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The Effect of Biofertilization, Organic Nutrient Spraying and Glutamic Acid on Some Lettuce Traits

Ayad Ali Hunaish Al-Samah *[1], Ridha Mustafa Abdul-Hussein Al-Ubaidy [2]
*[1]Shatra Technical College - Southern Technical University. [2] Department of Horticulture and Landscape Gardening - College of Agricultural Engineering Sciences - University. of Baghdad.

ABSTRACT

A field experiment was conducted over two agricultural seasons, in a field of the Organic Fertilizers Project located in the Bani Zaid district of Shatra, under the Directorate of Agriculture of Dhi Qar. The factorial experiment included three factors conducted within a Randomized Complete Block Design (R.C.B.D) with three replications, 36 treatments per replicate. The study aimed to evaluate the effects of reducing mineral fertilizer application by 50% on lettuce (*Lactuca sativa* L.) growth, focusing on the combined impact of biofertilization with mycorrhiza and *Azospirillum*, along with foliar applications of an organic nutrient solution (Forteorgan) and glutamic acid. Conducted over two agricultural seasons (2022-2023 and 2023-2024). The results indicated that the combined treatment of mycorrhiza and *Azospirillum* inoculation, coupled with foliar applications of 4 ml L^{-1} Forteorgan and 500 mg L^{-1} glutamic acid, significantly enhanced leaf nitrogen percent in both seasons. Additionally, this combination improved leaf phosphorus and potassium percent, as well as total chlorophyll concentration, suggesting that integrating biofertilizers with reduced mineral fertilizer application can effectively promote nutrient uptake and photosynthesis in lettuce plants.

Key words: Azospirillum, *Lactuca sativa*, Mycorrhiza, Reduced mineral fertilizer,

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INTRODUCTION

Lettuce (*Lactuca sativa* L.) belongs to the Asteraceae family, one of the largest plant families (Shi et al., 2022.). Lettuce is one of the most widely consumed leafy vegetable crops globally (Peiris and Weerakkody, 2015). It has shallow roots and requires high fertilization, particularly nitrogen. Nitrogen plays a critical role in amino acid synthesis, protein formation, plant growth, chlorophyll production, and increasing lettuce yield (Sukor, 2013). Lettuce, being a leafy vegetable, demands substantial nitrogen fertilization. However, the excessive use of mineral fertilizers in agricultural areas leads to groundwater and surface water contamination, highlighting the need for alternative techniques to reduce mineral fertilizer usage without compromising yield (Pandorf, et al., 2020). Biofertilization is considered one of the modern techniques to reduce the excessive use of mineral fertilizers and minimize pollution sources. This technique involves the use of beneficial microorganisms to enhance soil properties. The use of fungal and bacterial biofertilizers has become more widespread recently due to their low cost and environmental safety (Ali et al., 2022 ; Fall et al., 2023). Mycorrhizal fungi (*Glomus mosseae*) establish symbiotic relationships with plant roots, benefiting both organisms. These fungi receive sugars and growth factors from the plant while providing several benefits to the plant, such as increasing nutrient uptake (AjemaGebisa, 2024). Azospirillum has the ability to fix atmospheric nitrogen, produce plant hormones, and secrete compounds that improve soil structure and nutrient absorption

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(Helman, et al., 2011). Mineral fertilizers enhance the production of vegetable crops; however, excessive use of these fertilizers increases pollutant levels, leading to soil and groundwater contamination and higher nitrate concentrations in the leaves of vegetable crops (Saleh et al., 2010 ; Al-Ubaidy et al., 2017). The use of organic fertilizers can help mitigate these issues (Ekinci et al., 2020). Glutamic acid promotes vegetative growth and early production. The application of amino acids contributes to the growth and productivity of various agricultural crops (Saaseea and Al-A'amry, 2024). This research aims to rationalize the use of mineral fertilizers and investigate the effects of *Azospirillum lipoferum* bacteria, mycorrhizal fungi, organic nutrient sprays, and glutamic acid.

MATERIALS AND METHODS

This experiment was conducted during the Fall seasons of 2022-2023 and 2023-2024 in a Field of the organic fertilizer project, Directorate of Agriculture in Dhi Qar, located in Bani Zaid district, Shatra. Seeds of local lettuce cultivars were planted on October $_1$st, 2022, and October $_1$st, 2023, in well-prepared beds designed for lettuce seedling production. The factorial experiment included three factors conducted within a Randomized Complete Block Design (R.C.B.D) with three replications, 36 treatments per replicate. The total number of experimental units in were 108. Urea fertilizer was used as the nitrogen source, and di-ammonium phosphate (DAP) (46% P_2O_5, 18% *N*) was used as the phosphorus source. Half of the recommended fertilizer dose was applied to all experimental units (110 kg ha^{-1} urea and 60 kg ha^{-1} DAP were added before transplanting for all treatments) (Al-Mharib et al., 2019 ; Al-Zaidi, 2021). This procedure was followed for both agricultural seasons.

Experimental Treatments for Both Seasons

The first factor consisted of four levels of biofertilization: Application of 15 g per hole of peat moss mixed with mycorrhizal fungal inoculum (*Glomus mosseae*) at an inoculation density of 50 spores per gram of peat moss, applied 5 cm below the soil surface beneath the lettuce seedlings' roots. Additionally, 5 g per hole of *Azospirillum lipoferum* bacterial inoculum was applied at an inoculation density of 1.5×10^9 cells mL^{-1}, also beneath the roots of the lettuce seedlings (Issa and Muhaibis. 2020).

