

The role of the dupe interaction of *Tricoderma hirazanum* fungus and *Azotobacter* ssp bacteria with sulfur in the growth and yield of onions (*Allium cepa* L.) and some active compounds

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Abstract

A field experiment was conducted on onion plants at the spring season of 2025 to study the dupe interaction of three factors: the first factor was the fungus *Tricoderma hirazanum* at three levels (0, 2, and 4) kg.ha⁻¹ the second factor was inoculation with *Azotobacter* ssp at two levels (non inoculation and inoculation); and the third factor was agricultural sulfur at three levels (0, 30, and 50 kg.ha⁻¹). A B.R.C.D. design was used. After 90 days of planting, the following indicators were measured: wet weight of the onion (g), protein content (mg.g⁻¹), ascorbic acid (mg.kg⁻¹), *Azotobacter* ssp. 10⁷ CUF g⁻¹, and the dupe interaction treatment (T₄*B₁) inoculation with *Tricoderma hirazanum* 10⁻³ CUF g⁻¹ in the onion rhizosphere. *Tricoderma hirazanum* inoculation yielded 4 kg.ha⁻¹ + *Azotobacter* ssp inoculation. The highest significant increase was observed when the treatments reached 237.7 (g), 1.29 (mg.g⁻¹), 42.10 (mg.kg⁻¹), 4.10* 10⁷ CUF g⁻¹, and 71.71* CUF 10³ g⁻¹, compared to the control 168.7 (g), 0.83 (mg.g⁻¹), 24.67 (mg.kg⁻¹), 2.56* 10⁷ CUF g⁻¹, and 5.11* 10³ CUF g⁻¹, respectively. This experiment led to an increase in the productivity and quality of the onion crop and a reduction in environmental pollution resulting from the addition of mineral fertilizers.

Keywords: Ascorbic acid, *Azotobacter* ssp, onion, *Trichoderma harzianum*

Introduction

Onion (*Allium cepa* L.) has great economic value, as it is the second most grown crop in arid and semi-arid regions (Srinirasan *et al.*, 2024). It also contains vitamins, minerals, proteins, and carbohydrates, and is characterized by low fat and calorie content (Intiaj and Tae 2008). 70 mg per 100 g of dry onion contains 6.4 mg of ascorbic acid (vitamin C) and 8.6 mg of protein (Kumar *et al.* 2019; Bozókí, B. *et al.*, 2025). Onions require relatively large amounts of nitrogen because it is the essential element in plant metabolism. Sufficient nitrogen in the plant enhances metabolic pathways for fat synthesis, increases metabolic activity, boosts energy storage, and increases antioxidant compounds (Biti *et al.*, 2022; Zhao *et al.*, 2021). Mineral fertilization pollutes the soil; therefore, it is important to use fungal and bacterial biofertilizers. These contribute to supplying the soil and plant with nutrients, including nitrogen and other biologically

active substances (Bozókí, B., *et al.*, 2025), in a manner appropriate to the plant's growth period, resulting in healthy and safe food (Zaib, 2023; Ali Al-Husseni, *et al.*, 2021). The fungus *Tricoderma hirazanum* colonizes onion roots and secretes chemical compounds that dissolve mineral compounds in the soil and release nutrients into the soil solution for the plant to benefit from (Altomare, 2015; Maji, 2015). Among the substances secreted by *Tricoderma hirazanum* are several hormones such as BAP (nitrile aminourine), IAA (3-indoleacetic acid), GA₃ (gabarlin acid), and others, these hormones work to lengthen and branch the roots and increase the density of root hairs, thus absorbing water and nutrients more efficiently, which increases plant growth and production, *T. hirazanum* also produces a group of enzymes that increase the availability of phosphorus, potassium, and iron, and secretes iron-chelating substances called sideroforces, which chelate iron and zinc (Mishu, 2013;).

