Integrated application of Pseudomonas fluorescens, Wheat Residues and Organic Extract enhanced Soil Microbial Activity and Nutrient Content in Maize plant (Zea mays L.) under Saline Soil Conditions

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Abstract
This Pseudomonas fluorescens bacteria have an important role in agriculture as plant growth-promoting rhizobacteria (PGPR). Their combinations with organic extract should be studied to detect possible changes in the microbial activity in saline soils environments and maize yield. This study investigated the effects of Pseudomonas fluorescens, wheat residues, and organic extract on microbiological behavior and nutrient content in maize plants under saline soil conditions. The experiment included the following factors: the first factor was the addition of bacterial bio-fertilizer versus no addition, denoted as (B1 and B2), the second factor was the addition of wheat residues at a level of 20 tons/hectare, 10 tons/hectare, and no addition, denoted as (E2, E1, and E0) respectively, and the third factor was the use of organic extract at three levels: 200 ml/m², 100 ml/m², and no addition, denoted as (W2, W1, and W0) respectively .
Results indicated that the addition of bacterial biofertilizer produced the highest soil microbial respiration (7.162 mg CO2 g^{-1} dry soil) and nutrient concentrations in maize leaves (K: 1.76%, P: 0.449%, N: 1.750%). Furthermore, the addition of 20 tons/ha wheat residues enhanced soil microbial respiration (7.002 mg CO2 g^{-1} dry soil) and nutrient levels (K: 1.76%, P: 0.473%, N: 1.699%). Whereas, the application of organic extract at 200 ml/m² yielded similar benefits of soil microbial respiration (7.451 mg CO2 g^{-1} dry soil) and nutrient content (K: 1.75%, P: 0.44%, N: 1.736%). Interestingly, it was found that the highest soil microbial respiration rates was at B1E2W2 treatment (9.103 mg CO2 g^{-1} dry soil). Similarly, the triple interaction improved nutrient content (K: 1.96%, P: 0.55%, N: 2.110%) as such. It is concluded that the combination of the organic extract with bacterial inoculation as well as wheat residues enhanced microbial activity and plant growth in saline soils.

Keywords: Microbial activity, Nutrient content in maize, Plant growth-promoting rhizobacteria (PGPR), Saline soils, Zea mays.

Introduction

Soil salinity is one of the major obstacles affecting crop production worldwide. In recent decades, human activities have exacerbated soil salinity levels [1-2]. Additionally, water

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scarcity and the accumulation of ionic salts have compounded this issue [3-4]. Studies indicate that salinity has impacted over 800 million hectares of agricultural land globally [5]. Reports suggest that approximately 1-2% of fertile soil degrades annually due to salinity [6], and it is projected that within the next 35 years, about 50% of the Earth's land will be affected by varying degrees of salinity [7]. Furthermore, data suggests that in recent decades, the annual cost of land degradation due to salinity in irrigated areas is estimated at $27.3 billion [8]. Excessive salt in the soil has long-term, cumulative effects on crop yields [9.[

Most agricultural lands in arid and semi-arid regions, including Iraq, face significant challenges, such as reduced availability of essential nutrients and low organic matter content. These conditions lead to a substantial decrease in soil microbial biomass. Consequently, researchers worldwide have been developing long-term strategies to achieve sustainable agriculture and increase productivity. Enhancing agricultural output is not solely dependent on the use of mineral fertilizers, as their extensive use increases the risk of soil, water, and air pollution. Thus, the search for new sources and modern techniques to boost crop yield and quality has intensified, aiming to minimize the reliance on mineral fertilizers and reduce environmental degradation.

In response to these challenges, the global agricultural community has turned towards clean farming technologies to mitigate pollution. Utilizing natural resources, such as organic and biofertilizers, is a suitable alternative or complement to mineral fertilizers [10]. Biofertilizers, a recent advancement in agriculture, are preparations containing one or more beneficial microorganisms. These are applied to seeds, plants, or soil to enhance the physical, chemical, and biological properties of the soil. By maintaining nutrient balance in agricultural soils, these microorganisms convert nutrients into plant-available forms, thus supporting plant growth throughout its life cycle. This approach can reduce the dependency on mineral fertilizers and lower agricultural production costs [11.[

