

THE ROLE OF *PSEUDOMONAS FLUORESCENS* BACTERIA AND *TRICHODERMA HARZIANUM* FUNGUS IN THE INTERACTION WITH POULTRY MANURE IN THE GROWTH AND YIELD OF BASIL PLANTS (*OCIMUM BASILICUM* L.)

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Abstract

This study was conducted on basil (*Ocimum basilicum* L.) in the spring season of 2026 in Al-Qadisiyah Governorate - Nafar. The study aimed to determine the effect of three factors: the first factor was bacterial biofertilizer at two levels (Non inoculation and inoculation with *Pseudomonas fluorescens*); the second factor was fungal biofertilizer (no inoculation and inoculation with *Trichoderma harzianum*); and the third factor was poultry manure at three levels (3, 6, and 7) t. ha⁻¹, consisting of a single biofertilizer and a mixture of the fungus *Trichoderma harzianum* and the bacteria *Pseudomonas fluorescens* and the second factor was poultry manure (M) at three levels (2, 5, and 7) t.ha⁻¹. The (R.C.B.D.) experimental design was used in the field factorial experiment. The results showed that single inoculations and their dual and triple combinations achieved a significant increase in the studied traits: available NPK (mg/kg), bacterial number ($^{6}10^{*}C.F.U. g.soil^{-1}$), fungal number ($^{3}10^{*}C.F.U. g.soil^{-1}$), and leaf number(leaves.plant⁻¹), reaching (32, 49, 33, 65, 77, and 42)%, respectively, compared to the control. The results of the field experiment on basil confirmed that the economic costs of fertilizers can be reduced while maintaining soil health, thus enabling the use of sustainable Agriculture methods.

Keywords: NPK soil available, biofertilizer, sustainable agriculture, Basil plant.

INTRODUCTION

The productivity of different crops, both in quantity and quality, is of great importance to those interested and specialized in formulating agricultural policies around the world (5). The availability of nutrients in appropriate and environmentally safe quantities throughout the growth period is another challenge they face in order to achieve sustainable agriculture (24), in which natural organic or mineral materials and fungal, bacterial, or other inoculants are used to prepare the soil and the plant throughout the growth period and afterwards remain effective in the field soil (13). One of the problems of Iraqi soils is that they are calcareous soils with a high pH and calcium carbonate content, which causes alkalinity in the soil, causing phosphorus to fix or precipitate and nitrogen to volatilize as ammonia (25). *Pseudomonas fluorescens* bacteria produce organic acids that reduce the acidity of the medium, which encourages fungal growth and production and increases the availability of phosphorus in the soil on the one hand and micronutrients on the other (9). *Trichoderma harzianum* lives saprophytically on organic matter in the soil, primarily supplying the plant with nutrients and water, but it needs an environment that tends to be acidic (1). Both *Pseudomonas fluorescens* and *Trichoderma harzianum* are PGPF growth promoters that produce many growth regulators, vitamins, amino acids, siderophores, and other substances that promote plant and microbial growth. They can secrete many enzymes such as phosphatases, cellulase, and lipase, and also produce siderophores and IAA (5). Organic matter is one of the sustainable farming methods used in conjunction with biofertilization, as it contains 40-50% organic matter and contains micro and macro-nutrients for

the plant, enzymes and other substances beneficial to the plant (22). Organic matter is also characterized by its ability to store water and also by its ability to improve the physical, chemical, and biological properties of the soil, as it supplies the soil with important nutrients, organisms, enzymes, proteins, vitamins, and many compounds that biomass uses as a material subject to the activity of microorganisms (23, 13). A study was conducted in calcareous soil to inoculate pepper plants with three levels of *Trichoderma harzianum* fungus (0, 3, and 12 g.kg⁻¹ soil). The spore content in the inoculum was 2 x 10⁹ spore.kg⁻¹. Level 3 g.kg⁻¹ soil) resulted in the highest significant increase in plant height, dry shoot weight, and dry shoot weight, reaching (48, 35 and 38)%, respectively, compared to the control treatment. The increase in iron, magnesium, zinc, and copper in the root zone of the plant was also observed. It reached (38, 4, 30 and 40)% respectively compared to the control treatment. The researcher explained the reason as the ability of the fungus to supply the soil with micronutrients and nitrogen, and its ability to encourage the growth of lateral roots and increase their branching and spread in the soil, which led to an increase in its absorption of phosphorus and potassium and its supply to the plant (6,36,26). In a field experiment with barley, inoculating the field soil with *Trichoderma harzianum* spores led to a significant increase in the plant's nitrogen, phosphorus, and potassium content (6, 11, and 6)% respectively. Similarly, the soil's nitrogen, phosphorus, and potassium content (mg.kg⁻¹) increased significantly (7.5, 9, and 45)%, respectively when using *Trichoderma harzianum* as a bioinotropic compared to the control treatment. This increase was attributed to the fungus's enhanced nitrogen availability and the increased vigor and growth of the root system (33,19,4). A laboratory and field experiment was conducted using *Pseudomonas fluorescens*. The acids produced in the bacterial isolate used as a vaccine were measured: formic acid 152.88 (mg.L⁻¹), acetic acid