The use of bacterial inoculant as a biofertilizer contributes to increasing the quality of onions. A field experiment was conducted by dipping onion roots in a bacterial inoculant *Azotobacter* ssp with a density of 3×10^9 CUF, which led to an increase in the dry weight of the onion by 8-10% and an increase in the protein content of the onion by 56%, due to the effectiveness of the bacterial inoculant (Prab et al., 2013; Al-Juthery, et al., 2019). The sulfur content in the onion increased by 4% due to the inoculation of the onion plant with *Azotobacter* bacteria, sulfur deficiency reduces crop productivity and negatively affects crop quality and plant health, sulfur is of great importance in plant cellular metabolism (Verma et al., 2020). As it is involved in the composition of sulfur containing organic acids and sulfur containing amino acids with low molecular weights that have volatile aromas which determine the flavor and taste (Indira and Singh 2014). In a field experiment with two agents, the first agent was four levels of *T. hirazanum* inoculant (0, 1.5, 3, and 6 kg. ha⁻¹) and the second agent was five levels of sulfur (0, 25, 50, 75, and 100 kg. ha⁻¹). Inoculant *T. hirazanum* resulted in the highest significant increase in sulfur content in the bulb at the (3) kg. ha⁻¹ level, where the sulfur addition coefficient reached 0.13% compared to the control 0.10%. However, the highest sulfur content in the bulb was at the (50) kg. ha⁻¹ level, reaching 0.14% compared to the control 0.09% (Akber et al., 2024). In this study, *T. hirazanum* fungi, *Azotobacter* ssp bacteria, and agricultural sulfur were used to determine the effect of the dual interaction and to identify the optimal level between the levels of these factors in increasing the growth and productivity of onions (*Allium cepa* L.) and some active compounds under the agricultural environmental conditions of the Nafar region Al-Qadisiyah Iraq.

Materials and Methods

Azotobacter ssp inoculation

Azotobacter ssp bacterial culture was obtained from the Biotechnology Laboratory - Department of Agricultural Research - Ministry of Science and Technology at a density of (3×10^{12} Cuf ml⁻¹) and multiplied using 1000 ml of PDA liquid medium. The bacteria were counted in the rhizosphere of onion plants after 90 days of planting using (2 ml) of liquid medium (Sucrose mineral salts). After sterilizing it with an autoclave for (20 minutes) at a temperature of 121 °C and a pressure of 15 (pound.in⁻¹), the tubes were inoculated with (1 ml) of dilution of (10^7) and incubated for three days at a temperature of (28°C) (Kende, Z., et al., 2024). The brown film was found, which is an indicator of *Azotobacter* spp. bacteria, The number of bacteria was estimated by the dilution and counting

method on plates using the specialized medium for them (Becking 1981: Black 1965).

Isolation and counting of *Tricoderma hirazanum* fungal colonies

Dilutements (10^{-3} Cuf g⁻¹) were taken from the rhizosphere of onion plants after 90 days of cultivation and the number of *Tricoderma hirazanum* fungal colonies was estimated by the dilution and plate counting method using PDA medium as described in (Black, 1965).

Field Experiment and Experimental Design

A factorial experiment using the R.C.B.D. design was conducted in the field for onion plants in the Nafar - Al-Qadisiyah on 3/10/2025. *Tricoderma hirazanum* fungal inoculum was used at three levels (0, 2, and 4) kg.ha⁻¹, and three levels of agricultural sulfur (0, 30, and 50) kg.ha⁻¹. The third factor was inoculation with *Azotobacter* ssp bacteria at two levels (inoculation and non-inoculation). The field soil was plowed to a depth of (20) cm, perpendicular plowing, and then smoothed and leveled. The field was divided into three replicates, each containing 18 experimental units. The area of the experimental unit was (3 * 3) m². The bulbs were planted in rows within the experimental units, with 40 cm between rows and 15 cm between bulbs. Each row contained 18 bulbs, and there were 6 rows in total, resulting in 108 bulbs in experimental unit. Forty percent of the recommended fertilizer application rate of N-P₂O₅-K₂O (175-120-120 kg.ha⁻¹) was used. A fungal inoculant of *T. harzianum* was added at a rate of (1) g.m². 100 ml of gum arabic at a ratio of 1:10 (gum:water) was used to dip the onion roots into a (24)hour bacterial culture at a bacterial density of (3×10^{12} Cuf ml⁻¹) for (30) minutes to ensure increased adhesion of the bacterial inoculum to the onion seeds. The results were analyzed using the Genstat program (2012) and the LSD test was used at a significance level of 5%, (Omar, S., et al., 2022).