Bacteria such as Pseudomonas are known for secreting plant hormones that promote plant growth, including auxins such as IAA (Indol acetic acid) and cytokinins. They also produce the enzyme Acc-deaminase, which inhibits the presence of ethylene. Pseudomonas bacteria play a role in enhancing phosphorus efficiency and the availability of other nutrients [12]. To achieve integration in sustainable agriculture, attention has turned to the use of organic fertilizers for their significant role in improving the physical, chemical, and biological properties of soil. In recent years, the use of organic fertilizers such as wheat residue has gained importance due to its role in increasing plant productivity, improving soil properties, and increasing its content of essential nutrients and organic matter, which enhances the activities of live microbial biomass, reflecting positively on soil health, plant growth, and productivity. Additionally, the use of organic extracts prepared from wheat residue, containing humic acids and beneficial major and minor elements for plants, stimulates soil biological activity and plant enzymes, along with its effective role in improving the chemical and physical properties of soil.

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Therefore, the integration of organic and biofertilizers in saline soils will create a balanced environment for microbial activity and increase the release of nutrients, aligning with the plant's needs. The aims of this study were to investigate the effects of Pseudomonas fluorescens, wheat residues, and organic extract on microbiological behavior and nutrient content in maize plant under saline soil conditions .

Materials and Methods
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Field Experiment Site
A field experiment was conducted during the 2023 autumn cropping season in the Musayyib project in Babil Province. Soil samples were collected from the 0-30 cm depth at various locations within the field. The samples were thoroughly mixed to homogenize them and sieved through a sieve with a 2 mm opening diameter. A composite sample was then

prepared for conducting various chemical and physical analyses (Table 1

Table (1) Some Chemical and Physical Characteristics of Field Soil Before Planting

Property		Value	Unit
Soil Reaction (pH) 1:1		7.68	-
Electrical Conductivity (EC) 1:1		7.22	dS m^{-1}
Organic Matter (O.M)		6.4	g kg^{-1}
dissolved positive ions	Calcium (Ca^{2+})	16.39	mmol L^{-1}
	Magnesium (Mg^{2+})	10.4	
	Sodium (Na^{+})	21.87	
	Potassium (K^{+})	0.88	
dissolved negative ions	Chloride (Cl^{-})	54.17	
	Sulfate (SO_4^{2-})	12.01	
	Bicarbonate (HCO_3^{-})	4.56	
	Carbonate (CO_3^{2-})	Nill	
ready-made elements	Available Nitrogen (N)	31.12	mg kg^{-1} soil
	Available	23.16	

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	Phosphorus (P)		
	Available Potassium (K)	76.54	
	Sand	58	g kg^{-1} soil
	Silt	22	
	Clay	20	
Texture		Silt Clay Loam	
Microbial Counts			
Microbial Counts	Total Bacteria	2.7×10^6	CFU g^{-1} soil
	P. fluorescens	5×10^4	CFU g^{-1} soil
Fungal Counts	Total Fungi	1.7×10^3	CFU g^{-1} soil

Study Site Preparation

The required area for conducting the experiment was determined, and soil preparation for planting was carried out by performing perpendicular plowing, smoothing, and leveling operations. The land was divided into three sectors, with each sector divided into 18 experimental units measuring 2 m x 3 m. Each experimental unit was further divided into three rows (each row containing eight plants) with a spacing of 75 cm between rows and 25 cm between plants within the row. Three seeds were placed in each planting hole at a depth of 5-10 cm, leaving a distance of 1 m between experimental units and 2 m between sectors. Field ridges were created between the sectors.

Crop Cultivation and Fertilization

The field was fertilized uniformly for all experimental units at the same time. Urea fertilizer (N%46) was applied at a rate of 240 kg N ha-1 as a nitrogen source, and potassium sulfate fertilizer (K%41.5) was applied at a rate of 100 kg K ha-1 as a potassium source, split into two applications. The first application was applied at planting, and the second application was applied one month after planting. Additionally, superphosphate fertilizer (20% P) was applied at a rate of 80 kg P ha-1 as a phosphorus source, applied only once at planting. Fertilizers were applied according to the recommendations for saline soils [13.[

Yellow corn hybrid seeds (obtained from the Yellow and White Corn Department of the Agricultural Research Division - Ministry of Agriculture) were manually planted on July 20, 2023. 2-3 seeds were placed in each planting hole. The field was irrigated three times consecutively after planting, with one irrigation every 4 days due to high temperatures at the beginning of the fall season to ensure good seed germination.