The second factor was the organic fertilizer *Fertiorgan*, which contains: Organic matter 42%, calcium 1.68%, magnesium 1.2%, boron 0.012%, iron 0.024%, manganese 0.012%, and zinc 0.0024%. Lettuce crops were sprayed with three concentrations of this organic fertilizer.

The first spray occurred 15 days after transplanting

The second spray was 30 days after transplanting

The third spray was 45 days after transplanting

The third factor involved the application of glutamic acid as an amino acid treatment

Lettuce crop were sprayed with three concentrations of glutamic acid: 0 mg L^{-1} (C_0), 250 mg L^{-1} (C_1), and 500 mg L^{-1} (C_2) (Tunio et al., 2021).

The first spray was applied 18 days after transplanting.

The second spray was 33 days after transplanting.

The third spray was 48 days after transplanting.

The results were analyzed after the completion of the study's parameters using the statistical analysis program *GenStat 12*. The differences between means were tested using the Least Significant Difference (L.S.D.) test at a 0.05 probability level to compare the differences between treatments mean.

RESULTS AND DISCUSSION

The effects on the percentage Chemical indicators, the intensity of mycorrhizal colonization, lettuce plants.: The results in Table (1) dicate significant effects of the three-way interaction among biofertilization, organic nutrient foliar application, and glutamic acid on the percentage of nitrogen, phosphorus, potassium, calcium, mycorrhizal infection intensity, and total chlorophyll concentration (mg per 100 g fresh weight) in lettuce leaves for the fall seasons of 2022 and 2023 (Table 1). The treatment A3B2C2 exhibited the highest nitrogen percentages, with values of 3.117% and 2.991% for both seasons, respectively, while the control treatment

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A0B0C0 recorded the lowest nitrogen percentages of 2.363% and 2.235% for the same seasons. For phosphorus, A0B0C0 also showed the lowest values of 0.228% and 0.218% across both seasons. The treatment A3B1C2 significantly resulted in the highest phosphorus percentage, with values of (0.570% and 0.557%) for the two seasons, respectively. The treatments, A3B0C0 demonstrated a significant increase in potassium percentage, achieving the highest percentages of 4.68% and 4.473% for both seasons, while the lowest potassium percentages were recorded for A1B0C0 in the first season at 3.523% and for A0B0C0 in the second season at 3.346%. Additionally, A3B2C0 significantly outperformed in terms of calcium percentage, with a percentage of 1.403% in the first season. In the second season, the treatment A3B0C2 achieved the highest calcium percentage at 1.342%, while the lowest calcium percentages were observed in treatment A1B0C0, with values of 0.927% and 0.895% for the two seasons, respectively. For total chlorophyll concentration, the treatment A3B0C0 significantly excelled in the first season, with a value of 84.83 mg 100^{-1} g fresh weight, while in the second season, A3B2C1 achieved the highest chlorophyll concentration at 72.81 mg 100^{-1} g fresh weight. On the other hand, the treatment A1B0C2 recorded the lowest chlorophyll concentration at 65.61 mg and 61.02 mg 100^{-1} g fresh weight for the first and second seasons, respectively. Results Table (2) indicate significant differences between the two-way interactions of the study factors in terms of percentages and the concentration of the studied traits. Biofertilization and organic nutrient foliar application showed a significant superiority of treatment A3B2, which produced nitrogen percentages of 2.977% and 2.857% and calcium percentages of 1.358% and 1.301% for the two growing seasons, respectively. In contrast, the lowest nitrogen values were observed in treatment A0B1, with values of 2.476% and 2.358% for the two seasons, while the lowest calcium values were recorded in treatment A0B2, reaching 1.082% and 1.047% for the two seasons, respectively. The treatment A3B1 significantly excelled by providing the highest phosphorus percentages (0.423% and 0.411%) and potassium percentages (4.523% and 4.342%) for both seasons, compared to the lowest values in treatment A0B0, which had phosphorus percentages of 0.282% and 0.270% and potassium percentages of 3.773% and 3.582% for the two seasons, respectively.

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Table 1. Effect of the three-way interactions between biofertilization, organic nutrient foliar application, and glutamic acid on the percentages of nitrogen, phosphorus, potassium, and calcium, total chlorophyll concentration (mg per 100 g fresh weight) in lettuce leaves, and mycorrhizal root infection intensity for the Fall seasons of 2022 and 2023

