characteristics were estimated according to (20) (see Table 1).

Table 1: Some chemical and physical characteristics of field soil.

Characteristic	Value	Unit
EC_e	**1.71**	**ds m^{-1}**
pH	**7.35**	
O.M.	**5.30**	**g Kg^{-1}**
Sand	**687**	**g Kg^{-1}**
Silt	**202**	
Clay	**111**	
Soil Texture	**Loamy sand**	
Bulck density	**1.33**	**Mg m^{-3}**
Available N	**45.2**	**mg Kg^{-1}**
Available P	**10.83**	
Available K	**160.30**	
CEC	**17.45**	**Cmole Kg^{-1}**
Total bacteria	**10^6 * 2.6**	**cfu g^{-1} Soil**
Total fungi	**10^3 *1.3**	

The land was prepared by plowing with a rotary plow, smoothed using a hoe, and performing the necessary leveling process for the soil in a good and homogeneous manner. The field was divided into three sectors, each containing 16 experimental units of 7.5 m^2 each, with dimensions of 3 x 2.5 m and spaced 1 m apart. Organic fertilizer comprising mushroom farm waste (Table 2) was added to the experimental units, at two levels of 1 and 2%.

Mineral fertilizer treatments were added according to the fertilizer recommendation, consisting of 52 Kg P ha^{-1} and 60 Kg K ha^{-1} (5) while nitrogen fertilizers were added at a level of 200 Kg N ha^{-1} as recommended (4) at a rate of 25, 50, 75, and 100%. Di-ammonium phosphate fertilizer (DAP 20% P and 18% N) was mixed with the soil according to the treatment level, and potassium sulfate (50% K2O) added according to the treatment level in two batches, the first mixed with the soil during land preparation and the second fed to the plant 21 days after planting. Urea fertilizer (46%N) was also added according to the level of treatments and the amount of nitrogen from DAP fertilizer in three batches. The first DAP fertilizer was mixed with the soil at preparation, and the second and third after 21 and 35 days of planting. Bacterial fertilizers were added in two treatments during planting by contaminating the roots of the lettuce seedlings by dipping them in vaccines of isolates prepared according to the treatments.

The first treatment was a mixture of two vaccines *B. megaterium*, *A. chroococcum*, and the second *S. maltopilia* inoculum, obtained from the Soil Microbiology Laboratory in the Department of Soil and Water Resources, College of Agriculture, Anbar University. The isolates were activated in the Nutrient Broth medium and prepared at a density of 10^8 cfu ml^{-1}. Lettuce seedlings (FAJR) were planted on 10/10/2023 in four lines of 9 plants each in each experimental unit. The irrigation process was carried as crop needed, using the surface drip irrigation method, and compensating for any loss from the evaporation basin. Crop service operations were carried out whenever necessary. The plants were harvested on 12/2/2023, and samples were taken from them and the soil for measurement and analysis in the College of Agriculture laboratories.

Table 2: Used organic fertilizer characteristics.

Characteristic	Value	Unit
EC (1:10)	3.9	dsm^{-1}
PH	6.61	
N	1.58	%
P	0.35	
K	1.11	
C	35.6	
C:N	22.53	
Total bacteria	2.3*107	cfu g^{-1}
Total fungi	1.4*103	

The content of N, P, and K in the soil after harvest was estimated (20). Nitrates were determined in fresh leaves using the Kjeldahl device according to (29) while phosphate-dissolving bacteria were estimated by the method of dilution and plate-counting by growing them on the Pikovskaya medium according to (20). As for nitrogen-fixing bacteria, they were able to extract yeast from the mannitol sugar (9). The results were analyzed statistically by analysis of variance (ANOVA) at a probability level of 0.05 using the Genstat V. 12.1 program.