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144.98 (mg.L⁻¹), oxalic acid 135.68 (mg.L⁻¹), and citric acid 135.68 (mg.L⁻¹) at concentrations of 22.89 (mg.L⁻¹). In the field experiment, the soil content of nitrogen, phosphorus and potassium (mg.kg soil⁻¹) increased significantly at the end of the season by (22, 20 and 2)% respectively compared to the control, the researcher explained the reason for the increase as the ability of *Pseudomonas fluorescens* bacteria to secrete organic acids, which led to a local decrease in soil pH, thus increasing the availability of nitrogen, phosphorus and potassium (35,42,43). This study investigated the effects of *Pseudomonas fluorescens*, *Trichoderma harzianum*, poultry manure, and their binary and ternary interactions on reducing losses of added organic nitrogen and phosphorus and increasing potassium availability in calcareous soils. The study aimed to determine the growth potential of *Trichoderma harzianum* fungi in calcareous soils in interaction with *Pseudomonas fluorescens* and organic manure.

MATERIALS AND METHODS

Isolation of *Pseudomonas fluorescens* and production of bacterial inoculums

Then 10 grams of soil adjacent to the roots of wheat plants were taken and placed in 90 ml of physiological solution and shaken for 10 minutes. Then 1 ml of the solution was taken and distributed onto plates containing solid Pikovskaya medium and incubated at a temperature of 28°C for five days. The appearance of halos around the colonies indicated that the bacteria were *Pseudomonas* spp. as in (18). The following biochemical tests were then performed on the isolate: catalase test, oxidase test, urea analysis test, nitrate reduction test, Fuchs-Proscauer test, gelatin liquefaction test, starch analysis test, and indole test. According to (20), the isolate was identified as *Pseudomonas fluorescens*. Subsequently, 1 liter of sterile nutrient broth was prepared, and 1 ml of *Pseudomonas fluorescens* isolate inoculum was transferred to it. The inoculum was incubated at 30°C for 48 hours, and its density was estimated using standard titration methods. The density of the inoculum was $0.4 * 10^8$ Cfu.ml⁻¹. The following biochemical tests were then performed on the isolate: catalase test, oxidase test, urea analysis test, nitrate reduction test, Fuchs-Proscauer test, gelatin liquefaction test, starch analysis test, and indole test. According to (37), the isolate was identified as *Pseudomonas fluorescens*. Subsequently, 1 liter of sterile nutrient broth was prepared, and 1 ml of *Pseudomonas fluorescens* isolate inoculum was transferred to it. The inoculum was incubated at 30°C for 48 hours, and its density was estimated using standard titration methods. The density of the inoculum was $0.4 * 10^8$ Cfu.ml⁻¹.

Isolation and Production of *Trichoderma harzianum* Inoculum

1. Ten grams of wheat root soil were taken from a depth of 10 cm and placed in 10 ml of distilled water.
2. The mixture was shaken for 10 minutes, then 1 ml was taken and placed in 9 ml of sterile water to achieve a dilution of 10⁻⁸.
3. The 10⁻³ dilution was spread on a PDA plate, and streptomycin 75 mg.L⁻¹ was added to inhibit bacterial growth.