Treatment	N in leaves (%)		P in leaves (%)	(%)	K in leaves (%)		Ca in leaves (%)		Chlorophyll concentration (mg per 100 g fresh weight(		Mycorrhizal root infection intensity(%)	
Season	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
						A x B x C						
$A_0B_0C_0$	2.363	2.235	0.228	0.218	3.557	3.346	1.083	1.043	69.51	64.65	6.67	3.33
$A_0B_0C_1$	2.707	2.578	0.264	0.254	3.73	3.547	1.22	1.175	74.09	71.30	6.67	3.33
$A_0B_0C_2$	2.57	2.452	0.355	0.339	4.033	3.853	1.26	1.214	67.97	63.21	3.33	0
$A_0B_1C_0$	2.467	2.351	0.334	0.321	4.347	4.118	1.167	1.123	68.08	63.32	10	3.33
$A_0B_1C_1$	2.527	2.405	0.307	0.297	4.067	3.851	1.123	1.083	74.39	69.19	6.67	3.33
$A_0B_1C_2$	2.433	2.318	0.327	0.316	4.107	3.943	1.053	1.023	69.48	64.61	3.33	3.33
$A_0B_2C_0$	2.707	2.589	0.319	0.307	3.997	3.85	1.037	1.002	76.70	70.70	6.67	3.33
$A_0B_2C_1$	2.833	2.731	0.318	0.306	4.22	4.059	1.053	1.023	71.23	66.24	3.33	3.33
$A_0B_2C_2$	2.667	2.551	0.324	0.312	4.42	4.237	1.157	1.115	66.93	62.25	6.67	6.67
$A_1B_0C_0$	2.59	2.485	0.298	0.288	3.523	3.391	0.927	0.895	68.71	63.90	76.67	80
$A_1B_0C_1$	2.35	2.258	0.281	0.269	4.48	4.286	1.24	1.195	74.50	68.64	80	76.67
$A_1B_0C_2$	2.747	2.641	0.328	0.316	4.373	4.193	1.117	1.077	65.61	61.02	76.67	76.67
$A_1B_1C_0$	2.453	2.363	0.336	0.323	4.227	4.065	1.03	0.993	76.68	71.32	76.67	80
$A_1B_1C_1$	2.72	2.617	0.339	0.326	4.513	4.298	1.307	1.258	69.14	64.30	80	83.33
$A_1B_1C_2$	2.753	2.651	0.342	0.330	4.493	4.333	1.333	1.284	74.81	69.57	76.67	80
$A_1B_2C_0$	2.743	2.647	0.309	0.297	3.997	3.813	1.25	1.198	74.83	69.59	76.67	76.67
$A_1B_2C_1$	2.84	2.737	0.298	0.289	4.033	3.887	1.19	1.149	65.99	61.37	76.67	76.67
$A_1B_2C_2$	2.933	2.831	0.344	0.331	4.33	4.129	1.273	1.228	66.15	61.52	80	80.0
$A_2B_0C_0$	2.713	2.617	0.312	0.299	3.97	3.766	1.17	1.122	74.11	68.92	0	3.33
$A_2B_0C_1$	2.91	2.801	0.362	0.347	4.323	4.115	1.183	1.139	73.62	68.46	3.33	0
$A_2B_0C_2$	2.973	2.855	0.375	0.359	4.5	4.28	1.217	1.171	73.75	68.58	3.33	3.33
$A_2B_1C_0$	2.88	2.764	0.333	0.320	4.16	3.959	1.197	1.155	68.13	63.37	3.33	0
$A_2B_1C_1$	2.86	2.747	0.338	0.327	4.647	4.427	1.287	1.24	77.99	72.53	3.33	3.33
$A_2B_1C_2$	2.847	2.744	0.352	0.341	4.56	4.359	1.217	1.178	69.34	64.49	3.33	3.33
$A_2B_2C_0$	2.823	2.721	0.336	0.323	4.237	4.067	1.16	1.119	70.77	65.81	6.67	3.33
$A_2B_2C_1$	2.87	2.77	0.348	0.335	4.647	4.455	1.31	1.262	74.39	69.19	6.67	3.33
$A_2B_2C_2$	2.85	2.741	0.417	0.400	4.547	4.355	1.283	1.239	77.97	72.52	6.67	3.33
$A_3B_0C_0$	2.723	2.614	0.349	0.335	4.68	4.473	1.253	1.206	84.83	72.77	60	66.67
$A_3B_0C_1$	2.89	2.772	0.342	0.330	4.427	4.245	1.333	1.284	70.53	65.59	63.33	70.0
$A_3B_0C_2$	3.017	2.901	0.326	0.314	4.277	4.089	1.4	1.342	77.36	71.95	76.67	76.67
$A_3B_1C_0$	2.697	2.6	0.360	0.347	4.567	4.374	1.197	1.16	59.65	55.48	66.67	70
$A_3B_1C_1$	2.973	2.859	0.340	0.328	4.483	4.305	1.367	1.31	69.33	64.48	73.33	76.67
$A_3B_1C_2$	2.867	2.767	0.570	0.557	4.52	4.346	1.353	1.303	73.39	68.25	66.67	70.0
$A_3B_2C_0$	2.957	2.839	0.399	0.385	4.26	4.097	1.403	1.334	75.40	70.12	63.33	63.33
$A_3B_2C_1$	2.857	2.742	0.363	0.352	4.653	4.451	1.353	1.295	78.28	72.81	63.33	73.33
$A_3B_2C_2$	3.117	2.991	0.394	0.386	4.627	4.428	1.317	1.273	76.10	70.77	70	70.0
LSD 0.05	0.518	0.499	0.109	0.109	0.939	0.890	0.218	0.200	2.99	1.54	15.039	13.663

Biofertilization A: A0: No addition. A1: *Mycorrhizae* 15 g per hole. A2: *Azospirillum* 5 g per hole. A3: *Mycorrhizae* + *Azospirillum*.
Organic fertilization B: B0: Water spray only., B1: Spray 2 ml L^{-1}., B2: Spray 4 ml L^{-1}.
Glutamic acid C: C0: Water spray only., C1: Spray 250 mg L^{-1}., C2: Spray 500 mg L^{-1}.