Subsequently, irrigation was conducted as needed, with one irrigation per week. Thinning was performed 15 days after planting, leaving one plant per hole, resulting in a plant density of 1296 plants per hectare. Weeding operations were carried out manually periodically for all treatments. Corn stalk borer was controlled using 10% diazinon insecticide (5 kg ha-1) twice: first, 20 days after emergence, and second, 15 days after the first application (Ministry of Agriculture, 2006.(

An extract, consisting of humic acid, was applied at rates of 0, 100, and 200 ml/m2 in three doses: before planting, 30 days after planting, and 45 days after planting. Wheat straw compost, an organic fertilizer, was uniformly incorporated into the soil to a depth of 15 cm, applied 20 days before planting. Mean values were compared using the Least Significant Difference (L.S.D) test at a significance level of 0.05 [14.[

Experimental Design and Treatments:

The treatments were randomly distributed within a Completely Randomized Block Design (RCBD) with three replications. The experiment included three main factors divided into levels as follows:

First Factor: Organic Fertilizer (Wheat Straw Compost(

Three levels of organic residues were added at rates of (0, 10, and 20) tons per hectare, represented as (E0, E1, E2.(

Second Factor: Bacterial Inoculation

Two levels of bacterial inoculation with Pseudomonas, represented as (B0, B1.(

Third Factor: Wheat Straw Extract

Three levels of wheat straw extract were added at rates of (0, 100, and 200) ml/m2, represented as (W0, W1, W2.(

Thus, the total number of treatments = 2 × 3 × 3 = 18 (with three replications.(

Number of experimental units = Number of treatments × Number of replications

Number of experimental units = 18 × 3 = 54 experimental units.

Table (2) Chemical Characteristics of Wheat Straw Organic Fertilize

Property	**Unit of Measurement**	**Value**
Humic Acid	%	4.41
Fulvic Acid	%	3.83
Organic Carbon	g kg^{-1}	339
Total Nitrogen	g kg^{-1}	23.77
Total Phosphorus	g kg^{-1}	13.5
Total Potassium	g kg^{-1}	27.78

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Table (3) Some Chemical Characteristics of Wheat Straw Extract

Property	Unit of Measurement	Value
pH	-	7.51
EC	dS m^{-1}	2.84
Total Nitrogen	g kg^{-1}	28.57
Total Phosphorus	mg kg^{-1}	5.8
Total Potassium	g kg^{-1}	136.02
Organic Matter	%	2.39
Iron	mg kg^{-1}	93.6
Zinc	mg kg^{-1}	13.3
Manganese	mg kg^{-1}	20.5

Isolation and Identification of Pseudomonas Bacteria:

A soil sample was taken from the rhizosphere of Babil Governorate, specifically from Al-Musayyib Technical Institute. The soil sample was then thoroughly sieved to remove plant root residues and gravel. Fifty grams of the sieved soil were weighed, and distilled water was added to prepare a soil suspension with a volume of 250 ml. One milliliter of the soil suspension was taken and inoculated onto Petri dishes containing the following culture media. The Petri dishes were then incubated at a temperature of 27°C for 2-3 days until bacterial colonies appeared. Subsequently, the following tests were conducted to diagnose Pseudomonas bacteria:

Gram staining and microscopic examination.

Catalase test.

Oxidase test.

IMVIC tests (Indole forming, Methyl-red, Voges-Proskauer, and Citrate test) [15-17.[

The culture media used were:

Nutrient agar.

Specialized media for the growth of Pseudomonas bacteria: Chromoagar for Pseudomonas and Pseudomonas agar base.

Bacterial purification:

After the appearance of green colonies and conducting the aforementioned tests to confirm the bacterial genus, a sample was taken from the colonies and subjected to serial dilution until 106 dilution. Then, 1 ml of the final dilution was inoculated onto Petri dishes containing Pseudomonas agar base and incubated at 27°C for two days. This process was repeated several times until pure colonies appeared.

Preparation of bacterial suspension:

A sample was taken from the pure colonies and inoculated into tubes containing 10 ml of N broth culture medium. The tubes were then incubated at 27°C for two days. After

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incubation, the bacterial suspension was centrifuged at 300 cycles per minute for 5 minutes. The sediment was resuspended in normal saline solution and adjusted to a total volume of 100 ml. The concentration of the bacterial strain used for inoculation was calculated based on the number of growing colonies on the Petri dishes and the dilution factor [18.[

Therefore, the concentration of the bacterial strain used was 8 × 105 (c.f.u ml-1) for the vaccine.