4. The plates were incubated for 4 days at 27°C. Cottony colonies appeared and turned green (15).
5. A portion of the colony's edge (mycelium) was taken before it turned green and transferred to PDA medium. After incubation under the same conditions as before, homogeneous fungi appeared in the colonies and spores. The medium was then incubated on a tilted surface and stored at 4°C in a refrigerator (2,14)
6. Wheat seeds were used after being soaked overnight. 2% calcium carbonate was added, and the seeds were packed into heat bags equipped with a cotton vent bottle. The bags were then sterilized at 121°C and a pressure of 15 psi for 20 minutes.
7. The seeds were then contaminated with fungal growth and incubated. The bags were incubated at 27°C and shaken twice daily to distribute the growth. After 10 days, the growth covered the wheat seeds (7).
8. Then it was dried under a temperature of 35°C, the grains were ground and mixed with sterile peat moss as a carrier at a ratio of 1:20 (powder: dry matter). The density of the pollen was $2 * 10^8$ spore g⁻¹ (8).

Soil preparation and planting

The field was plowed, smoothed and leveled, then the field was divided into three replicates, each with 12 experimental units of 1*1 m. The plants were planted in rows, 20 cm apart, and 10 cm apart. The number of plants in the experimental unit was 40 plants. Local basil seeds were used after applying 6% gum arabic for 15 minutes, and poultry manure was added mixed with the surface layer to a depth of 15 cm. Planting took place on 15/3/2026, and the field soil and poultry manure were analyzed as shown in Table (1)

Table 1 shows some properties of soil and organic fertilizer (poultry manure)

Measured character	Value	Unit	
Soil	pH _{1:1}	7.49	-
	CaCO ₃	17	%
	O.M	0.5	
	N	22	mg kg ⁻¹
	P	3.11	
	K	187.26	
	Total bacteria	$1.75 * 10^7$	C.F.U g ⁻¹ Soil
	Total fungi	$1.35 * 10^3$	
poultry manure	pH	7.57	-
	N	3	%
	P	1.5	
	K	1.1	
	C	46.33	
were measured according to (3)			

Factors and Experimental Design

The field experiment used three replicates and three factors: the first factor was *Pseudomonas fluorescens* bacteria, the second factor was *Trichoderma harzianum* fungus, and the third factor was three levels of poultry manure (3, 6, and 7) t. ha⁻¹. The factorial experiment was conducted according to a randomized complete block design (RCBD), and 25% of the full recommended fertilizer equivalent (170-170-200) kg ha⁻¹ (N-P₂O₅-K₂O) was used. Statistical analyses were performed using GenStat 2007 software on a Windows 7 PC operating system. To study the type of significance between the different treatments, the LSD was used at a probability level of 0.05.

Measured Characteristics: The following characteristics were measured in plants 60 days after planting

1. Number of leaves (leave plant⁻¹)
2. Available NPK (mg.kg⁻¹) in the soil. Available nitrogen in the soil was estimated using a MicroKjeldahl apparatus. Available phosphorus was then extracted and estimated using sodium bicarbonate according to (Olsen's method) in alkaline soils using a spectrophotometer. Available potassium was extracted and estimated in the soil using ammonium acetate using a flame photometer as described in (3).
3. Count of *Pseudomonas fluorescens* bacteria (C f u g⁻¹ soil). Phosphate-solubilizing bacteria were counted by dilution and plate counting using King's B medium modified according to (3).
4. Preparation of *Trichoderma harzianum* fungi (C f u g⁻¹ soil). by dilution and plate counting using TSM medium, (3).

Results

Table (2). shows that the individual treatments of *Pseudomonas fluorescens* bacteria (P), *Trichoderma harzianum* fungus (T), and poultry manure (M) had significant effects ($P \leq 0.05$) on the available nitrogen content (mg.kg⁻¹) of basil soil. The highest effect was observed with the *Pseudomonas fluorescens* treatment (mg.kg⁻¹), compared to the control treatment (mg.kg⁻¹). However, for the duple interaction treatments (*Pseudomonas fluorescens* + *Trichoderma harzianum*), (*Pseudomonas fluorescens* + poultry manure), and (*Trichoderma harzianum* + poultry manure), the double interaction treatment (*Trichoderma harzianum* + poultry manure 7 t. ha⁻¹) gave the highest significant increase in the double interactions. The highest significant increase was observed in Mono interactions, reaching (mg.kg⁻¹) compared to the control treatment (mg.kg⁻¹). As for the triple interaction (*Pseudomonas fluorescens* + *Trichoderma harzianum* + poultry manure 7 tons ha⁻¹), it achieved the highest significant increase in all field trial treatments, reaching (mg.kg⁻¹) compared to the control (mg.kg⁻¹).