Measured Characteristics

Wet Weight of Onion (g) The bulbs were weighed using a scale.

Protein Estimation (mg.g⁻¹) The plant extract of the bulbs was prepared, and the % nitrogen in the bulbs was estimated using a microKulldhall apparatus. The % protein in the bulbs was calculated using the following equation: N % Concentration x 6.25 (Page et al., 1982).

Ascorbic acid (mg.kg⁻¹)

A standard ascorbic acid solution (500 µg.ml⁻¹) was prepared, and various concentrations were

obtained,(10) grams of the sample were mixed with (50) ml of phosphoric acid, filtered, and iodine solution was added, followed by drops of urea sulfate solution. Urea was then added to all standard solutions and samples, along with 2,4-dihydroxyphenylphosphatene, All were left for three hours at a temperature of 37°C, then placed in an ice bath.(5)ml of(85)% sulfuric acid was added to each sample, and the absorbance was measured using a spectrophotometer as in(Anal et al., 2019).

Results and Discussion

Table 1 indicates that bioinoculation with the fungus (T) *Tricoderma hirazanum* led to a significant increase in the weight of the fresh bulb, with the highest weight at level (4) kg.ha⁻¹ reaching 236.7 (g) compared to the control 171.3 (g). Similarly, bioinoculation with the bacterium (B) *Azotobacter* ssp achieved a significant increase in the weight of the fresh bulbs, reaching 210.7 (g) compared to the control 202.1 (g). The addition of agricultural sulfur at level (50) kg.ha⁻¹ also achieved the highest significant increase in the weight of the fresh bulb, reaching 217.3 (g) compared to the control 194.2 (g). As for the dual interactions in the field experiment, which are (T*S) inoculation with *Tricoderma hirazanum* fungus and agricultural sulfur, (T*B) inoculation with *Tricoderma hirazanum* fungus and *Azotobacter* ssp bacteria, and (S*B) *Azotobacter* ssp bacteria and the addition of agricultural sulfur, they achieved a significant increase in the weight of the onion. The highest dual interaction (T*S) inoculation with *Tricoderma hirazanum* fungus and agricultural sulfur gave a significant increase in the wet weight of the onion at (T₄*S₅₀), as the dual interaction treatment reached 250.0 (g) compared to the control 197.2 (g). The dual interaction (T*B) inoculation with *Tricoderma hirazanum* fungus and *Azotobacter* ssp bacteria gave the highest

significant increase in fresh onion weight at (T₄*B₁), with the dual interaction treatment reaching 237.7 g compared to the control treatment of 168.7 (g). As for the interaction (S*B) of *Azotobacter* ssp bacteria and the addition of agricultural sulfur, it gave the highest significant increase in fresh onion weight at (S₃₀*B₁), with the dual interaction treatment reaching 218.01(g) compared to the control 191.8 (g).

Table 2 indicates that bioinoculation with the fungus *Tricoderma hirazanum* (T) resulted in a significant increase in Ascorbic Acid (mg.kg⁻¹) in the bulb, with the highest level at 4 kg.ha⁻¹, reaching 42.00 mg.kg⁻¹ compared to the control 27.89 mg.kg⁻¹. Similarly, bioinoculation with the bacterium *Azotobacter* ssp (B) also achieved a significant increase in Ascorbic Acid, reaching 34.96 mg.kg⁻¹ compared to the control 33.70 mg.kg⁻¹. Furthermore, the application of agricultural sulfur at the 50 kg.ha⁻¹ level resulted in the highest significant increase in Ascorbic Acid, reaching 37.17 (mg.kg⁻¹) compared to the control 32.39 (mg.kg⁻¹).