Measurements:

- Electrical conductivity (ECe) was measured in the saturated extract of the soil using an EC-meter as described in [19.[
- Soil pH was measured in a soil-water suspension (1:1 ratio) using a pH-meter as described in [20] and referenced in [21.[
- Organic matter content was determined using the Walkley and Black method as described in [22.[
- Available nitrogen was extracted using KCl 2N solution as mentioned in [23.[
- Available phosphorus was extracted using a sodium bicarbonate (0.5 M) solution at pH 8.5 according to the Olsen method, and the color was developed using the molybdate-ammonium solution and ascorbic acid. Measurement was performed using a spectrophotometer at a wavelength of 882 nanometers as described in [20.[
- Potassium content was determined using a Flamphotometer as described in [20.[
- Available micronutrients were extracted from the soil using the DTPA compound method [24] and measured using atomic absorption spectrophotometry.
- Organic residues were measured based on the method described in [25.[
- The organic extract and its humic acid content were measured according to the method outlined in [20.[
- Proline content was estimated using the method described in [26.[

Results and Discussion

Effect of Interactions between Pseudomonas fluorescens, Wheat Residues, and Organic Extract on Soil Microbial Respiration (mg CO2 g-1 dry soil(

The results presented in Table 4 showed that bacterial biofertilization, wheat residues, organic extract, and their interactions significantly affected the rate of soil microbial respiration. The bacterial biofertilization treatment (B1) resulted in the highest respiration rate of 7.162 mg CO2 g-1 dry soil compared to the control treatment (B0), which showed a rate of 5.386 mg CO2 g-1 dry soil. The addition of organic residues also significantly impacted microbial respiration, with the highest rate observed in treatment E2, which recorded 7.002 mg CO2 g-1 dry soil, compared to treatments E1 and E0, which recorded 6.435 mg CO2 g-1 dry soil each. Furthermore, the addition of the organic extract significantly increased microbial respiration, with the highest value in treatment W2 at 7.451 mg CO2 g-1 dry soil, compared to treatment W0, which recorded 4.669 mg CO2 g-1 dry soil.

The dual interactions of the study factors also led to a significant increase in microbial respiration, with the highest rate observed in the B1E2 treatment, which recorded 7.878 mg CO2 g-1 dry soil, compared to the B0E0 treatment, which recorded 4.397 mg CO2 g-1

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dry soil. Similarly, the dual interaction of bacterial biofertilization with the organic extract significantly increased microbial respiration, with the highest rate observed in the B1W2 treatment, recording 8.191 mg CO2 g-1 dry soil, compared to the B0W0 treatment, which recorded 3.471 mg CO2 g-1 dry soil. Additionally, the dual interaction of the organic extract with wheat residues significantly increased microbial respiration, with the highest rate observed in the E2W2 treatment, recording 8.285 mg CO2 g-1 dry soil, compared to the lowest value in the E0W0 treatment, which recorded 5.386 mg CO2 g-1 dry soil.

The table also shows that the triple interaction of the study factors significantly affected the increase in microbial respiration rate, with the highest rate observed in the B1E2W2 treatment, which recorded 9.103 mg CO2 g-1 dry soil, compared to the lowest value in the B0E0W0 treatment, which recorded 2.443 mg CO2 g-1 dry soil.

It is observed from Table 4 that the impact of bacterial biofertilization, wheat residues, organic extract, and their interactions significantly increased the rate of soil microbial respiration. Bacterial biofertilization provides a direct energy source to microorganisms, thus increasing their activity. This result aligns with findings by [27], who reported an increase in soil microbial respiration rate. Additionally, the organic extract containing organic acids leads to an increase in total soil nitrogen, available phosphorus, and potassium, consequently enhancing microbial activity in the soil, which is linked to an increased microbial respiration rate. Bacterial biofertilization also enhances the ability of these bacteria to decompose wheat residues, release organic acids, and liberate CO2, indicating increased microbial activity. Adding wheat residues to the soil and their interaction with the other factors provides organic carbon, a significant energy source, substantially increasing the microbial respiration rate