Table (3) shows that Mono treatments of *Pseudomonas fluorescens* bacteria (P), *Trichoderma harzianum* fungus (T), and poultry manure (M) had significant effects ($P \leq 0.05$) on the available phosphorus content (mg.kg⁻¹) of basil soil. The highest effect was observed with the *Pseudomonas fluorescens* treatment, reaching 12.87 mg.kg⁻¹, compared to the control treatment of 8.90 mg.kg⁻¹. As for the dual interaction treatments (*Pseudomonas fluorescens* + *Trichoderma harzianum*), the treatment (*Pseudomonas fluorescens* + poultry manure), and the treatment (*Trichoderma harzianum* + poultry manure), the dual interaction treatment (*Trichoderma harzianum* + poultry manure 7 t. ha⁻¹) gave the highest significant increase in the dual interactions, reaching 31.56 (mg.kg⁻¹) compared to the control of 8.23 (mg.kg⁻¹). The triple interaction (*Pseudomonas fluorescens* + *Trichoderma harzianum* + poultry manure 7 t. ha⁻¹) achieved the highest significant increase in all field trial treatments, reaching 14.31 (mg.kg⁻¹) compared to the control of 7.34 (mg.kg⁻¹).

Table (4) shows that Mono treatments of *Pseudomonas fluorescens* (P), *Trichoderma harzianum* (T), and poultry manure (M) had significant effects ($P \leq 0.05$) on the available potassium content (mg.kg⁻¹) of basil soil. The highest effect was observed with the *Pseudomonas fluorescens* treatment, reaching 187.89 mg.kg⁻¹, compared to the control 149.83 mg.kg⁻¹. As for the dupal interaction treatments (*Pseudomonas fluorescens* + *Trichoderma harzianum*), the treatment (*Pseudomonas fluorescens* + poultry manure), and the treatment (*Trichoderma harzianum* + poultry manure), the dual interaction treatment (*Trichoderma harzianum* + poultry manure 7 t. ha⁻¹) gave the highest significant increase in the duple interactions, reaching 138.99 (mg.kg⁻¹) compared to the control treatment of 195.111 (mg.kg⁻¹). However, the triple interaction (*Pseudomonas fluorescens* + *Trichoderma harzianum* + poultry manure 7 t. ha⁻¹) achieved the highest significant increase in all field trial treatments, reaching 198.33 (mg.kg⁻¹) compared to the control 132.00 (mg.kg⁻¹). It is observed that Tables 2, 3, and 4 show almost the same trend in the effect of the studied factors. *Pseudomonas fluorescens* bacteria produce specialized enzymes such as phytase, which breaks down phytic acid (the organic phosphorus reserve).

Table 2. Effect of the study factors on available soil nitrogen (mg.kg⁻¹)

Ingluation <i>P. fluorescens</i> (f)	Ingluation <i>T. Harzianum</i> (d)	Munroe fertilizer t.ha ⁻¹ (M)			Mine(f)
		3	6	7	
Non Incolation	Non-Incolation	22.483	23.413	24.363	24.639
	Incolation	25.293	25.877	26.403	
Incolation	Non-Incolation	26.783	27.447	29.813	30.114
	Incolation	31.250	32.013	33.377	
Lsd=0.4282					Lsd=0.1748
Intraction <i>T. Harzianum</i> * M					Mine (d)
Non-Incolation <i>T. Harzianum</i>		23.888	24.645	25.383	25.717
Incolation <i>T. Harzianum</i>		29.017	29.017	31.595	29.036
Lsd=0.3028					Lsd=0.1748
Intraction <i>P. fluorescens</i> * M					
Non-Incolation <i>P. fluorescens</i>		24.633	25.430	27.088	Lsd= 0.3028
Incolation <i>P. fluorescens</i>		28.272	28.945	29.890	
Intraction <i>P. fluorescens</i> * <i>T. Harzianum</i>					
Non-Incolation <i>P. fluorescens</i> (f)	Non <i>T. Harzianum</i>	23.420			Lsd= 0.3028
	<i>T. Harzianum</i>	25.858			
Incolation <i>P. fluorescens</i>	Non- <i>T. Harzianum</i>	28.014			
	<i>T. Harzianum</i>	32.213			
Mine Munroe fertilizer t.ha ⁻¹ (M)		26.452	27.187	28.489	Lsd=0.2141