The duple interactions in the field experiment, it is (T*S) inoculation with *Tricoderma hirazanum* and agricultural sulfur, and (T*B) inoculation with *Tricoderma hirazanum* and *Azotobacter* ssp, and (S*B) *Azotobacter* ssp with the addition of agricultural sulfur, resulted in a significant increase in ascorbic acid. The highest of the duple interaction (T*S), inoculation with *Tricoderma hirazanum* and agricultural sulfur, resulted in a significant increase in fresh onion weight at (T₄*S₅₀), reaching 43.6 mg.kg⁻¹ compared to the control treatment of 24.67 mg.kg⁻¹. The duple interaction (T*B), inoculation with *Tricoderma hirazanum* and *Azotobacter* ssp, gave the highest significant increase in Ascorbic Acid at (T₄*B₁), as the dual interaction treatment reached 42.22 (mg.kg⁻¹) compared to the control 27.78 (mg.kg⁻¹), (Alabadi,

Table 1 - Effect of mono study factors and duple interaction on the weight of the wet onion (g)

trichoderma(T)kg.ht ⁻¹	sulfur (S) kg.ht ⁻¹	Inoculation Biological(B)		Mine T
0	0	Noincoulatin Aztobacter ssp (B ₀)	Inoculation Aztobacter ssp (B ₁)	171.3
2	30			214.2
4	50			236.7
Mine (B)		202.1	210.7	Lsd _T 13.69
Mine (s)		194.2	210.6	217.3
Intraction (T*S)		160.2	169.3	184.3
		198.8	226.0	217.7
		223.5	236.5	250.0
Intraction (T*B)		168.7	217.3	Lsd _{T*B} 14.02
		207.9	220.4	
		236.7	237.7	
Intraction (S*B)		191.8	217.3	Lsd _{S*B} 10.50
		203.9	218.1	
		216.6	210.7	
Lsd _S 10.24		Lsd _{T*S} 17.63		Lsd _B 7.77

Table 2 - Effect of mono study factors and duple interaction on Ascorbic Acid (mg.kg⁻¹)

Trichoderma(T) kg.h ⁻¹	sulfur (S) kg.h ⁻¹	Inoculation Biological(B)		Mine T
		Inoculation Azotobacter ssp (B ₀)	Noninoculation Azotobacter ssp (B ₁)	
0	0			27.89
2	30			34.61
4	50			42.00
Mine (B)		33.70	34.96	Lsd _T 0.218
Mine (S)		32.39	34.94	37.17
Intracction (T*S)		24.67	28.00	31.00
		32.67	34.33	36.83
		39.83	42.10	43.67
Intracction (T*B)		27.78	28.00	Lsd _{T*B} 0.689
		34.56	34.67	
		41.78	42.22	
Intracction (S*B)		32.22	32.56	Lsd _{S*B} 0.761
		34.89	35.00	
		37.00	37.44	
Lsd _S 0.407		Lsd _{T*S} 0.592		Lsd _B 0.550

L. et al., 2018). As for the interaction of *Azotobacter* ssp bacteria (S*B) and the addition of agricultural sulfur, it gave the highest significant increase in Ascorbic Acid at (S₃₀*B₁), as the dual interaction treatment reached 37.44 (mg.kg⁻¹) compared to the control 37.44 (mg.kg⁻¹).

Table 3 indicates that bioinoculation with the fungus (T) *Trichoderma hirazanum* led to a significant increase in protein (mg.g⁻¹) in the onion, with the highest increase at level (4) kg.ha⁻¹ reaching 1.12 (mg.g⁻¹) compared to the control 0.93 (mg.g⁻¹). Similarly, bioinoculation with the bacterium (B) *Azotobacter* ssp achieved a significant increase in protein, reaching 1.05 (mg.g⁻¹) compared to the control 1.00 (mg.g⁻¹). The addition of agricultural sulfur at level (50) kg.ha⁻¹ also achieved the highest significant increase in protein, reaching 1.05 (mg.g⁻¹) compared to the control 1.02 (mg.g⁻¹).

As for the dual interactions in the field experiment, which are (T*S) inoculation with *Trichoderma hirazanum* fungus and agricultural sulfur, (T*B) inoculation with *Trichoderma hirazanum* fungus and *Azotobacter* ssp bacteria, and (S*B) *Azotobacter* ssp bacteria and the addition of agricultural sulfur, they achieved a significant increase in protein. The highest dual interaction (T*S) inoculation with *Trichoderma hirazanum* fungus and agricultural sulfur gave a significant increase in protein of 1.24 (mg.g⁻¹) at (T₄*S₃₀), as the dual interaction treatment reached 1.29 (mg.g⁻¹) compared to the control 0.83 (mg.g⁻¹). As for the dual interaction (T*B) inoculation with *Trichoderma hirazanum* fungus and *Azotobacter* ssp bacteria, it gave the highest significant increase in protein at (T₄*B₁), as the dual interaction treatment reached 1.24 (mg.g⁻¹) compared to the control 0.92 (mg.g⁻¹). As for the interaction (S*B) of *Azotobacter* ssp

bacteria and the addition of agricultural sulfur, it gave the highest significant increase in protein (mg.g⁻¹) at (S₃₀*B₁), as the dual interaction treatment reached 1.15 (mg.g⁻¹) compared to the control 1.01 (mg.g⁻¹).