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Table 2. Effect of the two-way interactions between biofertilization, organic nutrient foliar application, and glutamic acid on the percentages of nitrogen, phosphorus, potassium, and calcium, total chlorophyll concentration (mg per 100 g fresh weight) in lettuce leaves, and mycorrhizal root infection intensity for the Fall seasons of 2022 and 2023

Treatment	N in leaves (%)		P in leaves (%)		K in leaves (%)		Ca in leaves (%)		Chlorophyll concentration (mg per 100 g fresh weight(		Mycorrhizal root infection intensity(%)	
Season	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
A x B												
A0B0	2.547	2.422	0.282	0.270	3.773	3.582	1.188	1.144	70.52	66.39	5.56	2.22
A0B1	2.476	2.358	0.323	0.311	4.173	3.971	1.114	1.076	70.65	65.71	6.67	3.33
A0B2	2.736	2.623	0.321	0.309	4.212	4.048	1.082	1.047	71.62	66.40	5.56	4.44
A1B0	2.562	2.461	0.302	0.291	4.126	3.957	1.094	1.056	69.61	64.52	77.78	77.78
A1B1	2.642	2.544	0.339	0.326	4.411	4.232	1.223	1.178	73.54	68.40	77.78	81.11
A1B2	2.839	2.738	0.317	0.306	4.12	3.943	1.238	1.192	68.99	64.16	77.78	77.78
A2B0	2.866	2.758	0.349	0.335	4.264	4.054	1.19	1.144	73.82	68.66	2.22	2.22
A2B1	2.862	2.752	0.341	0.329	4.456	4.248	1.233	1.191	71.82	66.79	3.33	2.22
A2B2	2.848	2.744	0.367	0.353	4.477	4.293	1.251	1.207	74.38	69.17	6.67	3.33
A3B0	2.877	2.762	0.339	0.326	4.461	4.269	1.329	1.277	77.57	70.10	66.67	71.11
A3B1	2.846	2.742	0.423	0.411	4.523	4.342	1.306	1.258	67.46	62.74	68.89	72.22
A3B2	2.977	2.857	0.385	0.374	4.513	4.325	1.358	1.301	76.59	71.23	65.56	68.89
L.S.D 0.05	0.299	0.288	0.063	0.063	0.542	0.514	0.126	0.116	1.726	0.89	8.683	7.888
A x C												
A0C0	2.512	2.392	0.294	0.282	3.967	3.771	1.096	1.056	71.43	66.22	7.78	3.33
A0C1	2.689	2.571	0.296	0.286	4.006	3.819	1.132	1.093	73.24	68.91	5.56	3.33
A0C2	2.557	2.44	0.335	0.323	4.187	4.011	1.157	1.117	68.13	63.36	4.44	3.33
A1C0	2.596	2.498	0.314	0.303	3.916	3.757	1.069	1.029	73.41	68.27	76.67	78.89
A1C1	2.637	2.537	0.306	0.295	4.342	4.157	1.246	1.2	69.87	64.77	78.89	78.89
A1C2	2.811	2.708	0.338	0.325	4.399	4.218	1.241	1.196	68.86	64.04	77.78	78.89
A2C0	2.806	2.701	0.327	0.314	4.122	3.931	1.176	1.132	71	66.03	3.33	2.22
A2C1	2.88	2.773	0.349	0.336	4.539	4.332	1.26	1.214	75.33	70.06	4.44	2.22
A2C2	2.89	2.78	0.381	0.367	4.536	4.331	1.239	1.196	73.69	68.53	4.44	3.33
A3C0	2.792	2.684	0.369	0.356	4.502	4.315	1.284	1.233	73.3	66.12	63.33	66.67
A3C1	2.907	2.791	0.348	0.336	4.521	4.334	1.351	1.296	72.71	67.62	66.67	73.33
A3C2	3	2.886	0.430	0.419	4.474	4.288	1.357	1.306	75.62	70.32	71.11	72.22
L.S.D 0.05	0.299	0.288	0.063	0.063	0.542	0.514	0.126	0.116	1.726	0.89	8.683	7.888
B x C												
B0C0	2.598	2.488	0.297	0.285	3.933	3.744	1.108	1.067	74.29	67.56	35.83	38.33
B0C1	2.714	2.602	0.312	0.300	4.24	4.048	1.244	1.198	73.18	68.50	38.33	37.5
B0C2	2.827	2.712	0.346	0.332	4.296	4.104	1.248	1.201	71.17	66.19	40	39.17
B1C0	2.624	2.52	0.341	0.328	4.325	4.129	1.147	1.108	68.14	63.37	39.17	38.33
B1C1	2.77	2.657	0.331	0.320	4.428	4.22	1.271	1.223	72.71	67.62	40.83	41.67
B1C2	2.725	2.62	0.398	0.386	4.42	4.245	1.239	1.197	71.75	66.73	37.5	39.17
B2C0	2.808	2.699	0.341	0.328	4.122	3.957	1.212	1.163	74.43	69.06	38.33	36.67
B2C1	2.85	2.745	0.332	0.320	4.388	4.213	1.227	1.182	72.47	67.40	37.5	39.17
B2C2	2.892	2.778	0.370	0.357	4.481	4.287	1.257	1.214	71.79	66.76	40.83	40
LSD 0.05	0.259	0.250	0.055	0.054	0.470	0.445	0.109	0.100	1.49	0.77	N.S	N.S

Biofertilization A: A0: No addition. A1: *Mycorrhizae* 15 g per hole. A2: *Azospirillum* 5 g per hole. A3: *Mycorrhizae* + *Azospirillum*.
Organic fertilization B: B0: Water spray only., B1: Spray 2 ml L^{-1}., B2: Spray 4 ml L^{-1}.
Glutamic acid C: C0: Water spray only., C1: Spray 250 mg L^{-1}., C2: Spray 500 mg L^{-1}.