Table 3. Effect of the study factors on available soil phosphorus (mg.kg⁻¹)

Inglutation <i>P. fluorescens</i> (f)	Inglutation <i>T. Harzianum</i> (d)	Munroe fertilizer t.ha ⁻¹ (M)			Mine(f)
		3	6	7	
Non Incolation	Non-Incolation	7.347	8.143	8.830	8.902
	Incolation	9.130	9.630	10.333	
Incolation	Non-Incolation	11.013	11.947	12.777	12.872
	Incolation	13.380	13.803	14.310	
Lsd=0.2300					lsd 0.1150
Intrraction <i>T. Harzianum</i> * M					Mine (d)
Non-Incolation <i>T. Harzianum</i>		9.180	10.045	10.803	10.009
Incolation <i>T. Harzianum</i>		11.255	11.717	12.322	11.764
Lsd=0.1626					Lsd=0.1626
Intrraction <i>P. fluorescens</i> * M					
Non-Incolation <i>P. fluorescens</i>		8.238	8.887	9.582	
Incolation <i>P. fluorescens</i>		12.197	12.875	13.543	
Intrraction <i>P. fluorescens</i> * <i>T. Harzianum</i>					Lsd= 0.1328
Non-Incolation <i>P. fluorescens</i> (f)	Non <i>T. Harzianum</i>	8.107			
	<i>T. Harzianum</i>	9.698			
Incolation <i>P. fluorescens</i>	Non- <i>T. Harzianum</i>	11.912			
	<i>T. Harzianum</i>	13.831			
Mine Munroe fertilizer t.ha ⁻¹ (M)		10.218	10.881	11.562	lsd 0.1150

Table 4. Effect of the study factors on available soil potassium (mg.kg⁻¹)

Inglutation <i>P. fluorescens</i> (f)	Inglutation <i>T. Harzianum</i> (d)	Munroe fertilizer t.ha ⁻¹ (M)			Mine(f)
		3	6	7	
Non Incolation	Non-Incolation	132.00	139.67	145.00	149.83
	Incolation	153.67	161.33	167.33	
Incolation	Non-Incolation	175.33	180.67	186.00	187.89
	Incolation	191.33	195.67	198.33	
Lsd=1.925					Lsd= 0.786
Intrraction <i>T. Harzianum</i> * M					Mine (d)
Non-Incolation <i>T. Harzianum</i>		153.67	160.17	165.50	159.78
Incolation <i>T. Harzianum</i>		172.50	178.50	182.83	177.94
Lsd=1.361					Lsd=0.963
Intrraction <i>P. fluorescens</i> * M					Lsd= 1.361
Non-Incolation <i>P. fluorescens</i>		142.83	150.50	156.17	
Incolation <i>P. fluorescens</i>		183.33	188.17	192.17	
Intrraction <i>P. fluorescens</i> * <i>T. Harzianum</i>					Lsd= 1.361
Non-Incolation <i>P. fluorescens</i> (f)	Non <i>T. Harzianum</i>	138.89			
	<i>T. Harzianum</i>	160.78			
Incolation <i>P. fluorescens</i>	Non- <i>T. Harzianum</i>	180.67			
	<i>T. Harzianum</i>	195.11			
Mine Munroe fertilizer t.ha ⁻¹ (M)		163.08	169.33	174.17	Lsd= 1.112