Table 4 indicates that bioinoculation with the fungus (T) *Trichoderma hirazanum* led to a significant increase in *Trichoderma* numbers, with the highest increase at level (4) kg.ha⁻¹, reaching 17.33 * 10³ Cuf g⁻¹, compared to the control 5.56 * 10³ Cuf g⁻¹. Similarly, bioinoculation with the bacterium (B) *Azotobacter* ssp achieved a significant increase in *Trichoderma* numbers, reaching 12.59 * 10³ Cuf g⁻¹, compared to the control 11.22 * 10³ Cuf g⁻¹. Furthermore, the addition of agricultural sulfur at level (50) kg.ha⁻¹ resulted in the highest significant increase in *Trichoderma* numbers, reaching 14.17 * 10³ Cuf g⁻¹. Compared to the comparator treatment 10.50*10³ Cuf g⁻¹.

As for the duple interactions in the field experiment, which are (T*S) inoculation with *Trichoderma hirazanum* fungus and agricultural sulfur, and (T*B) inoculation with *Trichoderma hirazanum* fungus and *Azotobacter* ssp bacteria, and (S*B) *Azotobacter* ssp bacteria and the addition of agricultural sulfur, they achieved a significant increase in the number of *Trichoderma*. The highest dual interaction (T*S) inoculation with *Trichoderma hirazanum* fungus and agricultural sulfur gave a significant increase in the number of *Trichoderma* at (T₄*S₅₀), as the dual interaction treatment reached 17.33* 10³ Cuf g⁻¹ compared to the control 2.67* 10³ Cuf g⁻¹. The duple inoculation (T*B) treatment, inoculating with *Trichoderma hirazanum* and *Azotobacter* ssp, resulted in the highest significant increase in *Trichoderma* numbers at (T₄*B₁), with the duple-inoculation treatment reaching 17.71 * 10³ Cuf g⁻¹ compared to the control 5.11 * 10³ Cuf g⁻¹. Similarly,

Table 3 - Effect of mono study factors and duple interaction on protein (mg . g⁻¹)

trichoderma(T) kg.ht ⁻¹	sulfur kg.ht ⁻¹ (S)	Inoculation Biological(B)		Mine T
0	0	Inoculation Azotobacter ssp (B ₀)	noninoculation Azotobacter ssp (B ₁)	0.93
2	30			1.10
4	50			1.12
Mine (B)		1.00	1.09	Lsd _T 0.009
Mine (s)		1.02	1.15	1.05
Intracction (T*S)		0.83	0.94	1.02
		1.05	1.10	1.14
		1.18	1.29	1.28
Intracction (T*B)		0.92	0.94	Lsd _{T*B} 0.02
		1.09	1.10	
		1.23	1.34	
Intracction (S*B)		1.01	1.03	Lsd _{S*B} 0.02
		1.09	1.10	
		1.13	1.15	
Lsd _S 0.004		Lsd _{T*S} 0.01		Lsd _B 0.015

Table (4) :Effect of mono study factors and duple interaction on the number of *Trichoderma* 10³ Cuf.