Results and Discussion

Effect of treatments on concentrations of N and P elements in the soil after harvest: The statistical analysis show that adding organic fertilizer (O) led to a significant increase in nitrogen and phosphorus availability in the soil (see Tables 3 and 4). Organic fertilizer (O2) at the 2% level treatment was superior reaching 113.63 and 24.88 mg kg^{-1} soil, respectively which was 25 and 27.2% higher than the 1% addition (O1) of 90.86 and 19.56 mg kg^{-1} soil, respectively. The results also show a significant effect of adding bacterial inoculants (B) on available nitrogen and phosphorus. The *S. maltopilia* (B1) treatment was superior in providing nitrogen and phosphorus, amounting to 103.16 and 22.63 mg kg^{-1} soil, respectively, while the *A.chroococcum* + *B.megatrium* (B2) treatment gave 101.32 and 21.8 mg kg^{-1} soil, an increase of 1.8 and 3.8%, respectively. Conversely, significant differences were found in the mean levels of mineral fertilizer (F), as the 100% addition treatment exceeded the fertilizer recommendation (F4) by providing nitrogen and phosphorus concentrations that reached 116.9 and 26.67 mg kg^{-1} soil, showing increased rates of 43.4 and 52.1%, 20 and 23.8%, and 3.5 and 9%, respectively, compared to the 25, 50 and 75% addition treatment of the F1, F2, and F3 fertilizer recommendation at 81.53 and 17.27, 97.48 and 21.22, and 113 and 24.10 mg kg^{-1} soil, respectively.

Regarding the bilateral interaction between organic fertilizer and bacterial inoculum (OB) (Tables 3 and 4), the O2B2 treatment was significantly superior to the other treatments, with the available nitrogen and phosphorus content reaching 119.00 and 25.84 mg kg^{-1} soil, while that for O1B2 reached 83.65 and 17.77. mg kg^{-1} soil, an increase of 42.3 and 45.4%, respectively. The binary interaction between organic and mineral fertilizer (OF) also show the O2F4 treatment being significantly superior providing nitrogen and phosphorus that reached 128.6 and 28.84 mg kg^{-1} soil, compared to the lowest O1F1 treatment reached 73.97 and 15.65 mg kg^{-1} soil, an increase of 73.9 and 62.8%, respectively. As for binary interactions between mineral fertilizer and bacterial inoculum, the B1F4 treatment was significantly superior to the others, providing nitrogen and phosphorus that reached 118.04 and 26.22 mg kg^{-1} soil, 42.9 and 62.8% above the lowest treatment of B2F1 at 82.59 and 18.44 mg kg^{-1} soil.

Statistical analysis of the triple interaction (OBF) also show major differences in available nitrogen and phosphorus values with the O2B2F4 treatment offering the highest amounts at 133.61 and 29.67 mg kg^{-1} soil, respectively, compared to the lowest treatment (O1B2F1) at 67.76 and 13.6 mg kg^{-1} soil, while the triple interaction treatment (O2B2F2) provided values of 117.7 and 26.56 mg kg^{-1} soil. These came close to the full fertilizer recommendation treatment (F4) which had available nitrogen and phosphorus amounting to 116.95 and 26.27 mg kg^{-1} soil, respectively. Hence, the use of mineral fertilization can be reduced by 50%.

Table 3: Effect of treatments on available nitrogen in soil after harvest (mg N kg^{-1} soil).

Organic Fertilizer (O)	**Biofertilizer (B)**	**Mineral Fertilizer (F)**				**Mean (O*B)**
		F1	F2	F3	F4	
O1	B1	80.17	90.55	109.05	112.49	98.07
	B2	67.76	76.19	92.53	98.11	83.65
O2	B1	85.01	105.48	118.92	123.6	108.26
	B2	93.19	117.7	131.49	133.61	119.00
L.S.D$_{O*B*F}$		3.323				1.662
Mean for Mineral Fertilizer (F)		81.53	97.48	113	116.95	
L.S.D$_F$		1.662				
Mean for Organic Fertilizer (O)		O*F				Mean (O)
		F1	F2	F3	F4	
O1		73.97	83.37	100.79	105.3	90.86
O2		89.1	111.59	125.21	128.6	113.63
L.S.D$_{O*F}$		2.350				1.175
Mean for Biofertilizer (B)		B*F				Mean (B)
		F1	F2	F3	F4	
B1		82.59	98.01	113.99	118.04	103.16
B2		80.47	96.94	112.01	115.86	101.32
L.S.D$_{B*F}$		2.350				1.175

Table 4: Effect of treatments on available phosphorous in soil after harvest (mg P kg^{-1} soil).