Table 5. Effect of study factors on bacterial counts in 10⁻⁷*C.F.U g⁻¹ Soil

Inglutation <i>P. fluorescens</i> (f)	Inglutation <i>T. Harzianum</i> (d)	Munroe fertilizer t.ha ⁻¹ (M)			Mine(f)
		3	6	7	
Non Incolation	Non-Incolation	2.500	3.503	4.267	4.186
	Incolation	4.407	5.210	5.227	
Incolation	Non-Incolation	5.307	5.757	6.593	6.342
	Incolation	6.677	6.527	7.190	
Lsd=0.4999					Lsd= 0.2041
Intrraction <i>T. Harzianum</i> * M					Mine (d)
Non-Incolation <i>T. Harzianum</i>		3.903	4.630	5.430	4.654
Incolation <i>T. Harzianum</i>		5.542	5.868	6.208	5.873
lsd =0.3535					Lsd= 0.2041
Intrraction <i>P. fluorescens</i> * M					Lsd=0.3535
Non-Incolation <i>P. fluorescens</i>		3.453	4.357	4.747	
Incolation <i>P. fluorescens</i>		5.992	6.142	6.892	
Intrraction <i>P. fluorescens</i> * <i>T. Harzianum</i>					Lsd= 0.2886
Non-Incolation <i>P. fluorescens</i> (f)	Non <i>T. Harzianum</i>	3.423			
	<i>T. Harzianum</i>	4.948			
Incolation <i>P. fluorescens</i>	Non- <i>T. Harzianum</i>	5.886			
	<i>T. Harzianum</i>	6.798			
Mine Munroe fertilizer t.ha ⁻¹ (M)		4.722	5.249	5.819	Lsd= 0.2500

They also produce low molecular weight siderophores that bind iron and other nutrients, taking them into the bacteria and bypassing pathogens. In other words, they redirect nutrients, binding iron to phosphorus, and then the iron is released from the iron-phosphorus bond (37, 41, 10). Furthermore, IAA production leads to increased root branching and elongation, resulting in a larger total root area available for nutrient and water absorption. This, in turn, increases the uptake of mineral nitrogen, phosphorus, and potassium by the plant, as well as micronutrients such as iron and zinc (43, 11, 26).

The fungus *T. harzianum* increases nitrogen utilization efficiency by mineralizing organic nitrogen present in poultry manure. It secretes enzymes (proteases, peptidases, and deaminases) that break down poultry manure residues and convert complex organic nitrogen into readily absorbable mineral forms, namely ammonium and nitrate (42, 39). Inoculation of plants with the fungus *T. harzianum* stimulates nitrate and ammonium transporters in root cell membranes, accelerating the rate of nitrogen uptake from the soil solution. The fungus also secretes organic acids such as gluconic, citric, fumaric, and succinic acids, which lower the pH locally in the plant's rhizosphere. Additionally, the fungus secretes acid phosphatases and phytases, which are specialized enzymes for breaking ester bonds in organophosphorus bound to organic matter and phytic acid. Due to the protons (^+H) in these organic acids, they attack the surfaces that bind to potassium ions between clay layers, leading to the uptake and release of potassium into the soil (37,29,30).The fungus produces compounds similar to IAA compounds that stimulate cell division in the roots, resulting in a change in the length of the main root, lateral roots, and root hairs. The root hairs reach places they have not reached before, where nutrients, especially phosphorus, are present in the normal root that is not infected with *Trichoderma harzianum* (41,16,27).

Table (5) showed the effects of Mono treatments of *Pseudomonas fluorescens* bacteria (P), *Trichoderma harzianum* fungus (T), and poultry manure (M) at a significance level of ($P \leq 0.05$) for the number of bacteria in 10^{-7} *C.F.U g^{-1} Soil. The highest increase was observed in the *Pseudomonas fluorescens* treatment, reaching $6.34 * CFU g^{-1}$ Soil compared to the control $4.18 * 10^{-7}CFU g^{-1}$ Soil. Among the binary interactions (*Pseudomonas fluorescens* + *Trichoderma harzianum*), (*Pseudomonas fluorescens* + poultry manure), and (*Trichoderma harzianum* + poultry manure), the binary interaction treatment (*Trichoderma harzianum* + 7 t ha^{-1} poultry manure) yielded the highest significant increase, reaching $6.89 * CFU g^{-1}$ Soil compared to the control treatment of $3.45 * CFU g^{-1}$ Soil. However, the triple interaction (*Pseudomonas fluorescens* + *Trichoderma harzianum* + poultry manure 7 t. ha^{-1}) achieved the highest significant increase in all field trial treatments, reaching $7.19 C.F.U g^{-1}$ Soil compared to the control $2.50 C.F.U g^{-1}$ Soil.