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Table (4): Effect of Pseudomonas fluorescens Interference, Wheat Residue, and Organic Extract Addition on Microbial Respiration in Soil (mg CO2 g-1 dry soil.(

Organic Fertilizers	Extract	Wheat Residues			B*W
		E0	E1	E2	
B0	W0	2.433	3.893	4.077	3.471
	W1	4.913	6.187	6.833	5.978
	W2	5.833	6.830	7.467	6.710
B1	W0	5.187	5.897	6.520	5.868
	W1	6.763	7.510	8.010	7.428
	W2	7.177	8.293	9.103	8.191

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0.05LSD	LSD: B*E*W = 0.582			LSD:B*W = 0.366
				B
B*E	4.397	5.637	6.126	5.386
	6.376	7.233	7.878	7.162
LSD 0.05	LSD:B*E = 0.336			LSD:B = 0.194
				W
W*E	3.815	4.895	5.298	4.669
	5.838	6.848	7.422	6.703
	6.505	7.562	8.285	7.451
LSD 0.05	LSD:W * E = 0.412			LSD:W = 0.237
Average E	5.386	6.435	7.002	
LSD 0.05	LSD : E= 0.237			

These findings align with [28], who reported that biofertilization plays a crucial role in decomposing wheat residues and increasing the availability of nutrients, including phosphorus, thereby creating a conducive environment for the growth of other microorganisms [29]. The increase in respiration rate is attributed to the colonization of bacteria in the rhizosphere, which stimulates physiological responses, resulting in increased root branching and biomass, thus affecting biological activity. The interactions among the study factors collectively increased the rate of soil microbial respiration.

Effect of Interaction between Pseudomonas fluorescens, Wheat Residues, and Organic Extract on Nitrogen Content in Leaves(%)

The statistical analysis results presented in Table 5 indicated that the addition of biofertilizer, wheat residues, organic extract, and their interactions had a significant effect on increasing the nitrogen concentration in maize leaves. The addition of biofertilizer significantly increased nitrogen concentration in the leaves, with the highest value recorded for treatment B1 at 1.750%, compared to treatment B0, which recorded 1.426%. The addition of wheat residues also significantly increased nutrient concentration in the leaves, with the highest value observed in treatment E2 at 1.699%, compared to treatments E1 and E0, which recorded 1.609% and 1.457%, respectively. Furthermore, the addition of the organic extract had a significant effect on increasing nitrogen concentration in maize leaves, with the highest value recorded for treatment W2 at 1.736%, compared to treatments W1 and W0, which recorded 1.620% and 1.409%, respectively.

Regarding the dual interactions of the study factors, they significantly increased

nitrogen concentration in the leaves, with the highest value observed in the B1E2 treatment at 1.869%, compared to the lowest value in the B0E0 treatment at 1.310%. The dual interaction of the organic extract and biofertilizer also significantly increased nitrogen concentration in the leaves, with the highest value observed in the B1W2 treatment at 1.950%, compared to the lowest value in the B0W0 treatment at 1.309%. Additionally, the dual interaction of wheat residues and organic extract significantly increased nitrogen concentration in the leaves, with the highest value observed in the E2W2 treatment at 1.878%, compared to the lowest value in the E0W0 treatment at 1.312%.

The table also shows that the triple interaction of the study factors had a significant effect on increasing nitrogen concentration in the leaves. The highest value was observed in the B1E2W2 treatment, which recorded 2.110%, compared to the lowest value in the B0E0W0 treatment, which recorded 1.190%, representing an increase of 43.6%.

Effect of Interaction between Pseudomonas fluorescens, Wheat Residues, and Organic Extract on Phosphorus Content in Leaves(%)

The statistical analysis results presented in Table 6 indicate that the study factors had a significant effect on increasing the phosphorus concentration in maize leaves. The addition of biofertilizer significantly increased phosphorus concentration in the leaves, with the highest value recorded for treatment B1 at 0.449%, compared to treatment B0, which recorded 0.387%. The addition of wheat residues also significantly increased phosphorus concentration in the leaves, with the highest value observed in treatment E2 at 0.473%, compared to treatments E1 and E0, which recorded 0.414% and 0.366%, respectively. Furthermore, the addition of the organic extract had a significant effect on increasing phosphorus concentration in maize leaves, with the highest value recorded for treatment W2 at 0.440%, compared to treatments W1 and W0, which recorded 0.420% and 0.393%, respectively.