Organic Fertilizer (O)	Biofertilizer (B)	Mineral Fertilizer (F)				Mean (O*B)
		F1	F2	F3	F4	
O1	B1	17.65	19.54	23.56	24.65	21.35
	B2	13.65	15.56	19.10	22.76	17.77
O2	B1	19.22	23.23	25.20	28.00	23.91
	B2	18.55	26.56	28.55	29.67	25.84
$L.S.D_{O*B*F}$		0.596				0.298
Mean for Mineral Fertilizer (F)		17.27	21.22	24.10	26.27	
$L.S.D_F$		0.298				
Mean for Organic Fertilizer (O)		O*F				Mean (O)
		F1	F2	F3	F4	
O1		15.65	17.55	21.33	23.71	19.56
O2		18.89	24.89	26.88	28.84	24.88
$L.S.D_{O*F}$		0.421				0.210
Mean for Biofertilizer (B)		B*F				Mean (B)
		F1	F2	F3	F4	
B1		18.44	21.38	24.38	26.33	22.63
B2		16.10	21.06	23.83	26.22	21.80
$L.S.D_{B*F}$		0.421				0.210

Tables 3 and 4 clearly show that adding organic, mineral fertilizer, and inoculum consisting of *S. maltopilia* bacteria and *A. chroococcum* + *B. megaterium* individually, or their binary and triple interactions, significantly affected available means of nitrogen and phosphorus in the soil, with the highest values achieved by O2B2F4 treatment. This is attributed to the role of organic fertilizers, which improve the physical, chemical and fertility properties of soil, and increase microbial activity, as reflected in the augmented nutrient availability for plants (6 and 11). Moreover, organic fertilizer functions to increase microbial organism activity and improve soil structure along with increasing the ability to retain water, leading to higher availability of the necessary elements, especially nitrogen and phosphorus (10). The addition of bacterial inoculum significantly affected the increase in available nitrogen and phosphorus in the soil after planting due to the role of the *S. maltophilia* bacteria in fixing free atmospheric nitrogen in the soil and providing it in an available form (21). The *A. chroococcum* bacteria also fixes nitrogen freely, in addition to its ability to produce growth regulators and increase nitrogen values in the soil (22). The higher availability of phosphorus means in the soil is due to the interactive effect between the bacteria *S. maltophilia* and *B. megaterium* in the process of dissolving phosphate compounds. Consequently, it has a direct effect on the production of some organic acids and the phosphatase enzyme, which contributes to the mineralization of organic phosphorus and phytase (27).

Effect of treatments on the concentration of NO_3 in leaves: Table 5 show that organic fertilization has a major role in reducing nitrate concentrations in leaves. The lowest NO_3 content, amounting to 1941 mg kg^{-1}, was recorded with the O1 treatment, while the O2 gave a mean of 2098 mg kg^{-1}. Adding bacterial inoculum significantly reduced mean NO_3 concentrations, with the B1 treatment registering 1896 mg kg^{-1}, 11.5% less than the 2142 mg kg^{-1} by the B2 treatment. Adding mineral fertilizer caused a significant difference between the means, as adding 100% of the recommended fertilizer amounts offered the highest mean NO_3 at 2373 mg kg^{-1}, while it was 1782, 1877 and 2046 mg kg^{-1}, respectively at 25, 50 and 75%.