Table (6) results showed the presence of effects in Mono treatments of *Pseudomonas fluorescens* bacteria (P), *Trichoderma harzianum* fungus (T), and poultry manure (M) at a significance level of ($P \leq 0.05$). *Trichoderma harzianum* fungus *C.F.U g^{-1} Soil. The highest increase was observed in the *Trichoderma harzianum* treatment, reaching $4.27* CFU g^{-1}$ Soil compared to the control treatment of $2.11* CFU g^{-1}$ Soil. Among the duple interactions (*Pseudomonas fluorescens* + *Trichoderma harzianum*), (*Pseudomonas fluorescens* + poultry manure), and (*Trichoderma harzianum* + poultry manure), the

binary interaction (*Trichoderma harzianum* + 7 tons ha^{-1} poultry manure) yielded the highest significant increase, reaching $5.04* CFU g^{-1}$ Soil compared to the control treatment of $1.52* CFU g^{-1}$ Soil. The triple interaction (*Pseudomonas fluorescens* + *Trichoderma harzianum* + poultry manure) also showed significant results. Poultry (7 t. h^{-1}) achieved the highest significant increase in all field trial treatments, reaching $5.80 C.F.U g^{-1}$ Soil compared to the control of $1.33 C.F.U g^{-1}$ Soil.

Tables 5 and 6 show the results of the effects of bacterial inoculation (P) *Pseudomonas fluorescens* and fungal inoculation (T) *Trichoderma harzianum*, and the addition of poultry manure (M) on the populations of *Pseudomonas fluorescens* and *Trichoderma harzianum*. If inoculation leads to a significant increase in the populations of *Pseudomonas fluorescens* and *Trichoderma harzianum*, this indicates the efficacy and viability of the inoculation used in the study. This is also due to the interaction of the inoculations with organic matter, which contains various nutrients, proteins, vitamins, acids, esters, and fats. These nutrients are utilized as biomass in the soil (42, 17, 30). The significant increase in organic matter levels reflects the importance of providing adequate quantities of essential materials to supply the biological community with carbon and nutrients such as iron, zinc, manganese, copper and boron required for sustaining reproduction and biological production, as poultry manure stimulates bacteria and fungi to secrete decomposition enzymes such as phosphatase, protease, urease and phytas (41,21,32). The results of the duple interaction also show an increase in the numbers of *Pseudomonas fluorescens* bacteria and *Trichoderma harzianum* fungus, reflecting the lack of influence of one organism on the other and their coexistence in one environment, benefiting from each other's products as a substance subject to the action of both of them, as both produce IAA, cedreforsate, humic, fulvic, oxalic, gluconic, and other organic acids (39, 40, 43).

Table (7) shows the effects of mono treatments of *Pseudomonas fluorescens* bacteria (P), *Trichoderma harzianum* fungus (T), and poultry manure (M) at a significance level of ($P \leq 0.05$) on leaf count in plants. The highest effect was observed in the *Pseudomonas fluorescens* treatment, reaching 33.78 (leaves.plant $^{-1}$) compared to the control treatment of 25.06 (leaves.plant $^{-1}$). This may be due to the bacteria's ability to increase the availability of phosphorus and nitrogen. Some recent research has indicated the potential of *Pseudomonas fluorescens* bacteria to fix atmospheric nitrogen (39,12)As for the dual interaction treatments (*Pseudomonas fluorescens* + *Trichoderma harzianum*), the treatment (*Pseudomonas fluorescens* + poultry manure), and the treatment (*Trichoderma harzianum* + poultry manure), the dual interaction treatment (*Trichoderma harzianum* + poultry manure 7 t. ha^{-1}) gave the highest significant increase in the duple interactions, reaching 35.33 (leaves .plant $^{-1}$) compared to the control treatment of 23.83 (leaves .plant $^{-1}$). The reason is due to the fungus's ability to utilize organic wastes, as they are rich in nitrogen, phosphorus, and potassium, which led to the accumulation of nutrients in the plant (34,38).However, the triple interaction (*Pseudomonas fluorescens* + *Trichoderma harzianum* + 7 t. ha^{-1} of poultry manure) achieved the highest significant increase in all field trial treatments, reaching 38.33 (leaves.plant $^{-1}$) compared to the control treatment of 22.00 (leaves.plant $^{-1}$).