Regarding the dual interactions of the study factors, they significantly increased phosphorus concentration in the leaves, with the highest value observed in the B1E2 treatment at 0.524%, compared to the lowest value in the B0E0 treatment at 0.353%. The dual interaction of the organic extract and biofertilizer also significantly increased phosphorus concentration in the leaves, with the highest value observed in the B1W2 treatment at 0.475%, compared to the lowest value in the B0W0 treatment at 0.369%

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Table (5): Effect of Pseudomonas fluorescens , Wheat Residue, and Organic Extract Addition on Nitrogen Percentage in Leaves.(%)

Organic Fertilizers	Extract	Wheat Residues			B*W
		E0	E1	E2	
B0	W0	1.190	1.320	1.417	1.309
	W1	1.350	1.470	1.523	1.448
	W2	1.390	1.527	1.647	1.521
B1	W0	1.433	1.517	1.577	1.509
	W1	1.620	1.837	1.920	1.792
	W2	1.757	1.983	2.110	1.950
0.05LSD		LSD: B*E*W = 0.141			LSD:B*W = 0.08
					B
B*E		1.310	1.439	1.529	1.426
		1.603	1.779	1.869	1.750
LSD 0.05		LSD:B*E = 0.08			LSD:B = 0.04
					W
W*E		1.312	1.418	1.497	1.409
		1.485	1.653	1.722	1.620
		1.573	1.755	1.878	1.736
LSD 0.05		LSD:W * E = 0.1			LSD:W = 0..05
Average E		1.457	1.609	1.699	
LSD 0.05		LSD : E= 0..05			

Additionally, the dual interaction of wheat residues and organic extract significantly increased phosphorus concentration in the leaves, with the highest value observed in the E2W2 treatment at 0.503%, compared to the lowest value in the E0W0 treatment at 0.346%.

The table also shows that the triple interaction of the study factors had a significant effect on increasing phosphorus concentration in the leaves. The highest value was observed in the B1E2W2 treatment, which recorded 0.550%, compared to the lowest value in the B0E0W0 treatment, which recorded 0.340%.

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Table (6): Effect of Pseudomonas fluorescens , Wheat Residue, and Organic Extract Addition on Phosphorus Percentage in Leaves(%)

Organic Fertilizers	Extract	Wheat Residues			B*W
		E0	E1	E2	
B0	W0	0.340	0.374	0.394	0.369
	W1	0.354	0.386	0.418	0.386
	W2	0.366	0.395	0.457	0.406
B1	W0	0.353	0.413	0.485	0.417
	W1	0.377	0.449	0.536	0.454
	W2	0.406	0.469	0.550	0.475
0.05LSD		LSD: B*E*W = 0.049			LSD:B*W = 0.028
					B
B*E		0.353	0.385	0.423	0.387
		0.387	0.443	0.524	0.449
LSD 0.05		LSD:B*E = 0.028			LSD:B = 0.016
					W
W*E		0.353	0.385	0.423	0.393
		0.365	0.417	0.477	0.420
		0.386	0.432	0.503	0.440
LSD 0.05		LSD:W * E = 0.035			LSD:W = 0..020
Average E		0.366	0.414	0.473	
LSD 0.05		LSD : E= 0.020			

Effect of Interaction between Pseudomonas fluorescens, Wheat Residues, and Organic Extract on Potassium Content in Leaves(%)

The statistical analysis results presented in Table 7 indicate that the addition of biofertilizer, wheat residues, organic extract, and their interactions significantly affected the increase in potassium concentration in maize leaves .

The addition of biofertilizer significantly increased potassium concentration in the leaves, with the highest value recorded for

treatment B1 at 1.76%, compared to treatment B0, which recorded 1.57%. The addition of wheat residues also significantly increased nutrient concentration in the leaves, with the highest value observed in treatment E2 at 1.76%, compared to treatments E1 and E0, which recorded 1.66% and 1.57%, respectively. Furthermore, the addition of the organic extract had a significant effect on increasing potassium concentration in maize leaves, with the highest value recorded for treatment W2 at 1.75%, compared to treatments W1 and W0, which recorded 1.66% and 1.58%, respectively.

Regarding the dual interactions of the study factors, they significantly increased potassium concentration in the leaves, with the highest value observed in the B1E2 treatment at 1.84%, compared to the lowest value in the B0E0 treatment at 1.46% .