The binary interaction between organic fertilizer and the bacterial inoculum show that the O1B1 treatment was superior giving it the lowest concentration of NO_3 at 1848 mg kg^{-1}, a 17.8% decline compared to the 2250 mg kg^{-1} of the O2B2 treatment. For the binary interactions between organic and mineral fertilizers there was marked superiority in the O1F1 treatment with the lowest NO_3 concentration at 1711 mg kg^{-1}, a 29.3% decrease over the O2F4 treatment at 2422 mg kg^{-1}. For bacterial and mineral inoculums, the B1 treatment excelled in all interactions with the mineral fertilizer in reducing the mean NO_3 in leaves compared to the B2 treatment. The B1F4 treatment gave a mean NO_3 amounting to 2241. mg kg^{-1}, a decrease of 10.5% compared to the B2F4 treatment at 2504 mg kg^{-1}.

There were significant differences in the NO_3 means for the triple organic, bacterial, and mineral fertilizer interactions. The O1B1F1 treatment lead the others giving a mean nitrates concentration of 1628, 36.3% less than the 2555 mg kg^{-1} mg of the O2B2F4 treatment and 21.6% less than the 2077 mg kg^{-1} of the O2B2F2 compared to the full fertilizer recommendation treatment of 100% which gave a nitrate concentration of 2373 mg kg^{-1}.

Table 5: Effect of fertilizer treatments on NO3 concentrations in leaves (mg kg^{-1}).

Organic Fertilizer (O)	Biofertilizer (B)	Mineral Fertilizer (F)				Mean (O*B)
		F1	F2	F3	F4	
O1	B1	1628	1731	1838	2193	1848
	B2	1794	1882	2010	2453	2035
O2	B1	1678	1817	1996	2289	1945
	B2	2028	2077	2340	2555	2250
L.S.D$_{O*B*F}$		128.3				64.2
Mean for Mineral Fertilizer (F)		1782	1877	2046	2373	
L.S.D$_F$		64.2				
Mean for Organic Fertilizer (O)		O*F				Mean (O)
		F1	F2	F3	F4	
O1		1711	1807	1924	2323	1941
O2		1853	1947	2168	2422	2098
L.S.D$_{O*F}$		90.8				45.4
Mean for Biofertilizer (B)		B*F				Mean (B)
		F1	F2	F3	F4	
B1		1653	1774	1917	2241	1896
B2		1911	1980	2175	2504	2142
L.S.D$_{B*F}$		90.8				45.4

Table 5 demonstrates that adding organic and bacterial fertilizers improved the qualitative characteristics and yield of the plant by reducing nitrate concentrations in the leaves. This may be due to their role in supplying the plant with nitrogen in the form of NH4, which reduces the absorption of nitrate forms and their accumulation in the plant leaves and reduces the activity of bacteria that biologically oxidize ammonia to nitrates in the plant atmosphere. This allows good growth without accumulation of any substance over the permissible limits in the plant. Therefore, the availability of organic and microbial fertilizers is a possible solution for reducing nitrate content in plants while promoting sustainable agricultural practices (28).

As for the high concentration of nitrates, the addition of mineral fertilizer is attributed to the rapid dissolution of this fertilizer, especially the nitrogenous, which produces ammonium in relatively large quantities. A large portion of it is biologically oxidized by nitrification bacteria and turns into NO3 which is absorbed by plants in larger quantities due to its ease of absorption. It then accumulates in plant leaves tissues, which the plant prefers to achieve osmotic balance with the roots, due to increased salinity or mineral fertilizer compounds. Such dissolution in the root environment is at a greater extent than in the case of organic fertilizers, which release ammonium gradually (14). Likewise, the decrease in nitrate concentration is attributed to the role of the bacterial inoculum in secreting numerous growth regulators and enzymes, most prominent being the nitrate reductase enzyme, as well as reducing the activity of nitrifying bacteria (25).