The treatments A3B0 and A3B2 significantly outperformed others in total chlorophyll concentration, reaching 77.57 mg and 71.23 mg 100^{-1} g fresh weight for the two growing seasons, respectively. These values were significantly higher than all other treatments except for A3B2, which did not differ significantly, achieving 76.59 mg 100^{-1} g fresh weight in the first season. In contrast, the treatment A3B1 exhibited the lowest total chlorophyll concentration, with values of 67.46 mg and 62.74 mg 100^{-1} g fresh weight for the two seasons, respectively. In terms of mycorrhizal infection intensity, treatments A1B0, A1B1, and A1B2 significantly surpassed other treatments, each showing an infection intensity of 77.78% for the first growing season. The lowest infection intensity was recorded in treatment A2B0, with a value of 2.22% for the first season. In the second season, treatment A1B1 excelled with an infection intensity of 81.11%, while the lowest

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intensities (2.22%) were observed in treatments A0B0, A2B0, and A2B1 during the second season. Regarding the interaction between biofertilization and glutamic acid application, treatment A3C2 significantly increased the percentage of nitrogen (3% and 2.886%), phosphorus (0.430 % and 0.419%), calcium (1.357% and 1.306%), and total chlorophyll concentration (75.62 mg and 70.32 mg 100^{-1} g fresh weight) for both seasons, respectively. In comparison, the control treatment A0C0 yielded the lowest percentages of nitrogen (2.512% and 2.392%) and phosphorus (0.294% and 0.282%) for the two seasons, respectively. Treatment A1C0 recorded the lowest potassium (3.916% and 3.757%) and calcium percentages (1.069% and 1.029%) for the two seasons, respectively. Treatment A2C1 significantly outperformed others, providing the highest potassium percentage (4.539%) in the first season, while treatment A3C1 showed a potassium percentage of 4.334% in the second season. Additionally, treatment A1C1 significantly enhanced root infection by mycorrhizal fungi, showing the highest infection percentage. In the interaction between organic nutrient spraying and glutamic acid, treatment B2C2 significantly increased the nitrogen percentage (2.892% and 2.778%) and potassium percentage (4.481% and 4.287%) for both seasons, respectively. Treatment B1C2 significantly enhanced phosphorus content (0.398% and 0.386%) for both seasons, while B1C1 significantly increased calcium percentage (1.271% and 1.223%) for the two seasons. In contrast, the lowest percentages of nitrogen (2.598% and 2.488%), phosphorus (0.297% and 0.285%), potassium (3.399% and 3.744%), and calcium (1.108% and 1.067%) were recorded in treatment B0C0 for both seasons. Treatment B2C0 significantly increased total chlorophyll concentration, with values of 74.43 mg and 69.06 mg 100^{-1} g fresh weight for the two seasons, respectively, while treatment B1C0 recorded the lowest chlorophyll concentration (68.14 mg and 63.37 mg $^{1-}$100 g fresh weight) for both seasons. The results of the statistical analysis in Table 3 for the individual factors of the study showed a significant superiority of treatment A3 in terms of the percentages of nitrogen (2.9% and 2.787%), phosphorus (0.383% and 0.370%), potassium (4.499% and 4.312%), and calcium (1.331% and 1.278%) for the two growing seasons, respectively, compared to the lowest values in the control treatment A0, where nitrogen percentages were (2.586% and 2.468%), phosphorus (0.309% and 0.297%), potassium (4.053% and 3.867%), and calcium (1.128% and 1.089%) for the two seasons. Additionally, treatments A3 and A2 significantly excelled in total chlorophyll concentration, with values of 73.88 mg and 68.21 mg 100^{-1} g fresh weight for the two seasons, respectively, while treatment A1 recorded the lowest values (70.71 mg and 65.69 mg per 100 g fresh weight for the two seasons). For mycorrhizal infection intensity, treatment A1 significantly outperformed the others, with values of 77.78% and 78.89% for the two growing seasons, respectively, whereas the lowest infection rates were recorded in treatment A2, with values of 4.07% and 2.59% for the two seasons. Regarding the foliar application of organic nutrients, treatment B2 showed the highest nitrogen values (2.85% and 2.741%) and calcium values (1.232% and 1.186%) without significant differences. However, B2 significantly increased the total chlorophyll concentration, with values of 72.89 mg and 67.74 mg per 100 g fresh weight for the two seasons. Treatment B1 significantly enhanced phosphorus percentage (0.357% and 0.344%) for the two seasons, while B0 had the lowest phosphorus percentage (0.318% and 0.306%) for both seasons. No significant differences were observed in the percentage of potassium or in the mycorrhizal infection intensity. However, the glutamic acid foliar application treatments indicated that C2 significantly increased phosphorus percentages (0.371% and 0.358%) and calcium percentages (1.248% and 1.204%) for the two growing seasons, respectively. The lowest phosphorus values were found in treatment C1 (0.325% and 0.313%) for both seasons, and the lowest calcium values were recorded in treatment C0 (1.156% and 1.113%) for both seasons. Treatment C1 outperformed others in terms of total chlorophyll concentration, with values of