The dual interaction of the organic extract and biofertilizer also significantly increased potassium concentration in the leaves, with the highest value observed in the B1W2 treatment at 1.87%, compared to the lowest value in the B0W0 treatment at 1.50%. Additionally, the dual interaction of wheat residues and organic extract significantly increased potassium concentration in the leaves, with the highest value observed in the E2W2 treatment at 1.85%, compared to the lowest value in the E0W0 treatment at 1.49%.

The table also shows that the triple interaction of the study factors had a significant effect on increasing potassium concentration in the leaves. The highest value was observed in the B1E2W2 treatment, which recorded 1.96%, compared to the lowest value in the B0E0W0 treatment, which recorded 1.40%.

The results in Tables (5, 6, and 7) show that the addition of bacterial biofertilizer, wheat residues, organic extract, and their interactions had a significant effect on the concentration of N, P, and K in the leaves. The observed increase in nutrient concentration can be attributed to the substantial role of biofertilizer in enhancing cell membrane permeability, photosynthesis, and root growth. Additionally, biofertilizer indirectly increases the efficiency of available or added fertilizers and directly improves all biological activities [30.[

The bacterial biofertilizer also plays a significant role in increasing the levels of sugars and amino acids, which are crucial in various biological processes, particularly cell division and expansion. Enhanced enzyme activity due to biofertilizer leads to the decomposition of organic compounds, releasing nutrients that become readily available for plant uptake. Amino acids, being essential nitrogen sources, contribute to protein and enzyme synthesis and provide energy, promoting vegetative and root growth. Consequently, nutrient translocation from the plant to the leaves increases, resulting in higher NPK concentrations in the leaves [31.[

The impact of biofertilizer on nitrogen, phosphorus, and potassium is also due to its ability to improve soil physical and chemical properties and fertility, thus enhancing nutrient absorption by plants. The response to biofertilizer was more pronounced when combined with the organic extract, which provides readily available nutrients [32-34]. The increase in nutrient concentration in the vegetative parts (leaves) is attributed to converting non-available forms into absorbable ones, aligning with findings by

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[35] who noted the significant role of biofertilizers in increasing average nutrient concentrations of nitrogen, phosphorus, and potassium in plants.

The presence of biofertilizer contributes essential nutrients, particularly nitrogen, which is fixed by Pseudomonas bacteria. These bacteria also promote the release of growth regulators like gibberellin, enhancing nitrogen utilization from the soil, reflected in the nitrogen content of the tubers .

Table (7): Effect of Pseudomonas fluorescens , Wheat Residue, and Organic Extract Addition on Potassium Percentage in Leaves (%).

Organic Fertilizers	Extract	Wheat Residues			B*W
		E0	E1	E2	
B0	W0	1.40	1.51	1.60	1.50
	W1	1.47	1.59	1.69	1.58
	W2	1.52	1.62	1.74	1.62
B1	W0	1.58	1.67	1.75	1.67
	W1	1.82	1.73	1.67	1.74
	W2	1.78	1.88	1.96	1.87
0.05LSD		LSD: B*E*W = 0.04			LSD:B*W = 0.023
					B
B*E		1.46	1.57	1.67	1.57
		1.68	1.76	1.84	1.76
LSD 0.05		LSD:B*E = 0.023			LSD:B = 0.013
					W
W*E		1.49	1.59	1.67	1.58
		1.57	1.66	1.75	1.66
		1.65	1.75	1.85	1.75
LSD 0.05		LSD:W * E = 0.028			LSD:W = 0..016
Average E		1.57	1.66	1.76	
LSD 0.05		LSD : E= 0.016			

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Moreover, the increased potassium and other nutrient concentrations in the leaves of maize inoculated with Pseudomonas fluorescens can be attributed to the successful colonization of maize roots by these bacteria. They produce the enzyme ACC-deaminase, which inhibits ethylene production, promoting root growth in inoculated plants. This, in turn, enhances the roots' ability to absorb potassium and other nutrients [36-37.[