Effect of fertilizer treatments on total yield (Mg ha^{-1}): Table 6 shows that adding organic fertilizer leads to a significant increase in the mean total yield of the plant, with the O2 treatment outperforming at 54.83 Mg ha^{-1}, an increase of 13% over the O1 treatment which gave 48.49 Mg ha^{-1}. A significant effect of adding biofertilizers also appeared, as the B1 biofertilizer treatment gave a mean total yield of 52.20 Mg ha^{-1}, while the B2 treatment gave 51.12 Mg ha^{-1}, an increase of 2.1%. On the other hand, the analysis reveals significant differences between the mineral fertilizer means, as the addition treatment (100%) gave a mean total yield of 56.56 Mg ha^{-1}, while the 25, 50 and 75% treatments had 42.09, 53.13, and 54.86 Mg ha^{-1} respectively, reflecting increases of 34.4, 6.4, and 3.1 %, respectively.

Regarding the dual interaction between organic fertilizers and biofertilizers, the O2B2 treatment was significantly superior providing the highest mean total yield of 56.50 Mg ha^{-1} a 23.5% increase over the 45.75 Mg ha^{-1} of the O1B2 treatment. As for the interaction of organic and mineral fertilizers combination, the O2F4 treatment was superior with a total mean yield of 59.34 Mg ha-1 compared to the O1F1 treatment, which gave 38.50 Mg ha^{-1}, an increase of 54.1%. As for the binary interaction between bio-mineral fertilizer, the B1F4 treatment was significantly superior giving a mean total yield of 56.82 Mg ha^{-1} a 37.3% increase over the B2F1 at 41.39 Mg ha^{-1}.

The statistical analysis of the triple interaction treatments show significant differences between the means of the total yield, with the O2B2F4 giving the highest at 61.38 Mg. ha^{-1}. The O2B2F2 achieved a mean total yield of 58.43 Mg ha^{-1}, an increase of 3.3% over the full fertilizer recommendation treatment which yielded 56.56 Mg ha^{-1}. Thus, the amount of mineral fertilization required can be reduced by 50%.

It is clear from Table 7 that adding organic, biological, and mineral fertilizers and their dual and triple interactions significantly affect mean total yield. The highest yield was recorded in triple interaction treatment involving the addition of 2% organic matter with the double biofertilizer *A.Chroococcum* + *B.megatrium* which outperformed the fertilizer recommendation by 100%. This is due to the role of the organic matter in improving physical, chemical, fertility and biological properties as seen in the soil's greater ability to retain water and improves soil aeration, in addition to containing some important elements for plants, most notably nitrogen, phosphorus, and potassium.

Moreover, it increases the activity and effectiveness of microorganisms present in the soil. Decomposed organic matter contain many growth regulators such as auxin, gibberellin, cytokinin, vitamins and amino acids, which enhance plant growth and production through their effect on cell elongation, and increasing the plant's ability to absorb nutrients (8). The previously mentioned biofertilizers also work to improve the soil's fertility and productivity properties, as well as its organic matter and nitrogen content and dissolving some compounds, such as those containing phosphorus. This they achieve by secreting organic acids and improving root growth and development, as well as augmenting the absorption of nutrients, which reflect positively in increased yields (15).

Table 6: Effect of fertilizer treatments on total yield (Mg ha^{-1}).

Organic Fertilizer (O)	Biofertilizer (B)	Mineral Fertilizer (F)				Mean (O*B)
		F1	F2	F3	F4	
O1	B1	40.52	53.39	54.74	56.34	51.25
	B2	36.47	46.30	48.98	51.23	45.75
O2	B1	45.07	54.42	55.86	57.31	53.16
	B2	46.32	58.43	59.85	61.38	56.50
L.S.D$_{O*B*F}$		0.778				0.389
Mean for Mineral Fertilizer (F)		42.09	53.13	54.86	56.56	
L.S.D$_F$		0.389				
Mean for Organic Fertilizer (O)		O*F				Mean (O)
		F1	F2	F3	F4	
O1		38.50	49.84	51.86	53.78	48.49
O2		45.69	56.42	57.85	59.34	54.83
L.S.D$_{O*F}$		0.550				0.275
Mean for Biofertilizer (B)		B*F				Mean (B)
		F1	F2	F3	F4	
B1		42.79	53.90	55.30	56.82	52.20
B2		41.39	52.36	54.41	56.31	51.12
L.S.D$_{B*F}$		0.550				0.275