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72.79 mg and 67.84 mg 100^{-1} g fresh weight for the two seasons, while treatment C2 recorded the lowest values for total chlorophyll (71.57 mg and 66.56 mg per 100 g fresh weight for the two seasons). There was no significant effect of glutamic acid foliar application treatments on the percentage of nitrogen and potassium in the leaves, nor on the mycorrhizal root infection intensity.The biofertilizer (a combination of mycorrhizal fungi and *Azospirillum* bacteria) had a significant effect on the percentages of nitrogen, phosphorus, potassium, and calcium in both growing seasons. In a study on tomato plants, it was observed that inoculation with mycorrhizae and *Azospirillum* improved plant growth and increased the accumulation of certain nutrients in the leaves, such as phosphorus and potassium (Pokluda et al., 2021). Similarly, in a study on lettuce, it was found that inoculating lettuce with mycorrhizae at a low phosphorus fertilization level significantly increased the nitrogen and phosphorus percentage in the leaves (Cela et al., 2022). Additionally, Solórzano-Acosta et al. (2023) reported similar results when inoculating Avocado plants with mycorrhizae, which led to an increase in potassium percentage. This could be attributed to the ability of mycorrhizae to enhance the efficiency of plants in absorbing nutrients through the symbiotic relationship with plant roots. Mycorrhizae stimulate the roots to absorb both mobile and immobile nutrients, such as nitrogen, phosphorus, potassium, calcium, sulfur, and manganese from the soil (Yang et al., 2023). This could be attributed to the penetration of mycorrhizal hyphae through the cortex of plant root cells, forming branched structures called *arbuscules*. These hyphae help the host plant acquire nutrients, particularly phosphorus, potassium, calcium, zinc, and copper, which are otherwise difficult to access.

Table 3. Effect of the individual treatments of biofertilization, organic nutrient foliar application, and glutamic acid on the percentages of nitrogen, phosphorus, potassium, and calcium, total chlorophyll concentration (mg per 100 g fresh weight) in lettuce leaves, and mycorrhizal root infection intensity for the Fall seasons of 2022 and 2023

Treatment	N in leaves (%)		P in leaves (%)		K in leaves (%)		Ca in leaves (%)		Chlorophyll concentration (mg per 100 g fresh weight(		Mycorrhizal root infection intensity(%)	
Season	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023	2022	2023
						A						
A0	2.586	2.468	0.309	0.297	4.053	3.867	1.128	1.089	70.93	66.16	5.93	3.33
A1	2.681	2.581	0.319	0.308	4.219	4.044	1.185	1.142	70.71	65.69	77.78	78.89
A2	2.859	2.751	0.353	0.339	4.399	4.198	1.225	1.18	73.34	68.21	4.07	2.59
A3	2.9	2.787	0.383	0.370	4.499	4.312	1.331	1.278	73.88	68.02	67.04	70.74
LSD 0.05	0.173	0.166	0.036	0.036	0.313	0.297	0.073	0.067	1.00	0.51	5.013	4.554
						B						
B0	2.713	2.601	0.318	0.306	4.156	3.965	1.2	1.155	72.88	67.42	38.06	38.33
B1	2.706	2.599	0.357	0.344	4.391	4.198	1.219	1.176	70.87	65.91	39.17	39.72
B2	2.85	2.741	0.348	0.335	4.331	4.152	1.232	1.186	72.89	67.74	38.89	38.61
LSD 0.05	N.S	N.S	0.032	0.031	N.S	N.S	N.S	N.S	0.863	0.45	N.S	N.S
						C						
C0	2.676	2.569	0.326	0.314	4.127	3.943	1.156	1.113	72.28	66.66	37.78	37.78
C1	2.778	2.668	0.325	0.313	4.352	4.16	1.247	1.201	72.79	67.84	38.89	39.44
C2	2.814	2.704	0.371	0.358	4.399	4.212	1.248	1.204	71.57	66.56	39.44	39.44
LSD 0.05	N.S	N.S	0.032	0.031	N.S	N.S	0.063	0.058	0.863	0.45	N.S	N.S

Biofertilization A: A0: No addition. A1: *Mycorrhizae* 15 g per hole. A2: *Azospirillum* 5 g per hole. A3: *Mycorrhizae* + *Azospirillum*.
Organic fertilization B: B0: Water spray only., B1: Spray 2 ml L^{-1}., B2: Spray 4 ml L^{-1}.
Glutamic acid C: C0: Water spray only., C1: Spray 250 mg L^{-1}., C2: Spray 500 mg L^{-1}.