The addition of wheat residues has also had a significant impact on increasing the concentrations of these nutrients in the leaves. One of the components of the residues, as indicated in Table (2), is their nitrogen, phosphorus, and potassium content, which has led to higher nutrient concentrations in the leaves. The availability of these readily accessible nutrients aids the plant in developing a robust vegetative system, thereby increasing the amount of absorbed nutrients and their accumulation in the vegetative parts. This supports the plant in performing its biological activities, resulting in an increased amount of synthesized materials in the leaves [38.[

Furthermore, the role of organic matter in providing the plant's needs for major and minor elements by supplying the required levels of nitrogen, phosphorus, and potassium and enhancing their absorption by the plant is crucial for optimal growth, which, in turn, reflects on yield attributes. The use of organic fertilizers, which include a significant number of organic acids, has an effective impact on the availability of nutrients to the plant [39]. Decomposed organic fertilizer plays a significant role in supplying the plant with nutrients by improving the physical and chemical properties of the soil and increasing the number and activity of microorganisms, which mineralize elements and make them available to the plant [40.[

Microorganisms produce CO2, which forms carbonic acid (H2CO3) when dissolved in water, lowering the soil pH and increasing the availability of most elements to the plant. Organic fertilizers also hold nutrients by adsorbing them onto the surfaces of humus colloids. Additionally, organic acids act as chelating agents for elements, preventing their fixation [41.[

The increased availability of nutrients in the leaves with the addition of organic extract can be attributed to the role of this extract, which contains humic and fulvic acids, in improving the biological, chemical, and physical properties of the soil, thus enhancing plant growth and the absorption of some nutrients in maize, including nitrogen [42]. Moreover, these added organic acids contribute to increasing the root system of the plant and the release of potassium and other nutrients from their minerals into the soil solution, enhancing nutrient absorption. They also lower the pH of the medium or contain phenolic or carboxyl groups with a chelating property for calcium, releasing phosphorus and serving as a rich source of phosphorus, thereby increasing its availability and absorption [43.[

The effect of the interaction between biofertilizer and organic extract in increasing nutrients in the leaves can be attributed to the addition of the extract containing humic and fulvic acids to the soil, leading to the formation of natural chelates that contribute to the release of various elements from soil

minerals in the root zone. This release increases with the addition of organic acids to the soil. Humic acids also increase the population of microorganisms in the soil, especially in the surface layer surrounding the roots, which produce substances that increase the availability of some nutrients, thus enhancing their absorption by the plant, as confirmed by previous studies [44.[

The effect of the triple interaction of the study factors significantly increased the concentrations of the primary nutrients in the maize leaves. This can be attributed to the components of organic matter and organic extract, specifically humic and fulvic acids, which play a role in determining the quantity and stability of certain elements in the soil. Humic and fulvic acids have a positive impact on nutrient absorption by the plant by enhancing the availability and mobility of nutrients, particularly micronutrients. The amino group in humic acids can adsorb negative phosphate ions and improve their availability to the plant [45]. Additionally, the application of humic acids to the soil has been shown to significantly increase phosphorus availability by converting phosphate esters into inorganic phosphorus, thereby enhancing its absorption [46.[

Furthermore, humic acids may inhibit harmful bacteria in the rhizosphere, while promoting and increasing the activity and numbers of beneficial microorganisms, which enhances phosphorus availability and uptake [47]. The addition of humic acid also provides carbon as an energy source and nitrogen as an essential nutrient, which boosts the growth and activity of microorganisms, including nitrogen-fixing organisms already present in the soil. This increases the availability of nitrogen in the soil, leading to higher nitrogen uptake by the plant [48]. Moreover, these acids enhance the absorption of monovalent ions such as ammonium and potassium by accelerating the active uptake by plant roots, as confirmed by previous studies [49, 50.[

Conclusion

The study demonstrated that the application of bacterial bio-fertilizer, wheat residues, and organic extract significantly enhanced microbial activity and nutrient content in maize plants grown in saline soil. Among the treatments, the use of organic extract (W2) was found to be more effective than wheat residues (E2) in promoting soil microbial respiration and nutrient uptake. The highest benefits were observed when the organic extract was used in conjunction with bacterial inoculation (B1), indicating that this combination optimally supports soil health and plant growth. Notably, the triple interaction of bio-fertilizer, wheat residues, and organic extract (B1E2W2) resulted in the highest soil microbial respiration rates and nutrient. These findings suggest that integrating organic extract and bacterial inoculation, particularly alongside wheat residues, is highly beneficial for improving the resilience and productivity of maize in saline soils.

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