Effect of different fertilizer treatments on the logarithm of the phosphate-dissolving bacteria numbers in soil: Table 7 shows that organic fertilization has a major role in increasing the number of bacteria that dissolve compounds containing phosphorus. This is seen in the treatment of adding organic fertilizer O2 which significantly exceeded the mean logarithm of the bacterial population by log 7.715 colony forming units (cfu) g^{-1} soil, an increase of 2.1% compared to the O1 treatment of log 7.557 cfu g^{-1} soil. Conversely there were no significant differences between the biofertilization treatments in the mean logarithm of the number of phosphate-dissolving organisms. The results show that adding mineral fertilizer produced significant differences between the means, with the 100% recommended fertilizer addition giving a mean logarithm of the number of bacteria at log 7.708 cfu g^{-1} soil, a 2% increase over the 25% addition rate at log 7.557 cfu g^{-1} soil.

Further, the results of the dual interaction between organic fertilizer and biofertilizer showed the O2B2 treatment being significantly superior at a mean log of 7.739 cfu g^{-1} soil, an increase of 2.7% over the O1B2's 7.535 cfu g^{-1} soil. The interaction between the two fertilizers also showed the O2F4 treatment being considerably better at a mean log of 7.798 cfu g^{-1} soil, compared to the lowest O1F1 treatment at log 7.500 cfu g^{-1} soil, an increase of 4%. However, there are no significant differences between the mean numbers of phosphate-dissolving bacteria when interacting with the biological and mineral fertilizers.

The triple interaction between organic, biological and mineral fertilizers showed significant differences between the mean bacteria numbers with O2B2F4 treatment giving the highest at log 7.843 cfu g^{-1} soil, 4.9% above the lowest treatment of O1B2F1 at log 7.480 cfu g^{-1} soil.

Table 7: Effect of different fertilizer treatments on the number of phosphate-dissolving bacteria in soil (log cfu g^{-1} soil).

Organic Fertilizer (O)	Biofertilizer (B)	Mineral Fertilizer (F)				Mean (O*B)
		F1	F2	F3	F4	
O1	B1	7.520	7.560	7.600	7.633	7.578
	B2	7.480	7.500	7.560	7.600	7.535
O2	B1	7.593	7.693	7.727	7.753	7.692
	B2	7.633	7.727	7.753	7.843	7.739
$L.S.D_{O*B*F}$		0.104				0.018
Mean for Mineral Fertilizer (F)		7.557	7.620	7.660	7.708	
$L.S.D_{F}$		0.018				
Mean for Organic Fertilizer (O)		O*F				Mean (O)
		F1	F2	F3	F4	
O1		7.500	7.530	7.580	7.617	7.557
O2		7.613	7.710	7.740	7.798	7.715
$L.S.D_{O*F}$		0.073				0.036
Mean for Biofertilizer (B)		B*F				Mean (B)
		F1	F2	F3	F4	
B1		7.557	7.627	7.663	7.693	7.635
B2		7.557	7.613	7.657	7.722	7.637
$L.S.D_{B*F}$		N.S				N.S

The effect of different fertilizer treatments on the logarithm of number of nitrogen-fixing bacteria in soil: The statistical analysis evidenced that organic fertilizer plays a majority role in raising the number of nitrogen-fixing bacteria (Table 8). Adding organic fertilizer (O2) was superior with a mean bacteria number of log 6.751 cfu g^{-1} soil, 3.16% over the O1 treatment which had log 6.544 cfu g^{-1} soil. Adding biofertilizer also significantly increased the logarithm of the number of nitrogen-fixing bacteria. The B2 treatment was superior, giving a mean of log 6.855 cfu g^{-1} compared to the B1 which had 6.440 cfu g^{-1} soil. A significant mean difference was also seen when mineral fertilizer was added, with the recommended 100% fertilizer addition providing a log 6.703 cfu g^{-1} soil compared to log 6.573 cfu g^{-1} soil for the 25% addition, an increase of 2%.