Table 6. Effect of Study Factors on Fungal Counts (C.F.U g⁻¹ Soil)

Inglutation <i>P. fluorescens</i> (f)	Inglutation <i>T. Harzianum</i> (d)	Munroe fertilizer t.ha ⁻¹ (M)			ine(f)
		3	6	7	
Non Incolation	Non-Incolation	1.333	1.567	1.667	2.122
	Incolation	2.433	2.833	2.900	
Incolation	Non-Incolation	3.000	3.600	3.900	4.272
	Incolation	4.500	4.833	5.800	
Lsd=0.3304					lsd0.1349
Intracction <i>T. Harzianum</i> * M					Mine (d)
Non-Incolation <i>T. Harzianum</i>		2.167	2.583	2.783	2.511
Incolation <i>T. Harzianum</i>		3.467	3.833	4.350	5.883
Lsd= 0.1908					lsd0.1349
Intracction <i>P. fluorescens</i> * M					
Non-Incolation <i>P. fluorescens</i>		1.883	2.200	2.283	Lsd=
Incolation <i>P. fluorescens</i>		3.750	4.217	4.850	
					0.2337
Intracction <i>P. fluorescens</i> * <i>T. Harzianum</i>					
Non-Incolation <i>P. fluorescens</i> (f)	Non <i>T. Harzianum</i>	1.522			Lsd=
	<i>T. Harzianum</i>	2.722			
Incolation <i>P. fluorescens</i>	Non- <i>T. Harzianum</i>	3.500			0.2337
	<i>T. Harzianum</i>	5.044			
Mine Munroe fertilizer t.ha ⁻¹ (M)		2.817	3.208	3.567	lsd0.1652

Table 7. Effect of study factors on the number of (leaves .plant⁻¹)

Inglutation <i>P. fluorescens</i> (f)	Inglutation <i>T. Harzianum</i> (d)	Munroe fertilizer t.ha ⁻¹ (M)			Mine(f)
		3	6	7	
Non Incolation	Non-Incolation	22.00	22.67	24.33	25.06
	Incolation	25.67	27.33	28.33	
Incolation	Non-Incolation	29.67	30.67	33.00	33.78
	Incolation	34.67	36.33	38.33	
Lsd=1.594					Lsd=0.651
Intracction <i>T. Harzianum</i> * M					Mine (d)
Non-Incolation <i>T. Harzianum</i>		25.83	26.67	28.67	27.06
Incolation <i>T. Harzianum</i>		30.17	31.83	33.33	31.78
Lsd= 1.127					
Intracction <i>P. fluorescens</i> * M					Lsd= 0.651
Non-Incolation <i>P. fluorescens</i>		23.83	25.00	26.33	Lsd=
Incolation <i>P. fluorescens</i>		32.17	33.50	35.67	
					1.127
Intracction <i>P. fluorescens</i> * <i>T. Harzianum</i>					
Non-Incolation <i>P. fluorescens</i> (f)	Non <i>T. Harzianum</i>	23.00			Lsd=0.920
	<i>T. Harzianum</i>	27.11			
Incolation <i>P. fluorescens</i>	Non- <i>T. Harzianum</i>	31.11			Lsd=
	<i>T. Harzianum</i>	36.44			
Mine Munroe fertilizer t.ha ⁻¹ (M)		28.00	29.25	31.00	Lsd= 0.797

This may be due to the fact that the 7 t.ha⁻¹ of poultry manure level is the most suitable level that does not cause toxicity to *Pseudomonas fluorescens* bacteria and *Trichoderma harzianum* fungus, and the ability of the two organisms to make the most of the organic matter and the various nutrients it contains, which are supplied to the plant and are reflected in the nutritional status and accumulation of food within the plant (37,38).

Conclusion

The results of the experiment showed that the individual treatments of *Pseudomonas fluorescens* (P), *Trichoderma harzianum* (T), and poultry manure (M), as well as the duple and triple treatments, had effects on the studied traits: available NPK in the root zone of the plant, *Pseudomonas fluorescens* bacterial count, *Trichoderma harzianum* fungal count, and the number of leaves in basil plants. The results of the study showed that the triple interaction (*Pseudomonas fluorescens* + *Trichoderma harzianum* + 7 t.ha⁻¹ of poultry manure) achieved the highest significant increase in all the traits measured in the field experiment. This interaction reflects the importance of bio-fertilization and organic fertilization in soil and plant health. The use of this interaction led to a 75% reduction in the recommended mineral fertilizer.

This reduces pollution on the one hand and economic costs on the other. We also call for the importance of repeating such field experiments to clarify the role of different bio-fertilizer and organic fertilizer interactions in basil growth and yield.

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