trichoderma(T) kg.ht ⁻¹	sulfur (S) kg.ht ⁻¹	Inoculation Biological(B)		Mine T
0	0	Inoculation Azotobacter ssp (B ₀)	noninoculation Azotobacter ssp (B ₁)	5.56
2	30			14.33
4	50			17.33
Mine (B)		11.22	12.59	Lsd _T 0.796
Mine (s)		10.50	12.56	14.17
Intracction (T*S)		2.67	5.50	8.50
		12.00	14.83	16.17
		16.83	17.33	17.83
Intracction (T*B)		5.11	6.00	Lsd _{T*B} 0.92
		14.00	14.67	
		17.56	17.71	
Intracction (S*B)		10.33	10.67	Lsd _{S*B} 0.731
		12.44	12.67	
		13.89	14.44	
Lsd _S 0.313		Lsd _{T*S} 0.805		Lsd _B 0.556

the duple inoculation (S*B) treatment, inoculating with *Azotobacter* ssp and adding agricultural sulfur, resulted in the highest significant increase in *Trichoderma* numbers at (S₃₀*B₁), with the duple inoculation treatment reaching 14.44 * 10³ Cuf g⁻¹ compared to the control 10.33 * 10³ Cuf g⁻¹.

Table 5 indicates that bioinoculation with the fungus (T) *Trichoderma hirazanum* led to a significant increase in the number of *Azotobacter* ssp bacteria at 10⁷ Cuf g⁻¹, with the highest concentration at level (4) kg.ha⁻¹ (3.88 * 10⁷ Cuf g⁻¹) compared to the control 2.40 * 10⁷ Cuf g⁻¹. Similarly, bioinoculation with the bacteria (B) *Azotobacter* ssp achieved a significant increase in the number of *Azotobacter* ssp bacteria, reaching 2.92 * 10⁷ Cuf g⁻¹ compared to the control 2.88 * 10⁷ Cuf g⁻¹. The addition of agricultural sulfur at level (50) kg.ha⁻¹ also achieved the highest significant increase in the number of *Azotobacter* ssp bacteria. It reached 2.96* 10⁷ Cuf g⁻¹

compared to the treatment of 3.02* 10⁻⁷ Cuf g⁻¹.

As for the dual interactions in the field experiment, which are (T*S) inoculation with *Trichoderma hirazanum* fungus and agricultural sulfur, and (T*B) inoculation with *Trichoderma hirazanum* fungus and *Azotobacter* ssp bacteria, and (S*B) *Azotobacter* ssp bacteria and the addition of agricultural sulfur, they achieved a significant increase in the numbers of *Azotobacter* ssp bacteria. The highest dual interaction (T*S) inoculation with *Trichoderma hirazanum* fungus and agricultural sulfur gave a significant increase in the numbers of *Azotobacter* ssp bacteria at (T₄*S₅₀), as the dual interaction treatment reached 4.11 * 10⁷ Cuf g⁻¹ compared to the control 2.56 * 10⁻⁷ Cuf g⁻¹. As for the dual interaction (T*B) inoculation with *Trichoderma hirazanum* fungus and *Azotobacter* ssp bacteria, it gave the highest significant increase in the number of *Azotobacter* ssp bacteria at (T₄*B₁), as the duple interaction treatment reached 4.97 * 10⁷ Cuf g⁻¹

Table 5 - Effect of mono study factors and dupe interaction on the number of *Azotobacter* ssp 10^{-7} CUF.

trichoderma(T) kg.ht ⁻¹	sulfur (S) kg.ht ⁻¹	Inoculation Biological(B)		MineT
0	0	Inoculation <i>Azotobacter</i> ssp(B ₀)	noninoculation <i>Azotobacter</i> ssp(B ₁)	2.40
2	30			2.43
4	50			3.88
Mine (B)		2.88	2.92	Lsd _T 0.15
Mine (s)		2.72	3.02	2.96
Intracction (T*S)		2.56	2.49	2.15
		2.27	2.48	2.54
		3.34	4.10	4.10
Intracction (T*B)		2.41	2.43	Lsd _{T*B} 0.25
		2.44	2.45	
		3.78	4.97	
Intracction (S*B)		2.63	2.82	Lsd _{S*B} 0.26
		2.06	2.98	
		2.95	2.97	
Lsd _S 0.14		Lsd _{T*S} 0.22		Lsd _B 0.18

g compared to the control $2.41 \times 10^{-7} \cdot \text{CUF g}^{-1}$. As for the interaction (S*B) of *Azotobacter* ssp bacteria and the addition of agricultural sulfur, it gave the highest significant increase in the number of *Azotobacter* ssp bacteria at (S30*B1), as the dual interaction treatment reached $2.06 \times 10^{-7} \cdot \text{CUF g}^{-1} \times 10^{-7} \cdot \text{CUF g}^{-1}$ compared to the control $2.98 \times 10^{-7} \cdot \text{CUF g}^{-1}$.