The dual interaction between organic fertilizer and biofertilizer under O2B2 treatment at log 6.906 cfu g^{-1} soil significantly outperformed the O1B1 treatment which had the lowest mean number of bacteria at log 6.284 cfu. g^{-1} soil. Also, the O2F4 significantly surpassed other treatments at log 6.814 cfu. g^{-1} soil compared to the lowest treatment for O1F1 at log 6.476 cfu g^{-1} soil, a 5.2% increase. For biofertilizer and mineral fertilizer interactions, the B2F4 treatment exceeded others with a mean bacteria number of log 6.913 cfu g^{-1} soil compared to the log 6.360 cfu g^{-1} soil for the B1F1 treatment.

For the triple organic, biological, and mineral fertilizers interactions, significant differences were found in the mean logarithms for bacteria numbers. The O2B2F4 treatment had the highest mean bacteria number at log 6.962 cfu g^{-1} soil compared to the lowest for O1B1F1 at log 6.201 cfu g^{-1} soil.

Table 8: Effect of different fertilizer treatments on the number of nitrogen-fixing bacteria in soil (log cfu g^{-1} soil).

Organic Fertilizer (O)	Biofertilizer (B)	Mineral Fertilizer (F)				Mean (O*B)
		F1	F2	F3	F4	
O1	B1	6.201	6.259	6.36	6.318	6.284
	B2	6.752	6.774	6.823	6.864	6.803
O2	B1	6.519	6.56	6.634	6.667	6.595
	B2	6.823	6.916	6.925	6.962	6.906
L.S.D$_{O*B*F}$		0.192				0.096
Mean for Mineral Fertilizer (F)		6.573	6.627	6.685	6.703	
L.S.D$_F$		0.096				
Mean for Organic Fertilizer (O)		O*F				Mean (O)
		F1	F2	F3	F4	
O1		6.476	6.517	6.591	6.591	6.544
O2		6.671	6.738	6.779	6.814	6.751
L.S.D$_{O*F}$		0.136				0.068
Mean for Biofertilizer (B)		B*F				Mean (B)
		F1	F2	F3	F4	
B1		6.360	6.410	6.497	6.492	6.440
B2		6.787	6.845	6.874	6.913	6.855
L.S.D$_{B*F}$		0.136				0.068

As Tables 8 and 9 show, adding organic matter and bacterial inoculum consisting of individual, dual or triple interactions significantly affected the number of bacteria that fix nitrogen and dissolve compounds containing phosphorus, especially at the triple interactions and at the highest levels. This has an important role in decomposing organic matter and providing the main nutrients to the plant. This increase may be due to the addition of organic fertilizers and because these organisms are heterotrophic, using this fertilizer as a source of energy and carbon thereby increasing their numbers in the area. The rhizosphere and organic fertilizer reduce the degree of pH to a suitable level for these organisms (19). Nutrients are also important for building their bodies thus leading to an increase in their numbers following increased mineral fertilization (12).

Conclusions

This study found an improvement in the quality of lettuce yield and a reduction in nitrates in the leaves within the recommended limits as a result of the use of integrated fertilization (organic fertilizer, *S. maltopilia bacteria* and *B. megatrium* + *A. chroococcum* in addition to mineral fertilization). Further, the 50% reduction in fertilizer required over the recommended amounts due to these treatments not only contribute to lower production costs but also help mitigate environmental pollution.

Supplementary Materials:

No Supplementary Materials.

Author Contributions:

Author Jassim Mohammed Mahmood methodology, writing—original draft preparation, Author and Author Waqas Al-Joboory and Idham Ali Abed writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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The authors declare no conflict of interest.

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