The hyphae absorb these nutrients and can extend up to 10 cm from the root surface, increasing the volume of soil available for phosphorus and other nutrients. Additionally, the hyphae produce the enzyme phosphatase, which mineralizes phosphate from organic sources, and they secrete organic acids around the mycelium near the roots, which can

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convert insoluble phosphates in the soil into soluble forms in the soil solution (Ali et al., 2022). These fungi stimulate the host plant to absorb nutrients from water and soil through multiple mechanisms. These include enzymatic processes like phosphatase, which dissolves phosphorus, and non-enzymatic mechanisms through chemical processes (such as Fenton chemistry), which promote nitrogen and carbon cycling (Tanwir et al., 2023). These results are consistent with findings from a study on lettuce inoculated with *Azospirillum* bacteria, which showed an increases in plant growth as well as an increase in the concentration of nitrogen, phosphorus, potassium, and calcium in the plant (Moreira et al., 2022 ; Da Silva Oliveira et al., 2023). This could be attributed to the ability of *Azospirillum* bacteria to fix nitrogen in the rhizosphere, thereby providing nutrients to plants and stimulating their growth. The bacteria also synthesize hormone-like substances that promote root growth, enhancing water and nutrient uptake, which in turn improves plant growth (Sivasakthivelan et al., 2023). Nitrogen fixation typically occurs through the enzyme nitrogenase, which is secreted by *Azospirillum* bacteria, providing a key source of nitrogen for plants (Naqqash et al., 2023). It was observed that treatment **A3** significantly outperformed the control treatment in terms of chlorophyll concentration for both growing seasons. The interaction between mycorrhizal fungi and *Azospirillum* bacteria increased vegetative growth indicators, as the combination of these microorganisms as a biofertilizer had a positive effect on promoting plant growth. The relationship between mycorrhizal fungi and *Azospirillum* bacteria is beneficial for encouraging plant growth and enhancing vegetative growth indicators, such as chlorophyll concentration (Haran, 2012 ; Ammar, 2024). There are also synergistic effects between mycorrhizal fungi and plant growth-promoting rhizobacteria in enhancing nutrient uptake and water retention in the rhizosphere (the root zone of the plant). These effects are carried out by the microorganisms individually or cooperatively, and in both cases, they provide essential growth requirements in the region influenced by root exudates (Alshegaihi et al., 2024). There is a strong connection between plant roots and mycorrhizal fungi, as these fungi enhance soil water retention and nutrient absorption. The fungal filaments (hyphae) increase the surface area of the roots, allowing them to benefit from root exudates by obtaining sugars from the host plant's roots. In return, the host plant's roots improve their ability to absorb water and nutrients from the soil. Mycorrhizae also help dissolve phosphates through the enzyme phosphatase, providing the essential phosphorus needed for plant growth and development (Bhantana et al., 2021). This could be attributed to the role of mycorrhizae in increasing the availability of nutrients like nitrogen, phosphorus, and potassium in the soil solution, thus enhancing plant absorption of these elements. Biofertilization, through various mechanisms such as enzyme and hormone secretion (like auxin), further facilitates nutrient uptake, which is reflected in the increased vegetative growth indicators (Balcı, 2023). This aligns with the findings of Chatzistathis et al. (2024) They reported that vegetative growth indicators in lettuce improved due to the use of plant growth-promoting bacteria and mycorrhizal fungi. Biofertilizers enriched the soil with essential nutrients like nitrogen, phosphorus, and potassium, improved soil aeration, and thus increased soil fertility and nutrient uptake, as well as the photosynthesis rates in lettuce plants. Nitrogen is the most important elements that encourage continuous plant growth and development, as it is an essential nutrient for plant growth. Without nitrogen, a plant couldnot complete its life cycle. *Azospirillum* bacteria perform biological nitrogen fixation in the root zone, providing an essential and readily available nutrient source near the plant roots (Al-Mashhadany, and Al-Mharib. 2023 ; Joe and A. Benson, 2024). The results also show that foliar application of organic nutrients significantly influenced the nutrient concentration and chlorophyll levels in the plant, with study indicators showing significant increases. Researches indicats that the method of application, such as foliar spraying, and the concentration of the organic