The results in Tables (1, 2, 3, 4, and 5) show that the mono inoculation of *Trichoderma hirazanum*, *Azotobacter* ssp, and agricultural sulfur led to significant increases in the studied traits: fresh onion weight (g), protein content (mg.g⁻¹), and ascorbic acid (mg.kg⁻¹). Specifically, the concentrations of *Azotobacter* ssp (10^7 CUF.g⁻¹) and *Trichoderma hirazanum* (10^3 CUF.g⁻¹) in the onion rhizosphere were significantly higher. The addition increase with the mono additions was less than the significant increase resulting from the interaction of these three factors. This is attributed to the important role of *T. hirazanum* in supplying a significant portion of the onion's nitrogen, phosphorus, potassium, and iron requirements by secreting various organic acids that dissolve non metallic compounds. Soluble and release nutrients from it to be available for absorption by the plant, as well as secreting many vitamins, enzymes and some growth hormones that encourage an increase in the number of branches and root density such as All, AG₃ and others (Kurrey, et al., 2018). Inoculation of onion plants with *Azotobacter* ssp bacteria led to an increase in all the study indicators, This bacterium works to fix atmospheric nitrogen in the soil, and nitrogen is very important in the metabolic activities of the plant, as it is a limiting factor for production (Mahato et al., 2009). Onions also need abundant amounts of nitrogen. The effectiveness of the bacterial inoculation was evident in increasing the traits studied above. This is

because *Azotobacter* ssp bacteria provide the bacteria with organic acids after their death and decomposition in the soil (Kushwaha and Hasan 2010). These acids also increase the availability of nutrients to the plant. This bacterium also produces many growth regulators, vitamins, hormones, and vitamins in the plant's rhizosphere (Raie et al. 2017; Jayathilake., 2006). Field experiments revealed that agricultural sulfur significantly increased the studied traits in onion plants. This is because sulfur lowers soil pH, thus increasing the availability of nutrients, which in turn enhances fungal activity and nutrient availability, such as nitrogen, phosphorus, and potassium. Conversely, high sulfur levels resulted in decreased sulfur content, possibly due to lower rhizosphere pH (Latif, 2013). This led to a decrease in protein content in the bulbs due to reduced nitrogen availability (Kushwaha, et al., 2010). In the opposite direction, high sulfur levels increased the number of *T. hirazanum* fungi due to increased acidity, there by enhancing their ability to thrive in acidic environments. The combined effect of these experimental factors resulted in a significantly higher increase in the studied traits than single application of any single agent (Mishu., 2013). This was attributed to the accumulation of nutrients in the plant and soil, which positively impacted on onion productivity in the experiment (Kumare et al., 2017). The positive interaction between the fungus *T. hirazanum* and the bacteria *Azotobacter* ssp led to the provision of nitrogen and other nutrients, increased the availability of beneficial compounds for plant growth, and boosted productivity (Altomare et al., 2015). This reflects the non antagonistic relationship between these microorganisms in the rhizosphere of onion plants. The application of these agents under the agricultural environmental conditions in our region resulted in a 60% reduction in the original

fertilizer recommendation, thus minimizing environmental pollution and improving soil health and yield. Further studies are needed to determine the effect of agricultural sulfur on the density and activity of important bacterial and fungal communities involved in biofertilization within the rhizosphere of crops.

Conclusion

The interaction between the study factors led to the highest significant increase in the studied traits in the treatment ($T_4 \times B_1$), the addition of *T. hirazanum* fungus at a level of $(4) \text{ kg.ha}^{-1}$ + *Azotobacter* ssp inoculation fresh onion weight (g), protein content (mg.g^{-1}), ascorbic acid (mg.kg^{-1}), *Azotobacter* ssp counts (10^7 CFU g^{-1}), and *Trichoderma hirazanum* counts (10^3 CFU g^{-1}) in the onion rhizosphere.

High sulfur levels led to a decrease in *Azotobacter* ssp inoculation counts and protein content, while onion weight, ascorbic acid, and fungal counts.

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