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nutrient play a key role in growth indicators. Foliar spraying was found to be more effective than other application methods in lettuce (Frasetya et al., 2021), This is also consistent with the conclusions of Al-Chalabi *et al.*, (2023). They found that foliar application of organic nutrients improves growth indicators, mineral concentration, and chlorophyll levels in lettuce under open field conditions. The application of biofertilizers and the foliar spraying of organic fertilizers had a positive effect on vegetative growth indicators and on the plant's concentration of nitrogen, phosphorus, and potassium (Mohammed et al., 2022). This could be attributed to the fact that organic nutrients increases the nutrient percentage in the plant, as elements like nitrogen, phosphorus, potassium, and other essential nutrients are needed for cell construction and the development of plant organs, along with chlorophyll, the primary pigment involved in photosynthesis. Consequently, growth indicators improve (AL-Ubaidy and AL-Zaidy, 2017 ; Ahmed et ali., 2021 ; Júnior et al., 2023). These results are consistent with a study on lettuce, which indicated that the use of organic nutrient foliar spraying increased root mass and nutrient absorption (Tunio et al., 2021). The obtained results clearly show the significant superiority of treatments involving the foliar application of glutamic acid in terms of total chlorophyll concentration in lettuce leaves. The use of glutamic acid on lettuce stimulates metabolic processes, leading to increased cell production and chlorophyll concentration by enhancing the efficiency of primary metabolite production. Glutamic acid has also been used in sustainable agriculture to reduce the use of mineral fertilizers, yielding positive results on lettuce (De Gregorio et al., 2023). In a study aimed at assessing the effect of glutamic acid foliar application on lavender (*Lavendula angustifolia* Mill.), the results indicated an increase in chlorophyll concentration (Hamada and Ahmed, 2022). Glutamic acid participates in various biological processes, including chlorophyll and proline synthesis. Chlorophyll is the pigment responsible for photosynthesis, while proline acts as a stress-inducing and stress-resisting agent (Franzoni et al., 2021). Glutamic acid has been used to enhance nutrient efficiency, reduce environmental impacts, act as a chelating agent for micronutrients, and serve as a nitrogen source. In one experiment, the use of glutamic acid produced positive results in stimulating lettuce growth in both hydroponic and conventional growing systems (Galieva et al., 2024). The significant increases in mycorrhizal root infection in treatment A1 (mycorrhizal inoculation) was confirmed by the results obtained. The rhizosphere, the zone of interaction between roots and soil, is an area where both affect each other. The higher mycorrhizal infection rate can be attributed to root exudates that contain chemical compounds promoting the symbiotic relationship between roots and mycorrhizae. These exudates include substances that encourage fungal spore germination, leading to infection (Dakora, 2003). Additionally, the host plant supplies the mycorrhizae with carbon-rich photosynthetic compounds, such as sugars, which are essential for completing the fungal life cycle (Ahammed and Hajiboland 2024). These findings align with the results of Al-Atawi (2021), who also reported increased mycorrhizal root infection in okra plants. These findings are consistent with the results of a study on lettuce inoculated with mycorrhizae (Avio et al., 2017). The infection of lettuce roots was significant even under hydroponic systems and phosphorus deficiency, which can be attributed to the inoculation treatment and the plant roots' ability to form a symbiotic relationship. This may be due to the root exudates, which provided a carbon source for the fungus (Cela et al., 2022). Similarly, these results align with a study on lettuce (Han et al., 2023).

CONCLUSION

The current study demonstrated that the combined application of biofertilizers, particularly the use of *Azospirillum* bacteria and mycorrhizal fungi, along with the foliar application of organic nutrients and glutamic acid, significantly enhanced the vegetative growth indicators, nutrient uptake, and chlorophyll content in lettuce. Mycorrhizae played a crucial role in improving nutrient availability, particularly phosphorus,

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potassium, and calcium, through symbiotic relationships with plant roots. The biofertilization treatments, in conjunction with organic nutrient spraying, promoted better root development and nutrient absorption, leading to increased growth and overall plant health. These results highlight the potential of sustainable agricultural practices, such as biofertilization and the use of organic compounds, to reduce reliance on chemical fertilizers while enhancing crop yield and quality.

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CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHOR/S DECLARATION

We confirm that all Figures and Tables in the manuscript are original to us. Additionally, any Figures and images that do not belong to us have been incorporated with the required permissions for re-publication, which are included with the manuscript.

AUTHOR'S CONTRIBUTION STATEMENT

All authors made equal contributions to the study design, methodology, experimental work, data analysis, and manuscript writing. All authors reviewed and approved the final version of the manuscript.

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تأثير التسميد الحيوي ورش المغذي العضوي وحامض الكلوتاميك في بعض صفات الخس

اياد علي حنيش السماح[1] ، رضا مصطفى عبدالحسين العبيدي[2]
[1]الكلية التقني الشطرة / الجامعة التقنية الجنوبية
[2]قسم البستنة وهندسة الحدائق / كلية علوم الهندسة الزراعية / جامعة بغداد

المستخلص

أجريت تجربة حقلية على مدى موسمين زراعيين في حقل مشروع الأسمدة العضوية الواقع في ناحية بني زيد / قضاء الشطرة، التابع لمديرية زراعة ذي قار. شملت التجربة العاملية ثلاثة عوامل, ضمن تصميم القطاعات العشوائية الكاملة بثلاثة مكررات، 36 معاملة لكل مكرر. بهدف تقييم تأثير تقليل استخدام الأسمدة المعدنية بنسبة 50% على نمو نبات الخس ، مع التركيز على التأثير المشترك للتسميد الحيوي باستخدام المايكورايزا والازوسبيرلم، بالإضافة إلى الرش الورقي بمحلول مغذي عضوي (*Forteorgan*) وحامض الكلوتاميك. أجريت التجربة على مدار موسمين زراعيين (2022-2023 و2023-2024). أظهرت النتائج أن المعاملة المشتركة لتلقيح المايكورايزا و*Azospirillum*، مع الرش الورقي بـ4 مل/لتر من *Forteorgan* و500 ملغم/لتر من حامض الكلوتاميك، أدت إلى زيادة معنوية في محتوى النيتروجين في الأوراق خلال كلا الموسمين. بالإضافة إلى ذلك، حسنت هذه المعاملة محتوى الفسفور والبوتاسيوم في الأوراق، وكذلك المحتوى الكلي للكلوروفيل، مما يشير إلى أن دمج التسميد الحيوي مع تقليل الأسمدة المعدنية يمكن أن يعزز بشكل فعال امتصاص العناصر الغذائية وعمليات التمثيل الضوئي في نبات الخس.

الكلمات المفتاحية: الازوسبيرلم ، *Lactuca sativa*، تقليل استخدام الأسمدة، المايكورايزا .